

Abstract Book



SETAC North America 46th Annual Meeting

16-20 NOVEMBER 2025

PORTLAND, OREGON

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THE ESSENCE OF
SCIENCE: CURIOSITY,
DISCOVERY AND SOLUTIONS

Abstract Book

SETAC North America 46th Annual Meeting

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This book comprises the abstracts from the 46th annual meeting of the Society of Environmental Toxicology and Chemistry – North America (SETAC North America), conducted from 16–20 November 2025 in Portland, Oregon.

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

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About SETAC

The Society of Environmental Toxicology and Chemistry (SETAC) is a global nonprofit professional society whose main objective is to advance environmental science and management through education, collaboration, communication and leadership.

The specific purposes of the society are:

- Provide life-long learning and professional development opportunities for all career stages,
- Foster collaboration by increasing membership, engagement, diversity and partnerships,
- Provide world-class science communication forums for members and stakeholders,
- Develop leadership of SETAC and its members in the environmental science and environmental management community,
- Participate in any and all other activities permitted under the Act and which are not inconsistent with the Corporation's main objective and qualifications as an organization described in Section 501(c)(6) of the Code.

These goals are pursued through the conduct of numerous programs including events, publications, awards and education programs.

SETAC members come 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 are an environmental professional with training in these or related disciplines and are engaged in the study, use or management of environmental resources, SETAC can be your professional home.

SETAC publishes peer reviewed scientific research in its journals Environmental Toxicology and Chemistry (ET&C) and Integrated Environmental Assessment and Management (IEAM).

About the Abstract Book

The titles, authors and abstracts of the presentations given at the meeting are summarized in this book. Acknowledgments and disclosures are not included for space considerations, though we recommend that authors include them in the presentations themselves. Abstracts are not peer-reviewed and therefore should not be cited.

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Environmental Quality Through Science®

Daily Schedule

MONDAY, 17 NOVEMBER		
TIME	GENERAL PROGRAM	ROOM
7:30-18:30	Coat and Luggage Check	Holladay Lobby
7:30-17:30	Badge Pickup and Help Desk	Pre-Function A
7:30-17:30	Speaker Ready Room	C125-126
8:00-8:30	New Member Welcome	Oregon Ballroom 202
8:30-9:15	Amy Cordalis, Author and Activist	Oregon Ballroom 203
9:15-10:15	Resume Development Mini Workshop	D135
12:30-13:30	Bayer Sponsored Session: Cross-Sector Science in an Uncertain Environment	D135
16:00-17:00	Carolinas Regional Chapter	D135
16:30-17:30	Aquatic Toxicity Testing Interest Group	D136
17:00-18:00	A4 Interest Group	D137
17:30-18:30	SETAC North America Regional Chapters Leadership Meeting	G130
17:30-18:30	Chemistry Interest Group	D135
17:30-19:00	Metals Interest Group	G131-132
17:30-19:00	Student/Mentor Mix and Mingle	C120-121
18:00-19:00	Biologics Interest Group	D136

TUESDAY, 18 NOVEMBER		
TIME	GENERAL PROGRAM	ROOM
7:20-8:30	Fun Run	Meet at Registration
8:00-18:30	Coat and Luggage Check	Holladay Lobby
8:00-17:30	Badge Pickup and Help Desk	Pre-Function A
8:00-17:30	Speaker Ready Room	C125-126
8:30-9:15	Kaci Buhl, Oregon State University	Oregon Ballroom 203
9:00-11:00	IBERA Information Breakfast Session	D135
12:00-13:30	Women in SETAC Luncheon	C123-124
12:15-13:15	Southeast Regional Chapter	G130
13:00-13:30	North America Student Advisory Council	D135
14:30-15:15	Mixer! EcoTALKS of Amphibians and Reptiles	D135
16:00-17:00	Endangered Species Interest Group	G130
16:00-17:00	Pacific Northwest Regional Chapter	D136
17:00-18:00	Plants Interest Group	D135
17:30-18:30	Wildlife Toxicology Interest Group	D136
17:30-19:00	Early Career Social	C120-121
18:00-19:30	Chesapeake-Potomac Regional Chapter	G130
19:00-22:00	Stumptown Science: An After-Hours Adventure at the Museum	Oregon Museum of Science and Industry (offsite)

WEDNESDAY, 19 NOVEMBER		
TIME	GENERAL PROGRAM	ROOM
8:00-18:30	Coat and Luggage Check	Holladay Lobby
8:00-17:30	Badge Pickup and Help Desk	Pre-Function A
8:00-17:30	Speaker Ready Room	C125-126
8:30-9:15	Eric Van Genderen, International Zinc Association	Oregon Ballroom 203
8:30-9:30	ASTM E50.47 Annual Meeting	G130
12:00-13:30	Student Lunch Seminar	C123-124
12:00-13:30	Trifluoroacetic Acid Mini-Workshop	G130
13:30-15:00	East Palestine, Ohio Rail Discussion	Hyatt, Columbia 2 (offsite)
16:00-17:00	Animal Alternatives Interest Group	G130
16:30-17:30	Plastics Interest Group	D135
16:30-17:30	Special Session: Supporting Environmental Science Within an Adverse Geopolitical Climate	G131-132
17:00-18:00	Endocrine Disrupter Interest Group	D136
17:30-18:30	South-Central Regional Chapter	D137
17:30-19:00	Exposure Modeling Interest Group	D135
17:30-19:00	SETAC General Assembly	G131-132
19:00-22:00	Student Trivia and Mixer	Spirit of 77 (offsite)

THURSDAY, 20 NOVEMBER		
TIME	GENERAL PROGRAM	ROOM
8:00-16:30	Coat and Luggage Check	Pre-Function A
8:00-15:30	Badge Pickup and Help Desk	Pre-Function A
8:00-13:30	Speaker Ready Room	C125-126
12:00-13:30	Program Committee Luncheon (by invitation)	C123-124
15:30-16:00	Closing Reception	Oregon Ballroom Lobby
16:00-16:30	Closing Remarks	Oregon Ballroom 203

POSTER SESSIONS 9:15 -10:00 15:30 -17:30	
Poster sessions beyond those paired with oral sessions	
5.02.P-Mo	Advances in Human Health Risk Assessment
5.09.P-Mo	New Approaches, Data and Solutions Regarding the Environmental Risks of Sunscreens and Cosmetics
5.11.P-Mo	Pharmaceuticals in the Environment—Global Innovations in Risk Assessment, Regulation and Science
7.05.P-Mo	Science Translation in Regulatory Programs and Impacts to Local Water Quality Improvement Efforts
2.14.P-Mo	Aquatic Toxicology, Ecology and Stress Response
1.16.P-Mo	Environmental (Human and Ecological) Toxicology and Stress Response
3.05.P-Mo	Wildlife Toxicology, Ecology and Stress Response

POSTER SESSIONS 9:15 -10:00 15:30 -17:30	
Poster sessions beyond those paired with oral sessions	
2.10.P-Tu	New Advances in the Ecotoxicology of Coral Reef Organisms
6.05.P-Tu	Engineering, Remediation and Restoration
5.15.P-Tu	Environmental (Human and Ecological) Risk Assessment
8.04.P-Tu	Systems Approaches

POSTER SESSIONS 9:15 -10:00 15:30 -17:30	
Poster sessions beyond those paired with oral sessions	
4.03.P-We	Bioactivity-Driven Chemical Assessment: Enhancing Risk Evaluation of Complex Mixtures
1.04.P-We	Cell-Based Approaches for Ecotoxicity Assessments
1.07.P-We	Environmental Science Education
4.13.P-We	Greener and Smarter Sample Preparation for PFAS and Contaminants of Emerging Concern (CECs): Innovations and Sustainable Approaches
1.15.P-We	Quantitative Structure-Activity Relationship (QSAR) Models and Tools in Environmental Toxicology and Chemistry
4.20.P-We	Chemistry and Exposure Assessment
7.07.P-We	Policy, Management and Communication

LATE-BREAKING SCIENCE POSTER SESSIONS 8:00 -10:00	
Poster sessions beyond those paired with oral sessions	
2.15.P-Th	Aquatic Toxicology, Ecology and Stress Response
4.21.P-Th	Chemistry and Exposure Assessment
6.06.P-Th	Engineering, Remediation and Restoration
5.16.P-Th	Environmental (Human and Ecological) Risk Assessment
1.17.P-Th	Environmental (Human and Ecological) Toxicology and Stress Response
7.08.P-Th	Policy, Management and Communication
8.05.P-Th	Systems Approaches
3.06.P-Th	Wildlife Toxicology, Ecology and Stress Response



MONDAY PLATFORM SESSIONS 10:00 -12:00		
ROOM	SESSION TITLE	*Accompanied by a poster session
A105-106	1.05.T Empirical and Theoretical Methods to Scale Contaminant Effects: Molecular Impacts to Organism Health*	
A107-109	4.19.T Use of Chemical Hazard Assessments to Drive Informed Substitution in Alternative Assessments*	
B110-112	3.04.T Wildlife Toxicology Across the Full Annual Cycle*	
B113-114	1.06.T Enhancing Endocrine System Evaluation: Exploring Opportunities and Insights From Lessons Learned*	
B115-116	4.16.T Mass Spectral Solutions to Environmental and Toxicological Challenges*	
B117-119	5.08.T Beyond Direct Contact: Non-Traditional Exposure Scenarios in Human Health Risk Assessment	
Oregon Ballroom 201	4.08.A.T Environmental Exposure to PFAS From Non-Traditional Matrices: Exploring the Where and How?*	
Oregon Ballroom 202	2.08.T Investigating the Sources and Toxicity of Salt and Multi-Ion Mixtures to Support Resource Management*	
Oregon Ballroom 203	2.04.A.T Exposure and Effects of Micro- and Nanoplastics in the Environment*	
Oregon Ballroom 204	2.13.A.T Stormwater Runoff Impacts, Solutions and Innovative Research*	

TUESDAY PLATFORM SESSIONS 10:00 -12:00		
ROOM	SESSION TITLE	*Accompanied by a poster session
A105-106	5.06.T Almost All Things Related to Endangered Species Assessment*	
A107-109	3.02.T Ecotoxicology and Risk Assessment in Marine Coastal Ecosystems*	
B110-112	5.03.T Advances in Understanding Hydrocarbon Toxicity: Impacts, Mechanisms and Mitigation*	
B113-114	5.13.T Soil Contaminants: Fate, Bioavailability Environmental Toxicology in Ecological Risk Assessment*	
B115-116	1.02.T Applications of Integrative Omics in Ecotoxicology*	
B117-119	7.04.T Risk Communication—the Essence of Science Solutions*	
Oregon Ballroom 201	2.02.T Assessing Toxicity in Water and Aquatic Sediments*	
Oregon Ballroom 202	4.17.A.T Mercury Biogeochemistry, Exposure Pathways and Risk to Wildlife*	
Oregon Ballroom 203	4.12.A.T From Tread to Watershed: How Tire Wear Particles and Chemicals Affect Aquatic and Terrestrial Ecosystems*	
Oregon Ballroom 204	4.01.A.T Advancing Science Through Current Approaches in Environmental Monitoring*	

WEDNESDAY PLATFORM SESSIONS 10:00 -12:00		
ROOM	SESSION TITLE	*Accompanied by a poster session
A105-106	4.07.A.T Environmental Contaminants in Animals: Exposure, Pharmacokinetics and Risk Assessment*	
A107-109	2.11.A.T One Health: Detection, Fate, Effects, Monitoring and Management of Benthic and Pelagic Harmful Algal Blooms (HABs)	
B110-112	6.01.T Case Studies in Natural Resource Damage Assessment and Restoration	
B113-114	4.14.T Human Exposure to Organic Contaminants of Current and Emerging Concern*	
B115-116	4.11.T Fate and Effects of Metals: Biogeochemical Perspective	
B117-119	4.15.T In Memoriam: Katherine Peter—Expanding Environmental Horizons to Unknown Limits	
Oregon Ballroom 201	1.11.T Microbial Interactions With Environmental Contaminants: Molecular Mechanisms to Ecosystem Effects	
Oregon Ballroom 202	4.10.A.T Environmental Forensics*	
Oregon Ballroom 203	2.03.T Ever-Present but Unknown Consequences: Sources, Fate and Transport, and Effects of Microfiber Pollution*	
Oregon Ballroom 204	1.01.A.T Alternative Approaches and New Approach Methods (NAMs) that Refine, Replace or Reduce Animal Testing*	

THURSDAY PLATFORM SESSIONS 10:00 -12:00		
ROOM	SESSION TITLE	*Accompanied by a poster session
A105-106	1.13.T Pesticides as Multiple Stressors*	
A107-109	5.12.T Potential Environmental Impacts of CO2 Releases From Carbon Capture and Storage (CCS) Activities*	
B110-112	7.02.A.T Inspiring Next Generation of Young Scientists – High School Students Share Their Research	
B113-114	5.01.T Advances and Applications of Probabilistic Risk Assessment*	
B115-116	4.02.T Automation of Methods for Environmental Contaminants: PFAS, Dioxins, PCBs, Pesticides and Beyond*	
B117-119	1.10.T Innovative and Novel Applications of New Approach Methodologies: From Tool Development to Case Studies*	
Oregon Ballroom 201	2.06.T Fate and Effects of PFAS in Coastal Ecosystems*	
Oregon Ballroom 202	1.12.T Nano and Advanced Materials Safety: Research Progress, Applications and Challenges*	
Oregon Ballroom 203	6.02.T Interventions to Reduce Environmental Microplastics*	
Oregon Ballroom 204	4.18.A.T Occurrence, Fate and Effects of Contaminants in Domestic, Agricultural, Landfill and Industrial Waste*	

MONDAY PLATFORM SESSIONS 13:30 -15:30		
ROOM	SESSION TITLE	*Accompanied by a poster session
A105-106	1.03.T Beyond the Model Organism: New Approaches for Predicting Cross Species Toxicity*	
A107-109	7.01.T Indigenous Knowledge and Chemicals Management*	
B110-112	3.03.T Herplandia!*	
B113-114	5.04.T Advancing Bioaccumulation Science: Novel Approaches and Challenges*	
B115-116	8.02.T Unlocking Insights From Multivariate Environmental Data*	
B117-119	4.06.T Drop by Drop: Natural and Anthropogenic Drinking-Water Contaminant Exposures and Human Health Risk*	
Oregon Ballroom 201	4.08.B.T Environmental Exposure to PFAS From Non-Traditional Matrices: Exploring the Where and How?*	
Oregon Ballroom 202	2.05.T Fate and Effects of Metals: Aquatic Biological Perspective*	
Oregon Ballroom 203	2.04.B.T Exposure and Effects of Micro- and Nanoplastics in the Environment*	
Oregon Ballroom 204	2.13.B.T Stormwater Runoff Impacts, Solutions and Innovative Research*	

TUESDAY PLATFORM SESSIONS 13:30 -15:30		
ROOM	SESSION TITLE	*Accompanied by a poster session
A105-106	1.14.T Pollution Impacts on Endemic and Threatened Species—A Focus on Metals and Persistent Pollutants*	
A107-109	8.01.T Real World Pacific Northwest: What It Takes to Build Solutions for Endangered Species and Agriculture	
B110-112	3.01.T DDT+ : New Perspectives on a Historic Contaminant*	
B113-114	5.05.T Advancing Effect Modeling for ERA: Bridging Scales, Data Gaps and Regulatory Challenges*	
B115-116	1.09.T Immunotoxicological Investigations: From Molecules to Model Organisms, Wildlife, and the Environment*	
B117-119	7.03.T Navigating the Misinformation Maze: The Scientist's Mission to Preserve Scientific Integrity*	
Oregon Ballroom 201	5.10.T PFAS in Our Food: From Farms and Fields to Streams and Shores*	
Oregon Ballroom 202	4.17.B.T Mercury Biogeochemistry, Exposure Pathways and Risk to Wildlife*	
Oregon Ballroom 203	4.12.B.T From Tread to Watershed: How Tire Wear Particles and Chemicals Affect Aquatic and Terrestrial Ecosystems*	
Oregon Ballroom 204	4.01.B.T Advancing Science Through Current Approaches in Environmental Monitoring*	

WEDNESDAY PLATFORM SESSIONS 13:30 -15:30		
ROOM	SESSION TITLE	*Accompanied by a poster session
A105-106	4.07.B.T Environmental Contaminants in Animals: Exposure, Pharmacokinetics and Risk Assessment*	
A107-109	2.11.B.T One Health: Detection, Fate, Effects, Monitoring and Management of Benthic and Pelagic Harmful Algal Blooms (HABs)	
B110-112	6.03.T Natural Resource Damage Assessment: Applied Toxicology for Injury Determination*	
B113-114	2.12.T Sediment Matters: Sediment Toxicity and Bioavailability Assessment, Remediation and Recovery*	
B115-116	2.01.T Assessing Contaminant Effects in Ecosystems With Multiple Stressors*	
B117-119	2.07.T Innovative Toxicity Endpoints: Behavior and Beyond*	
Oregon Ballroom 201	5.14.T Trifluoroacetic Acid in the Environment—Here Today, More Tomorrow*	
Oregon Ballroom 202	4.10.B.T Environmental Forensics*	
Oregon Ballroom 203	7.06.T The Growing Interface Between Science and Policy: Challenges and Strategies for Making Progress*	
Oregon Ballroom 204	1.01.B.T Alternative Approaches and New Approach Methods (NAMs) that Refine, Replace or Reduce Animal Testing*	

THURSDAY PLATFORM SESSIONS 13:30 -15:30		
ROOM	SESSION TITLE	*Accompanied by a poster session
A105-106	5.07.T Assessing Risks to Non-Target Terrestrial Invertebrates: Current Status and Future Refinements*	
A107-109	2.09.T Large River Monitoring: Programs, Design, Methods and Interpretation*	
B110-112	7.02.B.T Inspiring Next Generation of Young Scientists – High School Students Share Their Research	
B113-114	6.04.T Remediating Sediment Sites by Modifying Bioavailability, Degradation and Accessibility*	
B115-116	4.09.T Environmental Fate: Elucidating the Mechanisms and Kinetics of Chemical Transformation Products*	
B117-119	4.05.T Contaminant Property Measurement and Estimation: Methods and Applications*	
Oregon Ballroom 201	1.08.T From Acclimation to Adaptation: Mechanisms, Costs and Consequences of Acquired Pollution Resistance*	
Oregon Ballroom 202	8.03.T Where the Alternatives Meet the Road: Finding Substitutes for 6PPD in Vehicle Tires*	
Oregon Ballroom 203	4.04.T Connecting Plastics and Plastic-Associated Chemicals, From Analytical Methodologies to Ecotoxicology*	
Oregon Ballroom 204	4.18.B.T Occurrence Fate and Effects of Contaminants in Domestic, Agricultural, Landfill and Industrial Waste*	

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PLATINUM LEVEL



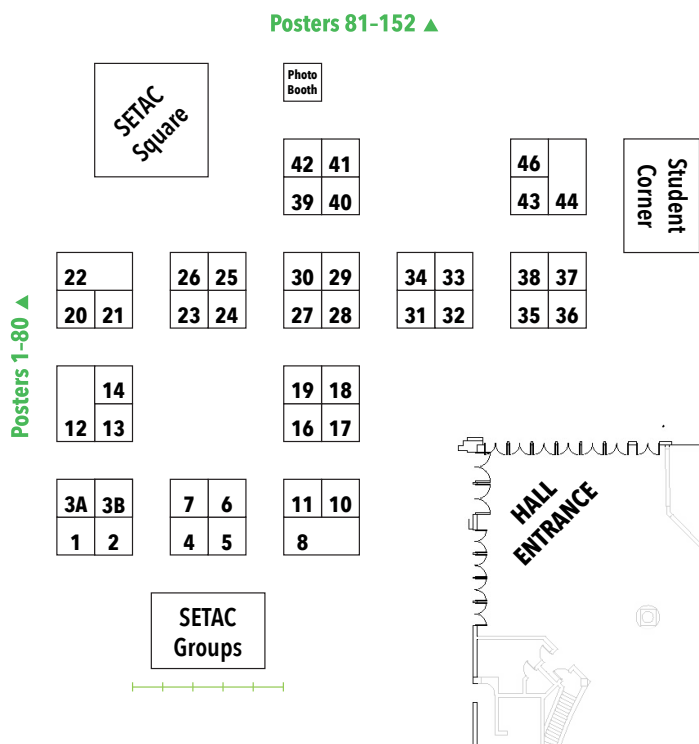
GOLD LEVEL



SILVER LEVEL

Exhibitors and Floor Plan

BOOTH NO.	EXHIBITOR LISTING
44	Agilent
4	ALS USA Environmental Labs
7	Aqua Science LLC
37	Baylor University
34	Aquatic Enterprises Inc.
25	Blue Frog Scientific
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40	TDI-Brooks
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20	Tentamus N.A.
29	Tetra Tech
13	Texas Tech University Department of Environmental Toxicology
31	UCT
16	Waters Corporation
35	Wellington Laboratories



1.01.A.T Alternative Approaches and New Approach Methods (NAMs) that Refine, Replace or Reduce Animal Testing

1.01.A.T-01 Study on extracellular metabolomics-based early indication of drug-induced liver injury (DILI) using HepaRG cell line

Dahyeon Hwang and Tae-Yong Jeong, Hankuk University of Foreign Studies, South Korea

Liver plays a fundamental role in metabolism of chemicals produced both in and out of the body. The liver has a unique metabolic mechanism that is the major organ for the degeneration of xenobiotics introduced through the gastrointestinal tract. Among various chemical substances metabolized, pharmaceutical substances that are increasing in industrial and residential usages are of high importance for their interference effects within the liver due to the enzymatic reactivity and chemical species diversity. Currently, drug-induced liver injury (DILI) is a concern in the drug development process. In vitro tests apply cytotoxicity, oxidative stress, and other molecular dysfunction as endpoints; however, these assays take a relatively long period and harvests exposed cells that limit observation of time- and physiology-dependent cellular and molecular responses. Therefore, in this study, we applied extracellular metabolomics, a non-destructive metabolomics methodology, to a traditional cytotoxicity assay for investigating whether the known DILI and non-DILI drugs show distinct profiles in the extracellular metabolome. We hypothesize that changes in abundance of liver cell-derived biomolecules will be correlated with the magnitude of subsequent hepatotoxicity. We additionally expected that whether a drug is DILI or not could be predictable from the metabolomic profile patterns. HepaRG cell line was used for this experiment. Three DILI and non-DILI pharmaceutical substances were respectively selected, and exposure tests were conducted at concentrations showing high and low degree of hepatotoxicity. The exposed cells and the cell culture media were sampled 5 days later, pretreated, and analyzed using LC-QTOF-MS/MS. As results, we identified biomolecular differences in abundance for both intra- and extra-cellular, between the control and exposure groups. Furthermore, potential intracellular biomarkers for hepatotoxicity were identified through analysis of major metabolic pathways of biomolecules exhibiting significant differences between the groups. There were variations as well within the control group depending on the sampling times. These results contribute to the basis for discovering potential extracellular biomarkers that can indicate DILI and non-DILI hepatotoxicity. At the same time, the study reveals the potentials and limitations that require further studies.

1.01.A.T-02 Electrospun aligned nanofiber yarns constructed biomimetic M-type interface integrated into precise co-culture system as muscle-tendon junction-on-a-chip for drug development

Ting Li¹, Weiwei Su², Ling Wang³ and Yaobin Wu⁴, (1)Columbia University, (2)Department of Human Anatomy, School of Basic Medical Sciences, Southern Medical University, China, (3)School of Biomedical Engineering, Southern Medical University, Guangzhou, China, (4)University of Oxford, United Kingdom

The muscle–tendon junction (MTJ) is a highly specialized interface essential for force transmission between muscle and bone. Due to its complex microstructures and heterogeneous cellular composition, recapitulating the MTJ in vitro remains a significant challenge in musculoskeletal tissue engineering, providing promising in vitro models for understanding cell-cell interaction at the interface between muscle and tendon tissues. Herein, this study established a biomimetic muscle–tendon junction (MTJ) model incorporating a hierarchically organized “M-type” interface structure, fabricated via an interwoven waving technique, and subsequently integrated into a compartmentalized microfluidic co-culture system to construct a functional MTJ-on-a-chip 215. The hierarchically structured nanofibrous scaffold with a 3D “M-type” interface, designed to mimic the native enthesis, was fabricated by an interwoven electrospinning technique. The aligned nanofiber yarns with the scaffold were designed for guiding anisotropic cell alignment and providing biochemical and biophysical cues to induce spatially controlled differentiation into myogenic and tenogenic. This engineered MTJ tissue was further integrated into a microfluidic organ-on-chip 215, establishing a compartmentalized co-culture environment with different media perfusion to induce both muscle and tendon phenotypes. Besides, the MTJ-on-a-chip device further enables be utilized for evaluation of drug-induced toxicity and protective effects at the

muscle–tendon junction. These findings demonstrate that the interwoven nanofibrous scaffolds featuring a biomimetic 'M-type' interface significantly enhance engineered MTJ tissue formation. Moreover, the MTJ-on-a-chip with its precise co-culture system shows great potential as an in vitro 215 for musculoskeletal drug screening and mechanistic studies.

1.01.A.T-03 Leveraging common loss-of-function variants and 3D CRISPR screening to assess population variability and uncover physiologically relevant mechanisms of chemical susceptibility

Chan Hee Kim and Christopher Vulpe, University of Florida, USA

Toxicological risk assessment is essential for evaluating adverse effects of chemical exposures on human health and informing regulatory decisions. A major challenge lies in capturing inter-individual variability, particularly genetic susceptibility, which remains poorly addressed by current approaches. Concurrently, systems biology approaches, such as multi-omics, offer mechanistic insights into chemical toxicity but are largely correlative and do not establish causal relationships. To overcome these limitations, we developed an innovative, CRISPR-based functional toxicology framework aligned with New Approach Methodologies (NAMs) to directly interrogate the role of common genetic variants modulating toxicant responses. We focus initially on common human loss-of-function (LoF) variants in ~1500 genes (termed PopVar LoF) with aggregate mean allele frequency of LoF alleles of >0.1% in the gnomAD V3.0 database, which should capture potential population relevant LOF variants in people which could influence toxicant response. Unexpectedly, the PopVar LoF genes were significantly enriched in gene products in xenobiotic metabolism pathways and associated with metabolism-relevant phenotypes in GWAS and PheWAS databases. CRISPR screens revealed that <1 % of PopVar LoF genes were essential under basal conditions in a hepatic cell line (HepG2/C3A). To improve the physiological relevance of our framework and methodology, we further established a 3D CRISPR screening system using HepG2/C3A liver spheroids cultivated in a bioreactor (ClinoStar). Time-course CRISPR screens of HepG2/CA3 exposed to Doxorubicin grown as 3D spheroids revealed distinct gene essentiality profiles as compared to cells propagated in standard 2D culture, highlighting the physiology-dependent nature of gene function. The 3D system identified more biologically relevant pathways and candidate genes associated with known toxicological mechanisms of Doxorubicin than the 2D counterpart. Altogether, this integrated 215—combining population-informed CRISPR screening with physiologically relevant 3D spheroids—offers a relevant, scalable, and causal approach to investigate the role of common genetic variants in interindividual variation in chemical toxicity. This approach could be applied in a variety of NAMs to enhance our ability to evaluate population variability and susceptibility to environmental toxicants.

1.01.A.T-04 Machine-learning assisted predictive toxicity assessment using *C. elegans* as a new approach methodology

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Developmental and Reproductive Toxicity (DART) assessment is a critical component of product safety evaluation and is traditionally conducted using vertebrate models. However, *C. elegans* has emerged as a promising in vivo alternative, offering advantages such as a short lifecycle, conserved toxicological pathways, high genetic homology with humans, and the availability of diverse genetic backgrounds. Despite these benefits, its broader adoption has been limited by low-throughput methods and a narrow set of measurable endpoints. To address this gap, we have developed a microfluidic-based imaging platform and machine learning (ML)-based analysis pipelines that enable high-throughput, multiparametric analysis of sublethal phenotypes relevant to DART endpoints in *C. elegans*, significantly expanding its utility in toxicity testing. Using 2.5D U-Net architecture, we achieved precise segmentation of *C. elegans* bodies, with an average Dice score of 97.80, and quantified the body length, area, volume of treated organisms with high accuracy. High-resolution imaging enables us to assess reproductive toxicity in adult *C. elegans* by counting and classifying in utero embryos for different developmental stages. The in-utero embryo imaging approach demonstrated a high sensitivity for detecting reproductive toxicity by quantifying embryonic development compared to total embryo counting.

Combining multiple endpoints - body volume, motility, stress-induced autofluorescence, and embryonic phenotypes - improved assay sensitivity, reproducible results, high-statistical power, and predictiveness. We have validated our assay by performing multiparametric toxicity analysis of several agricultural chemicals, including mesotrione, propiconazole, dichlorvos, and chlorpyrifos. Our DART assay yielded highly reproducible results (4–16% coefficient of variation) having high concordance with rat data (Spearman rank correlation ~0.8). In addition, we have developed assays for rapidly assessing systemic toxicity (motility and food consumption) to complement the DART studies and act as internal acceptance criteria for the control groups. 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. Disclaimer/Disclosure A.B., S.M., and E.H. are co-founders of vivoVerse, LLC and are co-authors on several patents. Funding: NIMH (5R44MH118841), NIEHS (R43ES036387, R44ES033579, R43ES033579, and R43MH118841), and USDA (2024-33530-41929).

1.01.A.T-05 The alternative advantage: strengthening predictive power of fish gill cell assays through integrated approaches

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The development of alternative methods for aquatic toxicity assessment remains essential for reducing vertebrate testing while ensuring environmental protection. Building upon previous evaluations of the OECD 249 fish gill cell assay, this study expands our investigation to include additional challenging substance classes with enhanced in silico integration. We evaluated industrially chemicals with varying physicochemical properties to examine how structural characteristics influence the RTgill-W1 assay's predictive capacity, identifying key parameters affecting correlation between in vitro and in vivo toxicity data. We enhanced our assessment through comprehensive QSAR modeling to determine whether computational approaches could improve predictive accuracy when combined with in vitro results. Our previous results demonstrated reasonable interlaboratory reproducibility for the OECD 249 assay, confirming its robustness. However, correlation between in vitro and in vivo results varied between substance groups, with some showing excellent alignment while others displayed weaker correlation. We identified that high calcium content in cell medium may cause substance precipitation, affecting test outcomes. The assay showed good correlation for organic chemicals with baseline toxicity, though specific modes of action remain outside its applicability domain. Methodological challenges included disadvantageous ratios of chemical to adsorption surface and technical difficulties with plate pre-coating procedures that require further optimization. Our findings suggest the assay's applicability domain can be optimized through understanding how chemical properties influence bioavailability and cellular interactions. While offering faster testing times and animal welfare benefits, challenges remain regarding regulatory acceptance and capturing all modes of action relevant to in vivo fish toxicity. The integration of targeted in vitro testing with refined QSAR models offers a promising approach for more accurate toxicity predictions across diverse industrial chemicals. This work contributes to developing an integrated testing strategy combining in vitro, in vivo, and in silico methods to maximize predictive power while minimizing animal testing. By characterizing how chemical variations influence test performance, we provide insights for regulatory acceptance of alternative methods for industrial chemical assessment, particularly for challenging substance classes.

1.01.B.T Alternative Approaches and New Approach Methods (NAMs) that Refine, Replace or Reduce Animal Testing

1.01.B.T-01 Accelerated molecular dynamics and 476 screening to understand per- and polyfluoroalkyl substance (PFAS) interactions with transthyretin

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Thyroid disruption is one health concern caused by exposure to per- and polyfluoroalkyl substances (PFAS) that has been a focus of toxicological research. Transthyretin (TTR) transports thyroid hormone in the blood plasma and cerebrospinal fluid. Binding to TTR can cause the alteration of thyroid signaling and thus serves as an important indicator of the potential for exogenous chemicals to interfere with the thyroid system. While legacy PFAS such as perfluorooctanoic acid (PFOA) have been reported as TTR binders, the binding mechanisms and their thermodynamics responsible for competing with thyroid hormone binding have not been fully investigated. This study employed ligand Gaussian accelerated molecular dynamics (LiGaMD) to reveal the binding and unbinding mechanisms of long- and short-chain PFAS with TTR. The direction of the hydrophobic tail of PFOA toward the inside of the binding pocket during rebinding agrees with the available crystallographic structures, indicating the importance of the contribution of the hydrophobic interaction. While MD is a powerful tool in understanding molecular interactions, there is a lack of a comprehensive comparison of predicted binding energies by different physics-based simulation methods. Thus, energy calculations from LiGaMD, MM-GBSA in conventional MD, and molecular docking were conducted as representatives of simulation methods from high to low sampling efficiency. Notably, LiGaMD was reported to have good performance in energy prediction by sufficiently capturing the binding/unbinding events within a micro-scale simulation time. The calculated energies were compared with experimental values to identify an accurate and efficient method as a supplementing strategy in toxicological evaluation. The binding and dissociation pathways were further compared with diflunisal, a known TTR stabilizer with a fluorinated structure. Finally, representative conformations via clustering LiGaMD trajectory were utilized to compensate for the lack of protein flexibility in static docking methods. The developed docking-based 476 screening was performed on a large PFAS dataset to expand the scope of TTR binding beyond well-studied PFAS to include structurally diverse and industrially relevant PFAS with limited toxicological data for future evaluation and guiding replacement design.

1.01.B.T-02 In vitro potencies of chemicals for inhibition of thyroperoxidase in five fish species compared to mammalian potencies in the ToxCast database

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Pesticides, industrial chemicals, pharmaceuticals and personal care products, and other additives in consumer and industrial products can enter the aquatic environment and disrupt thyroid hormone (TH) signaling, leading to adverse outcomes in fish. Thyroperoxidase (TPO) is the key enzyme in TH synthesis, catalyzing the production of thyroxine, which is converted into the biologically active triiodothyronine. TPO inhibition disrupts TH signaling, impairing vertebrate growth, development, and metabolism. While TPO inhibition is a known mechanism of endocrine disruption, most available fish data is derived from zebrafish (*Danio rerio*), a non-native laboratory model species that may not represent the diversity of responses across species. Furthermore, mammalian-based in vitro data from programs like the United States Environmental Protection Agency ToxCast program provide limited insight into potential species-specific differences in fish. Although TPO function is conserved, responses to inhibition may vary across taxa. This study aimed to evaluate TPO inhibition across five native marine fish species for six environmental chemicals with a range of potencies. We adapted the Amplex UltraRed TPO (AUR-TPO) assay, originally developed for mammalian models, for use with microsomal fractions from the kidney tissue of each fish species. Chemicals were selected based on existing ToxCast AUR-TPO assay data from the mammalian rodent model, *Rattus norvegicus*. Fluorescence data were used to generate dose-response curves via a four-parameter logistic model, from which inhibitory concentration values were calculated. Thresholds for significant inhibition were identified using one-way ANOVA. Results of this study showed that compounds identified as TPO inhibitors in mammals also inhibited TPO activity in all tested fish species. Sensitivity to inhibition was comparable across species, supporting the hypothesis that susceptibility to TPO inhibition is conserved among vertebrates. These results suggest that mammalian in vitro data may be useful for predicting thyroid disruption in fishes. This study demonstrates the utility of adapting existing new approach methods to non-model aquatic species, providing a consistent 215 for

comparison among species. These findings support the use of cross-species extrapolation in ecological risk assessment and help reduce reliance on whole-animal testing in regulatory toxicology.

1.01.B.T-03 Designing system-level interventions for the adoption of NAMs

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The Collaboration to Harmonise the Assessment of Next Generation Evidence (CHANGE) is built upon the interdisciplinary collaboration which serves as a foundation for risk and safety research. CHANGE focuses on system factors as an area of research that could be further developed and customized for accelerating the effective use of NAMs. By “system factors”, we mean potentially modifiable aspects of the organizational environment in which NAM-based research is conducted, interpreted, and acted on. The system factors, typically including technical and social factors (e.g., people, resources, culture, processes, policies, and legislation), may provide new opportunities for effective implementation of NAMs in regulatory toxicology. The overall goal of CHANGE is to design system-level interventions for speeding up adoption of NAMs. To achieve this goal, CHANGE follows a three-phase methodological approach “explore—reflect—design”:

Explore: Identify factors that might matter for the uptake and effective use of NAMs through assessing and describing mental models of the regulatory toxicology system by people operating within it. Reflect: Prioritise the factors that actually do matter by challenging the mental models and hypotheses created in the explore phase. Design: Develop interventions addressing the factors that matter. System diagrams visualize processes and dynamic forces in the regulatory toxicology system and the interactions between these forces will be used to identify opportunities for improving the effective use of NAMs. System diagrams with feedback loops will be used to identify important interventions. Reinforcing feedback loops in the system diagram shows where small changes will be amplified into big changes. Balancing feedback loops in the system diagram shows forces that will counteract changes. The system diagrams also enable identification of potential unintended consequences and indirect effects of interventions. CHANGE includes an international group of people in the regulatory toxicology system having different roles (i.e., risk managers, risk assessors, and researchers), different specializations, working in different sectors with specific types of chemical substances covered by specific legislations. Initial results from the first exploratory phase looked at the interplay among actors, goals, infrastructure, processes, culture, and technical aspects.

1.01.P-We Alternative Approaches and New Approach Methods (NAMs) That Refine, Replace or Reduce Animal Testing

1.01.P-We-005 Innovate EcoSafety Summit: A New Forum for Advancing Environmental Risk Assessment

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The inaugural Innovate EcoSafety Summit was held in Reykjavík, Iceland, in October 2025. This collaborative workshop was developed to advance ecological risk assessment and hazard evaluation through a recurring, issue-focused forum designed to complement, rather than replace, existing efforts. The mission of the summit was to bring the community together around specific topical issues in a solutions-focused, collaborative setting aimed at producing actionable next steps and facilitating the coordination of future work. Organized by a diverse multi-sector planning committee and co-sponsored by the Health and Environmental Sciences Institute, the UK National Centre for the 3Rs, and the International Collaboration for Cosmetic Safety with support from

additional partners, the 2025 summit focused on leveraging NAMs to reduce and replace acute fish testing and to advance the science for evaluating chemical endocrine activity potential for fish and amphibians, focusing on reducing reliance on in vivo tests. The summit emphasized collaboration and discussion among attendees to identify key areas of future work that would help address knowledge gaps and further the implementation of NAMs. This presentation will summarize summit discussions, highlight key outcomes, and outline avenues for future work.

1.01.P-We-006 A novel metric to define the applicability domain of the fish embryo acute toxicity test as an alternative to the acute fish toxicity test

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The fish embryo acute toxicity (FET) test is considered a potential alternative to the acute fish toxicity (AFT) test, particularly from an animal welfare perspective. However, due to uncertainties in its applicability domain, it is primarily used as a range-finding or screening test. Further research is needed to better define this domain and support its use as a reliable alternative to the AFT test. In our previous study, we proposed a novel method using the LC5096 h / LC5024 h ratio - i.e., the ratio of LC50 96h to LC5024 h obtained from the FET test - as a metric to estimate the applicability domain of the FET test. This approach was based on the understanding that LC5024 h primarily reflects general cytotoxicity, as major organs are not yet fully developed at this stage. In contrast, LC5096 h reflects both specific organ toxicity and general cytotoxicity. Consequently, the LC5096 h / LC5024 h ratio was interpreted as an index of the contribution of cytotoxicity to the overall toxic response. The results showed that as this ratio decreases (indicating a lower contribution of cytotoxicity), the fold difference between FET and AFT increases. Nonlinear regression analysis supported this relationship, with a high R² value (0.91), and proposed a threshold of 0.2–0.3 for identifying chemicals with more than a 10-fold difference between the FET and AFT results. In this study, we conducted FET tests and obtained the LC5096 h / LC5024 h ratios for additional ten chemicals to verify the proposed threshold. The results demonstrated that the proposed approach using the LC5096 h / LC5024 h ratio was able to support the identification of chemicals with more than a 10-fold difference between the FET and AFT results. Additionally, we assessed the substitutability of the LC5024 h values obtained from the FET test with those obtained from the RTgill-W1 cell line assay (EC50gill) or predicted by QSAR models (LC50QSAR). Because LC5024 h values are often difficult to obtain due to the necessity of conducting tests at concentrations that exceed the water solubility limits of certain chemicals. The results demonstrated that LC5024 h values can be substituted with RTgill-W1 cell line assay results and QSAR-predicted values, suggesting a broader utility of the LC5096 h / LC5024 h ratio as a metric for defining the applicability domain of the FET test across a wider range of chemicals. **Disclaimer/Disclosure:** This research was performed by the Environment Research and Technology Development Fund (JPMEERF20235004) of the Environmental Restoration and Conservation agency provided by Ministry of the Environment of Japan.

1.01.V Alternative Approaches and New Approach Methods (NAMs) That Refine, Replace or Reduce Animal Testing

1.01.V-01 Relative sensitivity of bobwhite quail and mallard duck in reproduction toxicity testing for conventional pesticide registration

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The United States Environmental Protection Agency (USEPA) sets limits on pesticide exposure to terrestrial and aquatic ecosystems based on ecological risk assessments. The Agency typically requires in vivo reproduction toxicity testing with two bird species for each conventional pesticide; the bobwhite quail (*Colinus virginianus*) and mallard duck (*Anas platyrhynchos*) are commonly tested. We conducted a retrospective study

to evaluate the relative sensitivity of these two species in reproduction toxicity tests (guidelines OCSP 850.2300 and OECD 206) submitted to the Agency to determine if one species instead of two can provide sufficient information to characterize potential effects after accounting for the observed relative sensitivities of mallard duck and bobwhite quail. No observed adverse effect concentrations (NOAECs) from bobwhite quail and mallard duck reproduction studies were analyzed from ecological risk assessments published by the USEPA for 317 conventional pesticides. For each chemical, NOAECs were compared qualitatively and quantitatively to evaluate the relative sensitivity of bobwhite quail and mallard duck for all evaluated pesticides, within each of the major pesticide groups (i.e., fungicide, herbicide, and insecticide), and within toxicity bins. Preliminary analysis suggests that a single test indicating a hazard (definitive NOAEC and LOAEC with observed effects) may be sufficient to assess chronic risk to birds.

1.01.V-02 Detecting pulp and paper mill effluent toxicity using the RTgill-W1 assay

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Recently, the Canadian Environmental Protection Act was amended to phase out whole animal toxicity testing by 2035. As these tests currently use millions of vertebrates to inform several regulatory programs, the development of replacements is desperately needed. One of the most extensively used regulatory tests exposes rainbow trout (*Oncorhynchus mykiss*) to industrial and municipal wastewater effluents to determine their compliance with federal and provincial legislation surrounding acute toxicity. Our research explores an alternative approach, based on the OECD 249 and ISO 21115 methods, that uses cell viability indicators in a rainbow trout gill cell line (RTgill-W1) to assess the acute toxicity of effluent samples. Using pulp and paper mill effluents from 6 unique Canadian sources, we assess the robustness of this approach in detecting effluent toxicity through reference toxicant testing in non-toxic effluents (N= 42) and control media (N= 54). The RTgill-W1 assay was also compared to the 96-hr rainbow trout acute lethality test using effect-causing effluents (N=20), demonstrating the similar sensitivity of these toxicity tests to effluent samples. This research provides critical information about the sensitivity of a potential replacement to the most widely used test in effluent regulations globally. **Disclaimer/Disclosure:** Research is funded by a Mitacs Accelerate grant, IT# IT36750.

1.02.T Applications of Integrative Omics in Ecotoxicology

1.02.T-01 Toxicogenomics in ecological risk assessment: current landscape, research gaps and recommendations to increase confidence across sectors

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In recent years, toxicogenomic-based approaches (especially transcriptomics) in human health risk assessment of chemicals have been progressing rapidly. For example, the United States Environmental Protection Agency (USEPA) is employing approaches such as high-throughput transcriptomics profiling and the USEPA Transcriptomic Assessment Product (ETAP) for chemical prioritization and development of reference values for data-poor chemicals, respectively. In contrast, toxicogenomic applications in ecological risk assessment of chemicals are less established but there has been increasing focus on this topic in the last 5–10 years. Thus, to increase confidence and advance toxicogenomic approaches in ecological risk assessment, a landscape review of grey and peer-reviewed literature was conducted to understand current frameworks and applications for toxicogenomics in ecological risk assessment and identify research gaps that need to be addressed. To date, there are no published regulatory frameworks that call for the integration of toxicogenomics data in ecological risk assessment, albeit several jurisdictions are now allowing for inclusion of such data as additional lines of

evidence. The types of toxicogenomic applications in ecological risk assessment identified during the landscape review included derivation and evaluation of the protectiveness of transcriptomic points of departure (tPODs), Adverse Outcome Pathway (AOP) refinement, chemical grouping and read-across, and hazard classification. These toxicogenomic applications were primarily published by academic and government research organizations, whereas publications from industry, contract researchers and government regulatory departments were not identified. Research on these applications has mainly focused on the incorporation of tPODs in ecological risk assessment, which show significant promise to reasonably accurately estimate apical PODs. However, critical sources of uncertainty regarding the application of tPODs to ecological risk assessment remain, including the reproducibility and variability within and across species, life stages, exposure regimens, and transcriptomic technologies. Based on learnings from this review, a case study is being developed to address some of the research gaps identified, the status of which will be presented. For the gaps not addressed by the case study, a prioritization process is underway and will be presented as a topic of discussion.

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1.02.T-03 Optimizing ecotoxicology 'omics in *Fundulus heteroclitus* using long-read sequencing and advanced bioinformatic tools

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Next-generation sequencing (NGS) technologies are highly useful tools in ecotoxicology. Here, we show the utility of newer bioinformatic tools in our recent study of genomic, epigenomic and transcriptomic endpoints in the highly genetically diverse mummichog, or *Fundulus heteroclitus*. First, we leveraged long-read sequencing (Oxford Nanopore Technologies (ONT)) for our genomic and epigenomic datasets; we observed improved mapping efficiency for ONT (0.4–4.7%), as compared to our short-read transcriptomic datasets (X–X%). Second, we de novo assembled a reference genome from our study population (2,659 scaffolds, N50: 37.36 Mb, 12.7x coverage, BUSCO 98.2%), which improved mapping efficiency (100%) over alignment to the published reference genome derived from a related fish population (NCBI reference MU-UCD_Fhet_4.1: 1.07 Gb) (95.3–99.6%). As a result, we observed genetic variation and differential DNA methylation signals that were originally missed when aligning to the published reference. Third, we used the software package StringTie to identify both known and novel transcripts (n=54,341 – 61,234 novel transcripts), instead of the more commonly used FeatureCounts, which limits analyses to transcripts that map to annotated features (in our dataset, n=20,617–20919 RefSeq transcripts). In summary, newer bioinformatic tools can optimize ecotoxicology 'omics analyses in non-model species.

1.02.T-04 Study on the application of sediment metabolomics to the assessment of spill-derived phytoplankton population damage

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When chemical spills occur in aquatic systems, they cause not only visible damage to macroscopic organisms but also invisible harm to microorganisms such as phytoplankton. However, the magnitude of the damage to biological resources resulting from aqueous chemical spills is generally assessed through the observation of visible indicators. Phytoplankton communities contribute to the basis of the aquatic food web; therefore, it is crucial to develop methodologies for assessing damage to phytoplankton. When chemicals spill into the aquatic system, phytoplankton die and settle on the sediment. In this study, we simulated chemical spill conditions in a laboratory using water and sediment collected from a river stream. Subsequently, the metabolome of the sediment containing dead phytoplankton was profiled, as it is expected to provide insights into population-level damage and the timing of death. Nontargeted mass spectrometry-based metabolomics was applied using Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS). The data were analyzed with MS-DIAL and MetaboAnalyst 6.0. Partial Least Squares Discriminant Analysis (PLS-DA) results revealed distinct differences in metabolite abundances, depending on the extent of phytoplankton population-level

damage measured as algae growth rate inhibition. Additionally, cluster analysis and ANOVA-Simultaneous Component Analysis (ASCA) enabled the selection of 90 putatively annotated metabolites with potential as biomarker candidates indicative of phytoplankton population-level damage or the time of death. Comparison with previous studies suggests that the 90 metabolites may originate from dead algal cells and microbes in the sediment. Consequently, this study supports the potential application of metabolomics as a new and informative methodology for assessing phytoplankton damage that results from a chemical spill.

1.02.T-05 Adding behavior to the mix: mosaic integration of behavioral and omics endpoints in adult zebrafish exposed to long-chain PFAS

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Integration of behavioral assays that are unpaired to omics datasets creates issues in toxicological data analysis. Challenges that arise with such ‘mosaic’ study designs hinder our ability to understand the complex interactions between biological systems that we study. Here, we modify a multi-omics approach to integrate behavioral assays of different sample sizes to transcriptomics and metagenomics datasets. For one month, adult zebrafish were exposed to 5 concentrations of perfluorononanoic acid (PFNA), a potential neurotoxicant. To assess the neurotoxic effects resulting from this acute exposure, behavioral assays that evaluate swimming, shoaling, and response to predators were employed. The brain transcriptome and gut microbiome were analyzed to discover biomarkers of PFNA exposure and to determine their relationships. To integrate behavioral assays of different sample sizes to the transcriptomic and metagenomic datasets Data Integration Analysis for Biomarker Discovery using Latent Variable Approaches for Omics Studies (DIABLO) was performed with the mixomics package in R. Omics features were selected with a two-step process using Multivariable Associations with Linear Models (MaAsLin2) and Hierarchical-All-Against-All Association (HALLA), using gene count (transcriptomics) and phylum level relative abundance (microbiome). Samples were aggregated by concentration in each behavioral assay (~36 fish per concentration) and omics dataset (8 fish per concentration) for model testing. The performance of each model was evaluated using perf() function with leave-one-out method. Only models generated for the predator assays met the set block error rate cut-off (≤ 0.5) and were further analyzed. A predator assay that measures time spent in each of three zones (close, middle, far) in response to a predator video, had strong positive associations with atypical gut microbes and negative associations with genes. Correlation between time spent in the far zone and genes associated with immune function (gpx3 and otud6b) suggests a relationship between increased fear and impaired immunological response in PFNA exposed zebrafish that may help explain proliferation of atypical microbes in the gut. In conclusion, we demonstrate that multi-omic approaches can be modified to address mosaic study design in toxicology.

1.03.P-Mo Beyond the Model Organism: New Approaches for Predicting Cross Species Toxicity

1.03.P-Mo-025 In vitro assessment of thyroperoxidase inhibition by organic ultraviolet filters in red drum microsomes

Vann Lynne Boyte, Christopher Lowrey-Dufour and Jonathon Doering, Louisiana State University

Organic ultraviolet (UV) filters are compounds widely used to prevent damage from radiation in sunscreens, cosmetics, and industrial materials. However, these chemicals are frequently detected in aquatic environments due to recreational water use, wastewater discharge, and runoff. Some UV filters are suspected to be endocrine-disrupting chemicals (EDCs) that may interfere with thyroid hormone signaling, a vital process for vertebrate growth, development, and metabolism. This study investigated the potential of commonly used organic UV filters to inhibit thyroid peroxidase (TPO), an essential enzyme for thyroid hormone synthesis. This study used the Amplex UltraRed (AUR-TPO) assay to measure TPO activity in kidney microsomal fractions from red drum (*Sciaenops ocellatus*). This high-throughput assay uses a commercial reagent that reacts with hydrogen

peroxide, which TPO consumes to catalyze oxidation, generating a measurable fluorescent signal. When a compound acts as a TPO inhibitor, it interferes with this reaction, leading to a dose-dependent decrease in fluorescence. By measuring fluorescence across a range of concentrations, dose-response curves are generated to quantify the potency of TPO inhibition. This assay has been widely used and validated in mammalian systems to assess TPO activity and inhibition. Prior research has shown that multiple fish species exhibit similar responses to TPO inhibition, supporting the relevance of red drum as a model for screening for additional chemicals for TPO inhibition in fishes. Preliminary results indicate that several UV filters reduce TPO activity in a concentration-dependent manner. This work highlights the utility of in vitro assays to detect potential thyroid-disrupting compounds in the environment and contributes to the development of animal-alternative methods for ecological risk assessment.

1.03.P-Mo-026 Assessing the in vitro sensitivity to disruption of thyroid hormone receptors among fish species

Lauren Eagon, Vann Lynne Boyte, Cameron Hunter Collins and Jonathon Doering, Louisiana State University

Metamorphosis is a vital process characterized by the transition from larval to juvenile stage. Many contaminants found in surface waters can disrupt thyroid hormone (TH) signaling leading to disrupted metamorphosis. TH disruption has been well studied in mammals and amphibians, and amphibians are generally sensitive to metamorphic impacts from a range of chemicals. In fishes, TH disruption studies have focused on model species with simple metamorphosis, such as zebrafish (*Danio rerio*), which are relatively insensitive to disruption. However, some fishes complete a metamorphosis as complex as amphibians, and the limited research suggests that these species might be more sensitive to disruption relative to zebrafish. Therefore, the present study aimed to determine whether species differences in metamorphic complexity and sensitivity to disruption were related to inherent differences in sensitivity of the thyroid hormone receptor (THR). An in vitro THR transactivation assay was developed to determine the sensitivity of THR to disruption across four fishes with varying degrees of metamorphic complexity using a known agonist, triiodothyronine (T3). The THR alpha isoform was cloned from liver of each target species and inserted into an expression plasmid. The in vitro THR assay utilized COS-7 cells transfected with the THR construct for species of interest, a thyroid response element vector, a reporter vector containing a luciferase gene, and a pRL-TK internal control vector. Cells in 96-well plates were exposed to serial concentrations of T3, incubated in the dark, and read on a luminescence plate reader following the addition of a dual-luciferase agent to produce dose-response curves. The THR from gulf killifish (*Fundulus grandis*), spotted gar (*Lepisosteus oculatus*), southern flounder (*Paralichthys lethostigma*), and American eel (*Anguilla rostrata*) had median effective concentrations (EC50s) that ranged by just 8-fold, from 0.0001nM to 0.0008nM. This suggests minimal differences in inherent sensitivity of THR among species, including species with differences in metamorphic complexity. Ongoing research is investigating whether there are species differences in THR sensitivity using the model antagonist, Tetrabromobisphenol A. Characterizing the specific mechanisms for possible species differences in sensitivity to disruption of metamorphosis could be essential in assessing impacts that thyroid hormone disrupting chemicals pose to diverse populations of fishes.

1.03.P-Mo-027 From fish to frogs: Interspecies correlation estimation model to predict long-term aquatic amphibian toxicity from fish ELS endpoints

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In the scientific community there are ongoing efforts to use the predictive potential of fish toxicity data for understanding (sub)lethal effects across aquatic species and thereby reducing vertebrate testing. In this context, numerous studies already show that acute fish toxicity data can predict acute toxicity of pesticides to aquatic life

stages of amphibians. However, comparison of long-term toxicity between the two groups has been limited due to a lack of relevant amphibian data. We investigated the relative long-term sensitivity of *Xenopus laevis*, investigated in amphibian metamorphosis assays (AMAs), for 87 pesticides (33 herbicides, 28 insecticides, 26 fungicides) compared to various fish species in the fish Early Life Stage (ELS) test. Comparisons were based on metrics for survival, body weight and length, which were extracted from publicly available registration documents (i.e. EU or US regulatory authorities) or, in a few cases, from unpublished study reports. We assessed the statistical correlation using Pearson's correlation coefficient and linear regression, aiming to derive an interspecies correlation estimation (ICE) model to predict chronic aquatic amphibian toxicity from fish ELS endpoints. In an initial analysis, the AMA was found to be equally (within a factor of 3) or less sensitive than the fish ELS test for most pesticides (84%). Paired t-tests identified the ELS as significantly more sensitive than the AMA for all investigated metrics ($p < 0.05$). Strong and statistically significant correlations ($r_P \geq 0.78$) between endpoints of the two test systems were observed across all parameters and pesticide classes. Although the test design of the AMA is not suited for deriving No Observed Effect Concentration values for quantitative use in an environmental risk assessment, the dataset can be used to derive an ICE model to support the use of fish as a surrogate species for aquatic phase amphibians.

1.03.P-Mo-028 The drug transporter ABCB1 confers resistance to naturally produced marine organohalogenes in the sea urchin *Lytechinus pictus*

Evan Tjeerdema, Rachel Metry, Michelle Schorn-Alanjary, Sanjoy Adak, Bradley Moore and Amro Hamdoun, Scripps Institution of Oceanography

The drug transporter ABCB1 protects cells from toxic xenobiotics by actively effluxing them from the cell membrane and limiting their accumulation. It has long been hypothesized that certain environmental chemicals can inhibit ABCB1 and enhance the sensitivity of the cell to these foreign compounds in a phenomenon termed "chemosensitization". Recently, synthetic organohalogenes were shown to be transporter interfering chemicals (TICs) that directly bind and inhibit ABCB1. However, the identity of the toxic substrates ABCB1 recognizes and effluxes in the marine environment remains unknown. We propose that naturally produced marine organohalogenes (MOHs) are substrates of ABCB1, and that this drug transporter is effective at limiting their accumulation and toxicity. To test this, we developed a high-content screening pipeline using ABCB1-knockout embryos (KO) of *Lytechinus pictus*, enabling us to dose, image, and analyze thousands of individuals. We screened 68 purified MOHs produced by common marine bacteria including *Streptomyces* and *Pseudoalteromonas* species. To detect putative ABCB1 substrates, we compared the cytotoxicity of each compound to wildtype (WT) and KO embryos at 0.2 and 2.0 μM . Compounds were classified as putative substrates if KO embryos experienced $>33\%$ inhibition of cell division than WT embryos. This yielded 16 compounds: three pyrroles, one bipyrrole, nine phenyl pyrroles, one indole, and two hydroxylated PBDEs. Five of these MOHs have reported bioactivities and drive cytotoxicity, antimicrobial activity, thyroid hormone disruption, and mutagenesis. Full dose-response analysis showed WT EC₅₀ values ranged from 0.18–2.50 μM , compared to 0.015–1.86 μM for KO embryos, representing a sensitivity increase of 3.86–91.86% in the absence of ABCB1, depending on the compound. Additional studies to characterize differences in the cellular accumulation of these MOHs and their subtle effects on embryo development are underway. Naturally produced organohalogenes are known to bioaccumulate in marine predators including whales, dolphins, and tuna, as well as in human tissues. Importantly, MOHs frequently co-occur with TICs, potentially resulting in elevated accumulation and chemosensitization to MOHs by environmental ABCB1 inhibition. Identifying toxic ABCB1 substrates in the environment is therefore a critical step toward understanding and mitigating risks associated with drug transporter inhibition.

1.03.P-Mo-029 Predicting acetaminophen metabolism and toxicity in bird species

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Previous work has demonstrated that snake species are extremely sensitive to acetaminophen, the active compound in Tylenol. The underlying mechanism for this toxicity was determined to be the lack of phenol-type UDP-glucuronosyltransferase, and N-aminotransferase. To investigate if bird species could also be sensitive to acetaminophen, which is used to manage the invasive brown treesnake on Guam, a dosing experiment was conducted with chickens. PCR analysis for showed that chickens do have 4 isoforms of the glucuronosyltransferase (UGT) enzymes that were upregulated in response to the acetaminophen exposure. To explore if other bird species have UGT isoforms that can metabolize acetaminophen, liver samples were collected from a wide variety of coastal Carolinian bird species, including crow, wading birds, pelican, vulture, hawks and owls. Liver samples of 15 different species were tested for phenol-type UGT enzymatic activity and demonstrated that some birds have very good activity towards acetaminophen-like compounds, while others had no significant activity. Surprisingly, there did not seem to be a connection between phylogenetic relationship, or food preference, and the enzyme activity, which complicates extrapolation to protected species, like native Pacific Island bird species. Primers for the four UGT isoforms that were upregulated in chicken after acetaminophen exposure were tested in PCR and qPCR reactions on liver samples from all other collected bird species. While PCR analysis initially suggested that some of these primers would work in all 15 species, quantitative PCR showed only results for chicken and one of the owl species. Further work is pursued to evaluate UGT isoforms in published genomes for similarity between bird and mammalian isoforms that are active towards acetaminophen. Using 3D protein folding and AI-driven docking and homology models, conserved regions in the protein sequences were identified that are essential for substrate binding. Based on these sequences, degenerative PCR primers for UGT isoforms that are predicted to have enzymatic activity towards acetaminophen are designed and tested for use in a larger variety of bird species, thus providing a predictive tool for acetaminophen toxicity in all birds. **Disclaimer/Disclosure:** Funding: Joint Region Marianas/USMC (Marc Hall, Al Borja) through USDA-APHIS Cooperative Agreement AP21WSNWRC00C001

1.03.T Beyond the Model Organism: New Approaches For Predicting Cross Species Toxicity

1.03.T-01 Using a weight of evidence approach for species surrogacy in chemical hazard and risk assessment

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Species surrogacy approaches have a long history of use in ecotoxicology and ecological risk assessment. With the growing availability of new approach methods along with increasing regulatory pressure to move away from animal testing, there are more opportunities to leverage species surrogacy approaches in chemical decision-making. The appropriateness of a surrogate species relies on the wealth and value of data supporting a cross-species comparison. Aspects such as species life histories, toxicokinetics, toxicodynamics, and inter-species correlation data can all inform on the basis for using a surrogate species in ecological risk assessments. When there are multiple lines of evidence for cross-species evaluations, a weight of evidence approach should be used. A weight of evidence approach determines the relevance, reliability, and strength of effect for each line of evidence, and collectively considers the consistency and consilience of the whole data set. Ultimately, integrating multiple lines of evidence into species surrogacy approaches can lead to more robust and defensible decision-making in ecotoxicology and ecological risk assessments.

1.03.T-02 Advancing ecotoxicological risk assessment: Integrative approaches in species sensitivity distribution modeling

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Environmental pollutants significantly threaten aquatic and terrestrial ecosystems by impacting species at various life stages. Accurate ecological risk assessment requires understanding species sensitivity throughout these stages. This study addresses the challenge of integrating life stage-specific toxicity data into Species Sensitivity Distribution (SSD) models to improve risk predictions. We develop a novel approach that combines a comprehensive dataset lacking life stage details (Dataset 1) with a smaller, detailed dataset containing life stage-specific toxicity information (Dataset 2). Predictive toxicity models are first generated from Dataset 1 and then aligned with life stage categories using overlapping data from Dataset 2. For compounds only present in Dataset 1, we apply statistical extrapolation and interpolation to estimate life stage-specific toxicity values. Using this integrated dataset, SSD models are constructed for multiple species across three key life stages: Early Development, Growth and Maturation, and Reproductive stages. Preliminary results indicate that incorporating life stage analysis into SSD modeling enhances the ecological relevance and accuracy of risk assessments. These findings advance environmental toxicology by providing a more nuanced understanding of species vulnerability, facilitating better-informed regulatory decisions and ecosystem protection strategies.

1.03.T-03 Toxicity of diamide insecticides to the standard surrogate species *Daphnia magna* and a native cladoceran *Simocephalus vetulus*

Stephanie Eckard and John D. Stark, Washington State University

The cladoceran *Daphnia magna* is often used in toxicity tests as a model or surrogate species representing all freshwater invertebrates in risk assessments. 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 we may be underestimating risk to native organisms. Our research investigated the acute and chronic toxicity of two anthranilic diamide class ryanodine-receptor modulator insecticides, chlorantraniliprole (CHLOR, as the formulated product Altacor®) and cyantraniliprole (CYAN, as the formulated product Verimark®), to two species of Daphniids: the standard species *D. magna* and a native species *Simocephalus vetulus* collected and cultured from a local pond in Puyallup, WA, USA. We hypothesized *S. vetulus* would be more sensitive than *D. magna*, resulting in lower LC50 and EC50 values. However, we found no statistically significant difference in their acute lethal toxicities and found *D. magna* was more sensitive to CHLOR than *S. vetulus* when looking at immobility as an endpoint. In chronic toxicity tests, we found CHLOR and CYAN significantly decreased fecundity in *S. vetulus*, and the resulting NOEC and LOEC values were much lower compared to published data on *D. magna*. This suggests that *S. vetulus* may be more susceptible to these insecticides than *D. magna* under chronic exposure. Additionally, we looked at demographic differences between the two species by developing a complete cohort life table for *S. vetulus*. Vital rates calculated include net reproductive rate (R_0) = 232.47, generation time (T) = 23.84, and finite rate of increase (λ) = 1.257. These vital rates resulted in a lower population trajectory compared to *D. magna*, indicating that *S. vetulus* may be inherently more sensitive to stress. Life tables can be used to refine population modeling and risk assessments for aquatic invertebrates and food web interactions with predators like endangered salmonids in the Pacific Northwest, USA. Better understanding of differing species susceptibility to new insecticides can guide regulators to improve their screening methods and prevent the registration and/or use of pesticides that unintentionally harm the environment.

1.03.T-04 Honey bee cell culture as a non-intrusive tool for understanding non-target effects of fungicides ***Dalma Martinovic-Weigelt¹* and Michael Goblirsch², (1)University of St. Thomas, (2)U.S. Department of Agriculture, Agricultural Research Service**

Fungal pathogens erode gains in crop yield and produce quality and threaten global food security. Fungicides are an important tool for the control of plant fungal pathogens; however, their use, especially on pollinator-visited crops, can be 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 215 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 fungicides (boscalid, pyraclostrobin, chlorothalonil) adversely affected cell viability and mitochondrial performance. Use of AmE-711 as a 215 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.

1.03.T-05 Community toxicology- estimating sensitivity using eDNA meta-barcoding results

Andrew Deines, Aman Gill and Jordan Pitt, Exponent

Effects of chemicals of concern on aquatic communities are often assessed based on a low-resolution impression of toxicity to a limited number of species present in that community. For example, species sensitivity distributions (SSDs) are widely used to evaluate the proportion of a community which is vulnerable to a chemical at a given concentration, but the laboratory species that inform standardized toxicity studies may have little in common with in situ communities. The implicit assumption is that the sensitivities of the relatively small set of tested species can be extrapolated to many more species, and it is natural to base this extrapolation on taxonomic relatedness. At the same time, multiple studies have found low levels of phylogenetic conservation of toxin susceptibility among related species, and others report only low levels of phylogenetic signal in SSD models. Models that account for relatedness do not produce results much different than models that do not make such an accounting, suggesting that extrapolation from SSDs to related species maybe tenuous. We present a preliminary/proof of concept approach to assessing community toxicity by using phylogenetic eigenvector maps trained on standardized ecotoxicology studies to extrapolate toxicity results across specific communities consisting of many species detected by environmental DNA (eDNA) meta-barcoding sampling. We will discuss the remaining uncertainties in this approach and the next steps for development.

1.03.T-06 Methodology for surrogate assessment using SeqAPASS and phylogenetic analysis

Tamara Dawn Lunsman, Vince Kramer, Austin Cool and Amy Wang, Corteva Agriscience

Surrogate species are used in Environmental Risk Assessment (ERA) to represent a broader group of species when determining potential risk. These species are chosen for several reasons, but generally because they are easily reared in a laboratory setting and are considered representative of a group of organisms either based on their ecological role, taxonomy, or the potential exposure pathway. In the context of the Endangered Species Act (ESA) and crop protection products, endangered and threatened (i.e., listed) species cannot undergo toxicity testing so identification of appropriate surrogate species that align to listed species is critical to more accurately assess potential population-level effects and subsequently propose appropriate mitigations. As such, the selection of a surrogate species could be qualified by performing a study to check whether the choice of species was appropriate. This study explores the use of SeqAPASS in the identification of appropriate surrogate species. We look at three crop protection products and compare SeqAPASS results with a phylogenetic similarity analysis and ground truth that analysis to existing conventional toxicity data. This analysis can be used as a tool to qualify the risk determination in ESA assessments. It can provide supplemental information for decision-makers to ensure that the surrogates selected are representative of the species of concern. We show how additional species testing can be targeted to clades that have limited data or to clades with few representative species with conventional toxicity data.

1.04.P-We Cell-Based Approaches for Ecotoxicity Assessments

1.04.P-We-002 Modulation of ABC transporters by short-chain PFAS in a human liver cell-based assay *Gracen Elizabeth Collier and Ramon Lavado, Baylor University*

Both short- and long-chain PFAS are environmentally persistent and resistant to degradation, resulting in widespread accumulation and continuous human exposure, primarily at low doses. Although numerous health effects and cellular alterations linked to PFAS have been reported, the mechanistic pathways and specific protein-level impacts remain underexplored. Among emerging targets are ATP Binding Cassette (ABC) transporters — a superfamily of efflux proteins vital for the transport of endogenous molecules and pharmaceuticals. While prior research has shown that PFAS can alter ABCG2 gene and protein expression, the effects on other ABC transporters and functional transport remain unknown. To address this gap, we developed a model using differentiated human hepatocytes (HepaRG) to quantify ABC gene expression and functional transport activity following PFAS exposure. Cells were exposed to short-chain PFAS (PFBS, PFHxA, PFHxS, 6:2 FTOH) for either 48 hr or one hr. Post-exposure, ABC gene expression was analyzed via qPCR, or cells were incubated with established fluorescent ABC substrates (Calcein AM, CMFDA, Bodipy-Cholesterol) to assess substrate loading and efflux using a plate reader. Preliminary results show significant reductions in retained substrate fluorescence following 48-hr exposure to PFBS, PFHxA, PFHxS, and 6:2 FTOH (1 nM and 1 μ M), suggesting increased efflux activity. For example, all PFAS exposures led to a 42% or greater reduction in retained fluorescence using Bodipy-Cholesterol relative to DMSO control, while 48-hr exposure to both 1 μ M PFHxS and 1 μ M PFHxA led to a 75% or greater reduction in retained Bodipy-Cholesterol relative to control. In contrast, 1-hr exposures did not significantly affect transport. We anticipate qPCR results will confirm upregulation of ABC genes corresponding to these substrates, aligning with enhanced transporter activity and reduced intracellular retention. These findings suggest that low-dose, environmentally relevant exposures to short-chain PFAS can significantly alter ABC transporter-mediated substrate handling in human liver cells. This has potential implications for the hepatic transport of endogenous compounds and pharmaceuticals. Ongoing work aims to identify the specific ABC transporters most sensitive to disruption and to further elucidate the regulatory mechanisms underlying these PFAS-induced changes.

1.04.P-We-003 Impact of constant and sudden temperature changes on copper and cadmium toxicity in the RTgill-W1 cell model

Loshitha Bokumburegedara¹ and Matteo Minghetti², (1)Oklahoma State University, (2)Integrative Biology, Oklahoma State University

Rising global temperatures and the increasing extreme weather events due to climate change are altering aquatic ecosystems, potentially enhancing the toxicity of pollutants such as metals. The interplay between physiological responses to environmental parameters (e.g., temperature, osmolarity, and pH) and the toxicological responses is poorly understood. Understanding how environmental parameters influence metal toxicity is critical for developing realistic ecological risk assessments. This study examines the effect of temperature on the cytotoxicity of copper and cadmium using the RTgill-W1 cell line, a gill epithelial model derived from rainbow trout. The central hypothesis is that elevated or rapidly changing temperatures intensify cellular susceptibility to metal toxicity by compromising physiological stability and stress defense mechanisms. To test this, cells were exposed to copper and cadmium at three temperatures: 4°C (cold stress), 19°C (optimal), and 26°C (heat stress). Cytotoxicity was assessed using three endpoints: metabolic activity (AlamarBlue), membrane integrity (CFDA-AM), and lysosomal function (Neutral Red). Half-maximal effective concentration (EC₅₀) values were calculated to evaluate temperature-dependent toxicity. Copper consistently exhibited greater cytotoxicity than cadmium across all assays. At 4°C, toxicity was reduced, likely due to suppressed metabolic activity and lower metal uptake. In contrast, exposure at 26°C led to increased membrane permeability, lysosomal disruption, and a decline in cellular integrity, indicating heightened vulnerability to metal-induced stress under heat stress conditions. Future experiments will include exposures at 12 and 21°C, with sudden thermal shifts mimicking events like heatwaves or cold fronts. These studies will assess how rapid temperature changes affect toxicity.

and cellular acclimation. Na⁺/K⁺-ATPase activity and heat shock protein expression will be measured to further characterize responses to combined metal and temperature stress. These findings highlight the need to integrate temperature stress into toxicity assessments to understand interactions between environmental and anthropogenic stressors and fish acclimation to abrupt thermal changes. **Disclaimer/Disclosure:** Funded by Minghetti Lab, Department of Integrative Biology, Oklahoma State University

1.04.P-We-004 Analyzing the sensitivity of three rainbow trout cell lines through cytotoxicity and targeted gene expression for aquatic toxicity testing

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The International Organization for Standardization recently approved the RTgill-W1 cell line as an in vitro alternative for acute toxicity testing in fish. While RTgill-W1 reflects acute toxicity in rainbow trout (*Oncorhynchus mykiss*), it represents only one tissue type. Testing with various tissue types can elucidate tissue-specific mechanisms of toxicity that may be overlooked when using a single cell type. In this study, the rainbow trout cell lines RTL-W1 (liver), RTgutGC (intestine), and RTgill-W1 (gill) were exposed to a range of 3,4-dichloroaniline (3,4-DCA) and cadmium (Cd) concentrations. Cytotoxicity levels and effective concentrations 50% (EC50) of Cd and 3,4-DCA were determined using a multi-endpoint viability assay with alamarBlue, CDFA-AM, and neutral red, which are markers of cell metabolic activity, cell membrane integrity, and lysosomal integrity, respectively. Results indicated alamarBlue EC50 values of 72.5 µM, 92.6 µM, and 234.5 µM for RTgill-W1, RTgut-GC, and RTL-W1 cells, respectively. For 3,4-DCA, EC50 values were 442.8 µM, 622.3 µM, and 653.3 µM for RTgill-W1, RTgut-GC, and RTL-W1 cells, respectively. Across all cell types, RTgill-W1 was the most sensitive to both 3,4-DCA and Cd, with Cd exhibiting higher toxicity in all cell lines compared to 3,4-DCA. RTL-W1 cells displayed greater resistance to these chemicals, suggesting that, like tissues in vivo, this cell line may possess more robust detoxification mechanisms. To test this hypothesis, cells were exposed to the No Observed Effect Concentration (NOEC) and the EC10 of Cd (18.5 µM and 185 µM, respectively) and 3,4-DCA (0.445 µM and 6.67 µM, respectively) for 24 hr. Following exposure, total RNA from each cell type was extracted and reverse-transcribed for quantitative PCR (qPCR) analysis. Metallothionein and Cytochrome P450 1A messenger RNA levels were measured by qPCR as biomarkers of Cd and 3,4-DCA exposure and toxicity. At both NOEC and EC10 levels for 3,4-DCA and Cd, there was a significant upregulation of their respective genes, indicating that gene expression is a more sensitive endpoint compared with cytotoxicity assays. Additionally, RTgill-W1 cells showed the highest gene upregulation following 3,4-DCA exposure, confirming their greater sensitivity to this compound, while RTL-W1 cells were the most sensitive to Cd. These results suggest that molecular biomarkers can be valid and highly sensitive as endpoints for aquatic testing.

1.05.P-Mo Empirical and Theoretical Methods to Scale Contaminant Effects: Molecular Impacts to Organism Health

1.05.P-Mo-002 Effects of nitrite exposure on pregnancy and maternal transfer in *Xiphophorus couchianus*

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Worldwide, nitrogen-based fertilizers commonly used in agriculture leach into groundwater. This leads to an increasing water nitrate level (low toxicity) that can be reduced to nitrite (high toxicity) endogenously and exogenously. The impact of long-term exposure to current levels of nitrates and nitrites in drinking water on human health is not well understood. Additionally, there is no information available regarding the chronic effects of these contaminants on one of the most vulnerable groups: pregnant women. We hypothesized that chronic exposure to nitrite alters the maternal transfer of immune and endocrine factors to the embryos, which are key for development, causing immune and neurodevelopmental disorders in the offspring. To test our

hypothesis, we propose a new fish pregnancy model, the swordtail (*Xiphophorus couchianus*), a live-bearing fish with 30 30-day pregnancy cycles. We exposed swordtails to different nitrite treatments (0 mM, 0.01 mM, 0.1 mM, and 1 mM) for 30 days (5 females and 3 males/treatment, 3 replicates/treatment). After exposure, fish were sacrificed, and a subsample of muscle, gill, brain, intestine, liver, spleen, ovary, eggs, and embryos was taken for biochemical (nitrite/nitrate content and immune parameters) and histological analysis. Another subsample of head kidney, eggs, and embryos was used to extract RNA and analyze gene expression using TaqSec transcriptomic techniques. Nitrite levels in eggs and embryos increased with higher concentrations, while immune factor levels declined. The 0.1 mM concentrations yielded immunocompromised and malformed embryos and eggs, while the 1 mM concentrations resulted in infertility. Nitrite treatments also alter gene expression of maternal genes and their transfer to the embryo in the first stages of gestation. The data analysis showed that chronic exposure to nitrite affects the molecular transfer of the maternal factor that affects the reproductive physiology of the mother and the neural development of the embryo. These findings could have further implications for how prolonged nitrite exposure affects pregnancy in mammals, particularly in pregnant human women. Nitrite levels should, therefore, be routinely monitored in aquaculture and public and private drinking water to prevent exposure risk. **Disclaimer/Disclosure:** Research reported in this abstract was supported by NIGMS of the National Institutes of Health under award number R16GM153659

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

1.05.T-01 Characterization of PFAS mixtures in environmental matrices and uptake by biota in an AFFF-contaminated wetland

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Per- and polyfluoroalkyl substances (PFAS) are persistent industrial pollutants affecting aquatic ecosystems worldwide. However, PFAS toxicity and ecological risk to freshwater environments is not yet fully understood. Clark's Marsh near Lake Huron in Oscoda, Michigan is one natural area in the US that has been contaminated with PFAS due to long-term use of fire suppressant Aqueous Film-Forming Foams (AFFF) at an adjacent military base. To better understand the distribution of PFAS in the aquatic environment, we conducted a survey of PFAS in surface water, pore water, and plankton at Clark's Marsh using grab and passive samplers. To better understand the uptake of PFAS mixtures by aquatic organisms, we also conducted a 12-day caging experiment with juvenile and adult fathead minnows (*Pimephales promelas*) at Clark's Marsh and a nearby reference site. The concentration of ΣPFAS40 in Clark's Marsh surface and pore water was 1104.9 and 923.6 ng/L, respectively. Of the 40 PFAS analytes tested, PFOS, PFOA, and PFHxS were present in the highest concentrations in both surface water and pore water. Similarly, PFOS was present at the highest levels in both fish and plankton tissue with bioconcentration factors of 2.1 and 1.8, respectively. While PFOSA was present in relatively low concentrations within surface and pore water, this analyte readily accumulated within biota. The bioconcentration factor for PFOSA in fish and plankton tissues ranged from 3.1 to 3.7. Compared to the reference site, survival and growth of adult and juvenile fathead minnows was not significantly impacted at Clark's Marsh. However, reproduction of adult fathead minnows decreased at Clark's Marsh relative to the reference site over the 12-day period. These data will be integrated with molecular endpoints, suborganismal effects, and bioenergetic models to predict population-level responses. Together, these results provide greater information about the distribution, fate, and uptake of PFAS in aquatic environments and can help inform our understanding of the ecological risks of PFAS contamination in freshwater ecosystems.

1.05.T-02 A new toxicity testing framework: Interpreting complex PFAS mixtures for ecological risk assessment in *Daphnia magna*

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Environmental contaminants in aquatic ecosystems typically occur as complex mixtures, yet standardized toxicity tests are designed for single compounds under simplified conditions. These limitations hinder our ability to predict ecological risks from chemical mixtures, especially when interactions are non-additive or exhibit non-monotonic dose-response relationships. Aqueous film forming foams (AFFF), used extensively in firefighting and on military bases, release mixtures of per- and polyfluoroalkyl substances (PFAS) into the environment, providing a relevant test case for developing improved mixture toxicity testing approaches. Here, we present a novel experimental framework to assess the ecological risk of PFAS mixtures by connecting molecular, organismal, and kinetic data across biological levels of organization. Modified standardized toxicity tests were used to evaluate both lethal and sublethal effects (mortality, growth, reproduction) in *Daphnia magna* exposed to PFOS (perfluorooctane sulfonic acid) and a reconstituted PFAS mixture reflecting AFFF contamination (PFOS, PFHxS, PFOA, PFOSA). Life-cycle exposures were paired with transcriptomic profiling to link molecular responses to phenotypic outcomes. In parallel, bioaccumulation experiments quantified uptake and depuration of PFOS and the mixture to inform a toxicokinetic-toxicodynamic (TKTD) model. Findings revealed overlapping impacts on survival and reproduction, as well as non-monotonic dose responses that would not have been detected through traditional acute or chronic testing. While PFOS is often cited as the most toxic PFAS, the reconstituted mixture produced more severe effects. These results demonstrate that focusing solely on single compounds underestimates the ecological risk posed by PFAS mixtures. This framework supports both prospective and retrospective risk assessments, provides mechanistic insight through molecular data, and enhances our ability to evaluate the toxicity of environmentally relevant mixtures. It also lays the groundwork for reducing animal testing by identifying predictive molecular signatures and informing next-generation screening approaches for complex contaminants.

1.05.T-03 A Transcriptomic approach to assessing the interactive effects of PFHxS exposure and salinity stress on embryo-larval red drum (*Sciaenops ocellatus*) development

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Estuaries are critical habitats for many biological and ecological processes and serve as nursery grounds for early life stages of many ecologically and economically valuable fisheries species, including the red drum (*Sciaenops ocellatus*). Estuaries are dynamic systems that are subject to extreme salinity and temperature fluctuations as a result of tidal exchange, freshwater inflow, and evaporation. Anthropogenic influences (e.g., altered freshwater inputs, contamination) can interact to further intensify environmental stress, resulting in environmental conditions that exceed the tolerances of sensitive early life stage fish. Among emerging contaminants of concern in these systems are per- and polyfluoroalkyl substances (PFAS), a diverse class of persistent and bioactive chemicals used in a wide array of industrial and commercial products. PFAS are continuously introduced into coastal environments through both point and nonpoint sources and have been widely detected in sediments, surface waters, and biota. While more toxicological data are becoming available on PFAS exposure in estuarine systems, few studies have addressed how PFAS may interact with co-occurring stressors present in estuaries, such as hypersalinity and elevated temperatures. This study investigates the sublethal impacts of perfluorohexanesulphonic acid (PFHxS), hypersalinity, and temperature stress on red drum embryos and larvae from multiple spawns, allowing us to better capture the effects of genetic diversity on resilience. Developmental exposure to environmentally relevant concentrations of PFHxS resulted in morphological alterations (i.e., total length, body area, brain area, eye area, pericardial area) and changes in gene expression. TagSeq-based transcriptomic profiling was used to identify differentially expressed genes (DEGs) and potential downstream effects on key pathways involved in stress responses, development, and osmoregulation. By linking gene expression to observed morphological outcomes, this study provides insight into the mechanistic basis of PFAS toxicity in developing red drum under more realistic exposure scenarios.

Results contribute to a growing body of literature highlighting the importance of multi-stressor approaches in ecotoxicology, assessing sublethal endpoints, the inclusion of non-model species in toxicity testing, and the value of integrating molecular endpoints to assess ecological risk in vulnerable estuaries.

1.05.T-04 Perfluorooctanesulfonamide-induced epiboly delay is associated with decreased ATP Production within zebrafish embryos

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Per- and polyfluoroalkyl substances (PFAS) have been widely used within various industrial processes and consumer products, such as food packaging and fire-fighting foams. PFAS persistence within environmental media has led to ubiquitous exposure within humans and wildlife. We previously conducted a high-content toxicity screen of 38 different PFAS within the first 72 h of zebrafish development. This screen identified perfluorooctanesulfonamide (PFOSA) as the most potent developmental toxicant among the 38 PFAS tested. At non-lethal concentrations, exposure to PFOSA from 0.75 to 6 hr post-fertilization (hpf) – a developmental window that includes cleavage, blastulation, and early-gastrulation – resulted in a concentration-dependent delay in epiboly (epiblast cell migration) by 6 hpf. Therefore, for this study, we determined whether 1) PFOSA delays epiboly by inhibiting actin polymerization within the yolk, as epiboly progression is reliant on yolk-associated actin networks, and/or 2) PFOSA-induced effects are associated with a decrease in embryonic ATP, as PFOSA is a potent uncoupler of oxidative phosphorylation in vitro. Consistent with our previous study, PFOSA significantly delayed epiboly in a concentration- and time-dependent manner within zebrafish embryos by 4–6 hpf when exposure to PFOSA (6.25–50 μ M) was initiated at 0.75 hpf. Based on high-resolution confocal microscopy, PFOSA did not significantly decrease yolk-associated actin within PFOSA-exposed embryos with delayed epiboly, suggesting that PFOSA does not inhibit actin polymerization in vivo. However, PFOSA exposure resulted in a concentration-dependent decrease in embryonic ATP within 6-hpf embryos beginning at 25 μ M. Moreover, co-exposure of embryos to 5 mM ATP from 0.75–6 hpf partially mitigated PFOSA-induced delayed epiboly starting at 4 hpf, suggesting that PFOSA-induced epiboly delay is driven, in part, by a decrease in embryonic ATP. Overall, our findings to date suggest that PFOSA exposure during early embryonic development reduces ATP production in the absence of effects on actin polymerization in vivo, an effect that is associated with PFOSA-induced epiboly delay. Currently, we are quantifying 1) embryonic doses of PFOSA to determine if the rate of PFOSA uptake is associated with PFOSA-induced impacts on ATP and epiboly and 2) embryonic maternal and zygotic transcripts to determine if PFOSA delays the maternal-to-zygotic transition – a developmental landmark that precedes epiboly. **Disclaimer/Disclosure:** T32ES018827 - NIEHS T32 Training Grant D020000A015 - UC Riverside Seed Grant

1.06.P-Mo Enhancing Endocrine System Evaluation: Exploring Opportunities and Insights from Lessons Learned

1.06.P-Mo-004 Hepatic enzyme induction and its potential effect on thyroid hormone metabolism in the metamorphosing tadpole of *Xenopus laevis* (African Clawed Frog)

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Hepatic enzyme induction, an inherent defense system against xenobiotics, is known to simultaneously affect endocrine system functions in mammals under specific conditions, particularly thyroid hormone (TH) regulation. While this phenomenon has been studied extensively, the pathway leading to this indirect thyroid effect in mammals remains unclear in its applicability to amphibians, despite the importance of amphibian species in assessing thyroid-disruptive chemicals. We investigated the effects of three well-known mammalian enzyme inducers— β -naphthoflavone (BNF), pregnenolone carbonitrile (PCN), and sodium phenobarbital (NaPB)—on the gene expression of phase-I and phase-II metabolizing enzymes in *Xenopus laevis* tadpoles.

Waterborne exposure of tadpoles to BNF and PCN significantly induced the expression of both phase-I (cytochrome P450, CYP) and phase-II enzymes (UDP-glucuronosyltransferase, UGT and sulfotransferase, SULT), but in different patterns. In contrast to mammals, NaPB exposure induced CYP2B expression without affecting phase-II enzymes. Furthermore, an ex vivo hepatic enzyme activity assay confirmed that BNF treatment significantly enhanced phase-II metabolic activity (glucuronidation and sulfation) toward TH. These results suggest that certain mammalian enzyme inducers may have the potential to influence TH clearance in *X. laevis* tadpoles. Our findings provide insights into the profiles of xenosensing activity and enzyme induction in amphibians, which can facilitate a better understanding of the mechanisms of indirect effects on the thyroid system via hepatic enzyme induction in non-mammalian species.

1.06.P-Mo-006 Establishing an adverse outcome pathway from thyroid peroxidase inhibition to mortality via impaired myelination in zebrafish

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Thyroid hormones (THs) play a critical role in vertebrate neurodevelopment and their disruption has raised concern on neurotoxicity due to the widespread thyroid-disrupting chemicals (TDCs) in the environment. While the developmental effects of TDCs are increasingly recognized, the lack of mechanistic understanding from molecular targets to adverse outcomes highlights the need for a structured framework such as an adverse outcome pathway (AOP). Thyroid peroxidase (TPO) is a known molecular target, but the AOP linking TPO inhibition to impaired neurodevelopmental outcomes remains insufficiently defined. This study aims to establish and experimentally support a mechanistically plausible AOP in which TPO inhibition, serving as the molecular initiating event (MIE), leads to increased mortality through impaired myelination using zebrafish embryos. TPO is a key enzyme in thyroid hormone synthesis and a critical molecular target for endocrine disruption. To experimentally induce TPO inhibition, zebrafish embryos were exposed to propylthiouracil (PTU), a well-characterized chemical inhibitor of TPO activity. Molecular, cellular, and behavioral alterations were subsequently examined across the proposed AOP. To confirm TPO inhibition and downstream thyroid hormone disruption, we will quantify levels of T3 and T4. In parallel, expression changes in key genes related to oligodendrocyte development and myelin formation will be assessed to support molecular plausibility. Cellular-level impacts will be examined using transgenic zebrafish to measure fluorescent intensity for myelin-related mbp mRNA expression to quantify oligodendrocyte populations. G-ratio analysis will provide ultrastructural evidence of myelin sheath thinning in the central nervous system. These morphological changes will then be functionally linked to behavior through swimming performance assays, which serve as ecologically relevant indicators of neural circuit integrity and survival-related capacity. Given the time-sensitive role of THs during early development, the findings are expected to demonstrate that TPO inhibition-induced hypothyroidism disrupts oligodendrocyte maturation, resulting in hypomyelination, reduced locomotor ability, and ultimately increased mortality. This study is expected to establish a mechanistic basis for this AOP and provide empirical evidence for its key events, offering a pathway-based framework applicable to thyroid-related developmental neurotoxicity assessments. **Disclaimer/Disclosure:** This research was supported by a grant (RS-2024-00331685) from Ministry of Food and Drug Safety in 2024.

1.06.P-Mo-007 We have problems—Assay sensitivity, species sensitivity and relevance in endocrine data interpretation

Christopher J. Borgert, *Applied Pharmacology & Toxicology Inc.*

Test species are assumed less sensitive than target species, but for the only bona-fide human EDC (DES) and for human pharmaceuticals, the relative sensitivity of humans versus rodents does not support that assumption. DES data strongly refutes the assumption that humans are more sensitive than rodent test species. Data for finasteride are less clear, but nonetheless, do not support a 10-fold interspecies uncertainty factor for humans, as normally applied. Some of the physiological reasons that humans are less sensitive to endocrine effects than

rodents are known, and suggest some principles for the interpretation of data cross-species. For example, circulating estradiol levels are hundreds of times higher during human pregnancy than during rodent pregnancy, rendering rodent fetuses much more sensitive than human fetuses to exogenous estrogens. The physiology of each species should be considered to the greatest extent possible, not merely assumed based on policy; this is especially critical for endocrine assessments. Furthermore, *in vitro* assays for ED are intentionally irrelevant for humans and many other species because these assays are conducted under conditions that intentionally avoid the normal physiological milieu (e.g., components of serum and tissues). As such, they cannot be directly interpreted without additional biochemical physiological and kinetic context. This problem is not and cannot be avoided by simply conducting *in vitro* assays using cells from the species of interest. In fact, the endocrine machinery – e.g., hormone receptors – is often more similar across species than the physiological and biochemical conditions between them (e.g., circulating hormone levels; carrier protein characteristics and concentrations, etc.). To be useful, *in vitro* endocrine assays must be interpreted with the physiological context to the greatest extent possible. The physiological and biochemical basis of endocrine potency thresholds – the Human-Relevant Potency Threshold (HRPT) – has been documented for ER α agonism and antagonism, and suggests a methodology for providing the necessary relevance. The method will be illustrated using examples from the recent literature, including ways to evaluate kinetic thresholds that improve relevance evaluations. Challenges for extending these methods to other endocrine MoAs will be explained and discussed. The solution to these challenges requires only readily-obtainable data.

1.06.T Enhancing Endocrine System Evaluation: Exploring Opportunities and Insights from Lessons Learned

1.06.T-01 Guidance on concentration setting for endocrine screening: A data-driven approach

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Testing chemical-induced endocrine activity and adverse effects in fish and amphibian *in vivo* test guideline studies plays a pivotal role in identifying endocrine disruptors in regulatory assessments. A key consideration in such testing is the setting of test concentrations. In theory, a “perfect” concentration range would be high enough to reveal any endocrine-mediated effects, but not so high that generalized toxicity (including mortality) is a confounding factor in identifying such effects as endocrine-specific. The concept of the “maximum tolerated concentration” (MTC; adapted from the Maximum Tolerated Dose used in mammalian toxicology) was developed to guide the selection of test concentrations. However, this concept has been inconsistently applied and is sometimes poorly defined within test guidelines. The lack of clear and consistent definitions for the MTC can lead to inappropriate test concentration setting, potentially invalidating the study and hindering effective endocrine assessment. This presentation provides recommendations for best practice on how to appropriately establish and interpret the MTC to guide concentration setting for endocrine disruption screening studies, with particular attention to practical limitations (e.g., functional solubility and solvent use) and biological factors (e.g., differential sensitivity between species and life stages). To help inform this discussion, we conducted a comprehensive retrospective data analysis of standard regulatory fish acute and chronic studies, Amphibian Metamorphosis Assays, and Fish Short-Term Reproduction Assays for 32 pesticide active substances. Based on this retrospective analysis, MTCs based on 1/10 LC₅₀ were generally less likely to result in confounding effects than 1/3 LC₅₀. However, concentration setting should be guided by a data-driven weight-of-evidence approach, utilizing all available data, including evaluating functional solubility, potential differences in life stage or species sensitivity (when using previously generated toxicity information), and the adequacy of any range-finding or tolerability experiments used to inform the selection of test concentrations.

1.06.T-02 The importance of historical control data to evaluate the performance of endocrine screening assays: A case study with the extended amphibian metamorphosis assay

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The Amphibian Metamorphosis Assay (AMA) was established as a standardized test guideline with the primary purpose of evaluating test materials for potential endocrine activity in the Hypothalamus-Pituitary-Thyroid axis of developing African clawed frogs (*Xenopus laevis*). The Extended Amphibian Metamorphosis Assay (EAMA) is similar to the AMA with the termination timepoint at a fixed developmental stage (i.e., Nieuwkoop and Faber (NF) stage 62) rather than the fixed exposure time (i.e., 21-days). This presentation will summarize EAMA control data for twenty-two control datasets (including five solvent control data) from seventeen studies conducted at two different laboratories. The termination endpoints in the EAMA, such as whole-body wet weight, snout-vent length, hind-limb length, and normalized hindlimb length exhibited lower variability in comparison to endpoints collected at test termination in the standard AMA, with coefficients of variation approximately half of those observed in the AMA. Regression analyses were conducted for continuous endpoint measurements at NF stage 62. Wet body weight vs. snout-vent length showed the highest average coefficient of determination of $R^2 = 0.71$. Time to NF stage 62 was generally less correlated to morphometric endpoints ($R^2 \leq 0.40$), which may reflect plasticity of amphibian metamorphosis. Histopathological investigations of thyroid glands at the termination time point of the EAMA indicate that mild and moderate follicular cell hypertrophy and mild follicular cell hyperplasia occur regularly as a part of normal development among NF stage 62 control tadpoles. In summary, the control performance of seventeen EAMAs indicates a robust baseline that can be used to refine the performance criteria of the assay and aid in the interpretation of the results of future fixed stage test designs.

1.06.T-04 Metamorphosis as an endpoint for thyroid disruption in fishes

Lauren Eagon, Vann Lynne Boyte, Cameron Hunter Collins, Jonathon Doering, Nonnie Cook and Christopher Lowrey-Dufour, Louisiana State University

Metamorphosis is a vital biological process characterized by the transition from larval to juvenile stage of development and is most commonly associated with amphibians. Pesticides, chemical additives, pharmaceuticals, and personal care products found in surface waters can disrupt thyroid hormone (TH) signaling leading to accelerated, delayed, or prevented metamorphosis. TH disruption has been well studied in mammals and amphibians, and amphibians are generally sensitive to metamorphic impacts from a wide range of chemicals. In fishes, TH disruption studies have focused on small model species with simple metamorphosis, such as zebrafish (*Danio rerio*), which are relatively insensitive to disruption. However, some fish species complete a metamorphosis as complex as amphibians, and it is unknown whether these fishes with dramatic transformations are more sensitive to chemical disruption relative to zebrafish. The present study aimed to develop a fish metamorphosis toxicity assay to compare metamorphic chemical disruption in vivo for alligator gar (*Atractosteus spatula*), a species with dramatic transformation, and zebrafish. Fishes were exposed to sub-lethal concentrations of a known thyroid agonist, triiodothyronine (T3) at 1.2, 0.2, 0.033, 0.0056, and 0 ppm. Exposure took place for the duration of the metamorphic period, from approximately 5 to 25 days after hatch. Endpoints included metamorphic milestones, mortality, growth, and molecular endpoints including gene expression. At the completion of the alligator gar exposure, metamorphosis was impacted at all treatment levels, with exposed groups exhibiting higher mortality, increased snout-to-body proportion, and changes to color development. The lowest observed effect concentration was 0.0056ppm, although future testing will examine lower concentrations. Based on existing literature, zebrafish experience malformations at 0.0098ppm T3 exposure, suggesting that alligator gar are more sensitive to thyroid disruption and experienced more detrimental impacts with increased mortality. For a more direct comparison, zebrafish will be exposed under the

same conditions and examined at similar endpoints. Fishes with a more complex metamorphosis are an overlooked group in ecological risk assessments, and here we see that they are more sensitive than model species. This group represents valuable species both economically and ecologically whose populations may be impacted by metamorphic disruption.

1.06.T-05 Updates on endocrine WoE, check-box approaches and application of synthetic methodologies

Christopher J. Borgert, *Applied Pharmacology & Toxicology Inc.*

For more than a decade, Weight of Evidence (WoE) has been the standard methodological category for evaluating Endocrine Disruption (ED). WoE methods consider all data pertinent to satisfying the ED definition and evaluate data with respect to relevance, reliability, strength, and coherence. WoE methods for EDC also consider whether a causal relationship exists between an endocrine mode of action (MoA) and adverse effects. Hypothesis-Driven WoE (HD-WoE) approaches have the advantage of providing transparency and internal validation. Transparency is ensured with respect to the hypotheses under evaluation, the types of data that constitute “evidence,” the methods to search and identify evidence, evaluate data quality, “weight” various types of evidence, test the data against the hypotheses, and for reaching overall conclusions. HD WoE methods compare patterns of responses for known effectors of each hypothesized MoA (e.g., estrogen agonist; androgen antagonist), ensuring validation. Since the initial formulation of HD WoE methodologies fifteen years ago, refinements now allow assessing the strength by which a test chemical acts via an endocrine MoA (potency), how the kinetic behavior of the chemical can inform the relevance of the dose-response for mechanistic steps and apical responses, whether alternative non-endocrine MoA are more plausible causes of adverse effects, and how uncertainties in the various components of the WoE analysis can be quantified. These improvements are illustrated by recently-published WoE evaluations for octamethylcyclotetrasiloxane (D4), ethylbenzene, and styrene. As well, challenges have been identified for rigorous interpretation of various gold-standard assays such as vitellogenin production in male fish, and data-quality issues have been identified for some high-profile high-throughput assays. Despite improvements and identification of challenges, some contend that WoE methods are too slow and resource-intensive. As short-cuts, check-box approaches have been developed, such as the Key Characteristics. Despite a lack of validation and performance testing, KC approaches are promoted by some among academia and the regulatory community, but little consideration has been given to scientific integrity and epistemic validity issues. Arguments for and against WoE versus check-box approaches will be discussed.

1.06.T-06 Advancing effect modeling in the context of endocrine-disrupting chemicals

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The potential impacts of endocrine-disrupting chemicals (EDCs) on wildlife populations have been of concern for a number of years. For vertebrate wildlife, current legislation and guidance in the European Union (EU) severely restrict or prevent the use of EDCs, unless it can be demonstrated that their (individual-level) effects are not adverse at the population level. A recent review (Hazlerigg et al. 2025; <https://doi.org/10.1093/inteam/vjae039>) and an ongoing SETAC initiative (MAPPED - use of Models for Assessing PoPulation relevance of Endocrine Disrupting effects) address how population models can be used to determine the population relevance of endocrine-mediated effects of chemicals. This talk will highlight some of the insights gained from these analyses as well as challenges related to data gaps and limitations of the standard tests that provide input for the models. Whereas some regulatory jurisdictions use a risk-based approach to assess the relevance of EDC effects for wildlife populations, some EU regulations require that a hazard-based approach be used, which presents additional challenges. We conclude that population modeling is the most promising option for assessing the population relevance of EDCs, and we propose some immediate next steps for moving these efforts forward.

1.07.P-We Environmental Science Education

1.07.P-We-112 Redesigning a classic chemical kinetics experiment into a student-led inquiry-based laboratory module teaching the tools of environmental fate assessment

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Assessing the fate of a chemical contaminant in the environment is a complex process that requires interdisciplinary thinking, careful experimental design, and a clear understanding of the fundamental chemistry behind reaction kinetics and mechanisms. Preparing students to tackle this kind of research challenge is difficult, and learning by doing is often the most successful approach. However, time and resource limitations in undergraduate laboratories preclude the kind of complex experiments required for full environmental fate assessment. Here we present a laboratory module intended to simulate the analysis of the transformation processes in a wastewater treatment plant with a focus on student-led experimental design. Students are asked to determine the rate law and propose a corresponding reaction mechanism for the oxidation of Brilliant Blue FCF by hydrogen peroxide with the goal of optimizing a reaction process to remove this substance from contaminated water. To simulate the research process, they are not given an experimental protocol to follow. In teams, students write and implement an experimental procedure while remaining flexible enough to adapt to data collected in real time during the laboratory period. To succeed, they must (with course instructor guidance) determine the reaction order with respect to each species in the reaction, recognize that the reaction is pH sensitive, and understand the connection between these kinetic parameters and the overall mechanism of the reaction. Students are graded on a long-form laboratory report where they are asked to document this process and are not necessarily evaluated based on getting the correct answer, but that their answer is consistent with the data they collected and the rationale behind their experimental design. This laboratory module is simple to implement, requiring only UV-Vis spectrometers and pH meters along with safe and readily available reagents. It is also flexible, allowing incorporation into different course levels through adjusting the amount of information and guidance given to students, and may be extended to transformation product analysis or environmental photochemistry experiments for a more complete environmental fate assessment experience.

1.07.P-We-113 The search for undergraduate research opportunities transforms a former tertiary treatment wetland into an excellent outdoor classroom

Sarah Michele Harmon, University of South Carolina, Aiken

In 1993, the City of Augusta, Georgia, USA, converted old agricultural fields on property adjacent to a natural floodplain wetland into 12 constructed wetland cells that would serve as tertiary treatment before effluent was released to the permitted outfall on the Savannah River. As an innovative form of mitigation, the City of Augusta included an environmental education center on the property. This education center has grown in popularity over the years and now hosts hundreds of adults and children for outdoor environmental educational activities each year. At the same time, the area around the wetland cells has become a popular city-owned “green space” for birders and hikers. In 2020, we began undergraduate led research in this 1,100-acre wetland habitat. Finding the ideal source of real-world pollution research for undergraduates can be a challenge, but this area provides an artificial “worst case” scenario for students to refine the techniques and tools that they will ultimately use to investigate actual problems in their communities. This nature park and green space provides easy access to treated municipal sewage in a “natural” habitat that combines freshly treated municipal sewage with a wetland habitat that attracts abundant wildlife. In this outdoor laboratory, undergraduate researchers have taken molecular techniques into the field to track fecal contamination to human or wildlife sources; discovered pockets of antibiotic resistance in the wetland; and investigated the potential for mercury methylation in the anaerobic layer of the sediment. Currently, a team of undergraduate researchers are using the area to investigate the presence of cyanobacteria and the potential for hazardous algal blooms by practicing microscopic algal identification, toxin quantification through ELISA, pigment comparison, and the presence and quantification of cyanobacterial genes related to microcystin, anatoxin, and saxitoxin production. While we were typically able to identify problem areas and “hotspots” in the wetland, we have also been able to demonstrate that the problems are resolved before flow reaches the river outfall.

1.07.P-We-114 Adventures in specs grading part 2: Introductory environmental science

Amanda D Harwood, Alma College

In recent years there has been an increased interest in novel grading strategies in undergraduate education that better emphasize student learning outcomes. One strategy is specifications grading. While there are many variations of 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) Multiple attempts on assessments. I have used this method in teaching several upper-level courses for third- and fourth-year students with great success as defined by improved understanding and performance as well as student preference for this method. Recently, I have shifted my introductory environmental studies course to this format with mixed results. While students appreciated the option to redo assignments very few engaged with this practice even on high impact assessments (e.g. exams). Students also seemed to be confused about the system and had difficulty estimating their grades. The participation in redoing assignments decreased over time which was the opposite of the more advanced courses. Generally, student understanding improved, but overall grades were similar to previous years. Although the majority of test cases of specifications grading take place in introductory courses with great success, some adjustments may need to be made for its success in this particular course.

1.07.P-We-115 Classroom demonstration used for the visualization of three environmental partition coefficients

Mitchell Lee Kim-Fu and Jennifer Field, Oregon State University

In environmental chemistry and toxicology courses, chemical partitioning is a fundamental concept needed for predicting the fate and transport of chemicals and is necessary for assessing environmental risk and exposure. Chemical partitioning offers students a practical framework to connect theoretical concepts with environmental challenges surrounding organic pollutants. However, visualizing and understanding partitioning behavior can be challenging in classroom settings. This study describes a simple, hands-on activity using commercially available food-grade menthol crystals to demonstrate air-water (KAW), octanol-water (KOW), and octanol-air (KOA) partition coefficients in a single-beaker setup. In this activity, students begin with food-grade menthol dissolved in water. Using the freely accessible data from PubChem, a KAW constant is calculated while simultaneously experiencing air-water partitioning directly through olfaction (smell). Next, vegetable oil, used as a safer proxy for octanol, is added to the menthol-water solution. Students observe the disappearance of menthol's scent, indicating preferential partitioning into the "octanol" phase. They then calculate KOW and KOA using values from PubChem and place their olfactory experience in the context of their calculated value. Additionally, this activity segues discussion toward physical-chemical relationships governing partitioning amongst the three coefficients, reinforcing principles such as intermolecular forces, thermodynamics, and solubility while integrating database navigation and interpretation skills. This activity was implemented in a combined undergraduate and graduate student toxicology course on organic chemical behavior in the environment. The activity received unanimously positive feedback. Students reported increased confidence in using chemical databases and a clearer conceptual understanding of partitioning in environmental contexts. Additionally, integrating a demonstration into the classroom setting increased student participation and discussion on atmospheric transport and deposition. Future ideas for modifying this activity to highlight the dynamic nature of aqueous solubility and provide opportunities for students to observe how changes in conditions affect these interactions are discussed.

1.07.P-We-116 Sailing scientists- working with young people to design STEM education materials

Katie Reilly¹, Liam Hanna², Ben Gilbert², Caitlin Vinicombe², David Tubbs¹, Sam Dobson², Tony Elgar² and Iseult Lynch³, (1)University of Birmingham, United Kingdom, (2)Sea Cadets UK, United Kingdom, (3)School of Geography, Earth and Environmental Sciences, University of Birmingham, United Kingdom

Societal interest in water quality has increased whilst water quality has been reported to decrease in the UK over recent years. The decline in water quality is often attributed to a combination of increased sewage overflows,

changes of land use, plastic mismanagement and introduction of new chemicals and materials into circulation. Young people are also often reported to be one of the groups that are most impacted by eco-anxiety, which is a term that is used to describe the hopelessness and frustration often felt by people when they are faced with environmental issues. Through this project, we worked with Sea Cadets UK, a charity working with young people aged 9-18 in the UK with a focus of maritime activities. The projects had a two-step approach to firstly; develop a practical workshop exploring data collection and advocacy and secondly; a sampling campaign to collect water quality data in environments that they use for boating. The aim of the project was to involve people in those environments with the experimental design and data collection for these sites, and to co-create a workshop to share this process and the results. Establishing the common ground of water quality assessment and the challenges with balancing different societal and environmental pressures for management plans also facilitated the further development of discussion on Advanced Materials risks and ecotoxicity methods used to assess these. Testing included temperature, pH and conductivity, nitrates and phosphates and a secchi disk to test the turbidity. Sampling took place at 8 sites across the UK including a mix of coastal, freshwater, brackish, estuarine and Sites of Special Scientific Interest. Partnership building was an important aspect in this project and the interdisciplinary expertise across the network was highlighted, spanning pedagogy, photography and social media which added value to the project and outputs that could not have been achieved by a dissemination model from the research team. In parallel, a practical workshop (230 attendees) was designed with the Sea Cadets Learning and Development team to facilitate discussion on environmental advocacy, the need for robust data and evidence and how to engage with decision makers (i.e. policy) going forwards. The workshop also included analysis of microplastics in water samples, which lead to discussion on analytical method development for particles, the challenges and the ecotoxicity risks and methods for freshwaters. **Disclaimer/Disclosure:** This work was funded through the Natural Environment Research Council's Engaged Environments bursary 2024.

1.08.P-Th From Acclimation to Adaptation: Mechanisms, Costs and Consequences of Acquired Pollution Resistance

1.08.P-Th-113 Later-life stress responses are compromised by developmental exposure to polycyclic aromatic hydrocarbons (PAHs) in fish evolved to resist PAH toxicity

Samantha E Phelps, Melissa Chernick, Richard Di Giulio and Nishad Jayasundara, Duke University

Evolved resistance to anthropogenic compounds has independently emerged in multiple populations across taxa. While such adaptations can confer survival advantages in polluted environments, they may come at the cost of decreased tolerance to other forms of environmental stress. In this study, we sought to determine whether evolved chemical resistance is associated with decreased phenotypic plasticity and reduced stress tolerance later in life following developmental exposures. We focused on a population of Atlantic killifish (*Fundulus heteroclitus*) that has evolved resistance to polycyclic aromatic hydrocarbons (PAHs) and examined the later-life mitochondrial effects of developmental exposure to PAHs. We collected killifish embryos from PAH-sensitive and PAH-resistant, populations and exposed them to PAHs throughout embryonic development and quantified embryonic mitochondrial function at 12 days post fertilization. Subsequently, after four months of rearing in clean water, we measured juvenile basal respiration, additionally testing their metabolic responses to thermal and hypoxia stress. Our study revealed that killifish from pollution-resistant populations exhibit distinct baseline metabolic responses to developmental PAH exposure across both early-life and juvenile stages. Notably, PAH exposure during development altered the metabolic response to thermal and hypoxia stress in both sensitive and resistant juvenile fish. These results suggest that early-life exposure to PAHs can reshape later-life phenotypic plasticity, even in populations that have evolved resistance to these pollutants.

1.08.P-Th-114 Characterization and validation of secondary reproductive consequences in male *Fundulus heteroclitus* in response to legacy contaminants

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Contaminant resistance is a well-documented phenomenon in the adaptive and resilient fish species *Fundulus heteroclitus*, commonly called the mummichog. Because of this, *F. heteroclitus* is one of the few abundant fish species in highly contaminated or modified rivers, particularly those with legacy pollution. They are commonly sampled to characterize impacts of contamination in these systems because of their ubiquity, however this also means that they are less sensitive to common toxicological endpoints like tumor proliferation or other histological changes. Despite this, potential evidence of secondary consequences in the form of male reproductive impairment has been seen in field-collected populations we have sampled from the Anacostia River, MD/DC, USA, and the Christina River, DE, USA as measured through sperm DNA damage quantified using single-cell gel electrophoresis (Comet Assay) and computer-assisted motility analysis (CASA). To better understand if these effects are related to resistance, we compared field-collected sperm characteristics with liver DNA damage in each fish from a variety of sites. As validation that this response is initiated as a result of contaminant exposure, we conducted a series of intraperitoneal (IP) injections of contaminants of interest to study systems, including Aroclor 1254, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), and creosote on naive, reference fish and sub-sampled them for the same parameters at 1, 3, and 6 weeks to monitor impacts. This will be complemented by injection of the suspected creosote-resistant population of *F. heteroclitus* in the Christina River. These field and lab studies taken together can serve as evidence of contaminant resistance-mediated secondary consequences in this species and if so, suggest that indirect reproductive impacts are an emergent concern for fish populations in historically contaminated environments.

1.08.T From Acclimation to Adaptation: Mechanisms, Costs and Consequences of Acquired Pollution Resistance

1.08.T-01 Adaptive transcriptomic responses to multiple pollutant classes in Atlantic killifish

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The composition of pollutants in urban estuaries varies based on historical and contemporary industrial activity and imposes strong selection pressures on urban estuarine organisms. In these environments, populations may evolve resistance to multiple contaminants with shared or distinct mechanisms of toxicity. In complex chemical environments, populations may be more likely to evolve resistance to multiple chemicals if they share some mechanisms of toxicity compared to chemicals with unique mechanisms of toxicity. Understanding the molecular basis and specificity of resistance is critical for predicting evolutionary outcomes in response to environmental change. Atlantic killifish, or mummichog (*Fundulus heteroclitus*) populations inhabit highly polluted urban estuaries and have rapidly and repeatedly evolved resistance to exposure-induced developmental cardiotoxicity. Atlantic killifish have evolved resistance in both dioxin-like compound (DLC)-dominated and polycyclic aromatic hydrocarbon (PAH)-dominated urban environments. These two chemical classes exhibit both shared and unique mechanisms of toxicity. To investigate the complexity of the molecular mechanisms underlying pollution resistance across chemical classes, we examined transcriptomic responses to two cardiotoxic pollutants during embryonic development in Atlantic killifish: 3,3',4,4',5-pentachlorobiphenyl (PCB-126, a DLC) and benzo[k]fluoranthene (B[k]F, a PAH). We compared transcriptomic responses between four populations, including two from heavily contaminated sites (PCB-contaminated and PAH-contaminated) with evolved resistance and two from nearby reference sites. For both pollutants, F2 embryos were assessed for a set of developmental heart defect phenotypes across a range of concentrations, coupled with RNA-seq analysis. Resistant populations demonstrated reduced cardiotoxic phenotypes in response to both classes of pollutant exposure relative to sensitive populations. Comparative transcriptomics revealed shared and unique molecular mechanisms underlying pollution resistance dependent on chemical class (PCB or PAH) and genetic background. These unique molecular mechanisms may underlie subtle phenotypic differences not detectable by

gross heart defect phenotype assessment. This work highlights how complex exposure histories contribute to complex evolved molecular responses and phenotypic outcomes in a wild species. **Disclaimer/Disclosure:** Supported in part by R01ES032323 and 5T32HL086350-17

1.08.T-02 Microbiota-mediated resistance: A co-evolved mechanism of pollution adaptation in Atlantic killifish (*Fundulus heteroclitus*)

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Mechanisms of evolved resistance to pollutants are traditionally studied through the lens of population genetics. Emerging evidence suggests that co-evolved symbiotic relationships between hosts and microbiota may play a critical role in shaping evolutionary adaptations. In polluted environments, microbial communities may biotransform toxicants or be reshaped by chemical exposure, indirectly influencing host function. However, whether host-microbiome interactions causally contribute to chemical resistance and long-term adaptation remains unclear. Atlantic killifish (*Fundulus heteroclitus*) inhabiting creosote-contaminated areas of the Elizabeth River, VA, including the former Republic Creosoting (REP) site, have evolved resistance to polycyclic aromatic hydrocarbons, such as benzo(a)pyrene (BaP), but exhibit fitness trade-offs. Notably, REP killifish harbor distinct gut microbiomes compared to a nearby reference population, King's Creek (KC), despite similar environmental microbial communities, suggesting host-driven microbiome selection. To test the role of the microbiome in pollution adaptation, we generated germ-free (GF) embryos from both populations and cross-conventionalized them with gut microbiota from field-dissected REP and KC adults. We assessed BaP-induced physiological responses (CYP1A induction, mitochondrial function, and larval behavior), performed RNA-sequencing, and profiled microbes and metabolites in gut inoculum. We reveal mitochondrial and behavioral resistance to BaP in REP embryos depends on REP-specific microbiota, as supported by metabolomic signatures. However, these microbes alone did not confer resistance in KC embryos, suggesting adaptive host-microbiome interactions have been integrated into the REP host. Transcriptomic analysis revealed REP embryos conventionalized with REP vs. KC microbiota showed differential expression of genes involved in mitochondrial function and xenobiotic metabolism in response to BaP. REP microbiota downregulated aryl hydrocarbon receptor (AhR) signaling - a chemical resistance mechanism - compared to KC microbiota, despite producing elevated levels of AhR-activating metabolites. Similar trends were observed in GF embryos, suggesting KC microbiota may induce a dysbiotic or maladaptive state, influencing sensitivity to xenobiotic-exposure. These findings suggest microbial divergence in REP killifish is a driver of chemical resistance and that these symbiotic relationships have co-evolved over generations. **Disclaimer/Disclosure:** Research performed under this study was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Numbers P42ES010356 and T32ES021432 (Duke University Program in Environmental Health). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

1.08.T-03 Exploring biotransformation of legacy pollutants in gulf killifish via waterborne and intraperitoneal injection exposure

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Polycyclic aromatic hydrocarbons (PAHs) drive evolutionary adaptation in aquatic species. Gulf killifish (*Fundulus grandis*) from the Houston Ship Channel (HSC) have developed resistance to PAH-induced cardiovascular teratogenicity through chronic exposure, linked to a deletion in the aryl hydrocarbon receptor (AHR) gene. This study characterized benzo[a]pyrene (BaP) biotransformation in PAH-adapted (Vince Bayou, VB) and non-adapted (Smith Point, SM) *F. grandis* populations, examining sex- and population-specific differences. Fish were exposed to BaP through two 15-day waterborne exposures (0, 10, 40, 100 µg/L) and one 96-hr intraperitoneal (IP) injection (Corn oil, 2.5 and 5 mg/kg). Waterborne BaP was extracted using C-18 filters and MeOH, followed by LC-FLD. Bile metabolites were extracted and analyzed by LC-HRMS to release free metabolites ("F"), following enzymatic treatment to release glucuronide ("G"), and sulfate ("S") conjugates.

Metabolite extraction and LC-HRMS analysis revealed four BaP phase I products, 1-OH-BaP, 9-OH-BaP, 7,8-BaP-diol, and 4,5-BaP-diol, and their phase II conjugates ("F", "G", and "S"). In the first waterborne exposure, 1-OH-BaP was present in all forms, peaking in SM males at 100 µg/L ("F" $p = 0.035$; "G" $p = 0.024$). 7,8-BaP-diol was most abundant in VB males, while 9-OH-BaP (mostly "S") was higher in SM males. Sulfated BaP appeared only at 100 µg/L, with the highest value in VB females (0.023 ng/µL). In the second exposure, all previous metabolites were confirmed, with 4,5-BaP-diol uniquely detected and significantly elevated in VB males at 100 µg/L (vs. VB-10 µg/L, $p = 0.014$; vs. VB-40 µg/L, $p = 0.026$). 9-OH-BaP occurred in all forms; 1-OH-BaP and 7,8-BaP-diol mainly as "G". In the IP exposure, only 7,8-BaP-diol-glucuronide and BaP ("F" & "G") were found. 7,8-BaP-diol-glucuronide was significantly higher in SM males at 5 mg/kg vs. SM-2.5 mg/kg ($p = 0.022$) and differed across VB-2.5 and SM-5 ($p = 0.0296$), and SM-5 vs. VB-5 ($p = 0.040$). "F" BaP reached 0.314 ng/µL in SM males at 2.5 mg/kg, and "G" BaP was highest in VB females (0.007 ng/µL). Overall, BaP metabolism in *F. grandis* exhibited route-of-exposure, dose-, sex-, and population-specific patterns, with distinct biotransformation profiles in both populations. Future research will assess the role of AHR deletions in metabolic responses. Integrating metabolic, genetic, and exposure data will enhance our understanding of adaptive mechanisms in contaminated habitats. **Disclaimer/Disclosure:** NIEHS Research Enhancement Award R15ES034885 funded this project

1.08.T-04 Epigenetic plasticity is a driver of heritable PAH tolerance in Atlantic killifish

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Heritable epigenetic adaptation to environmental stressors, in addition to genetic evolution, is a compelling but highly contested possibility. Previously, we showed evidence of a generationally heritable epigenetic memory at the cytochrome P450 1A (CYP1A) gene in wild Atlantic killifish (*Fundulus heteroclitus*) with evolved tolerance to PAH. This memory leads to blunted induction of CYP1A by PAH; this blunting that protects against polycyclic aromatic hydrocarbon (PAH)-induced cancer. Here, using Oxford Nanopore long-read sequencing in PAH-tolerant and sensitive wild-type embryos, we report PAH-induced loss of CYP1A gene body methylation in PAH-sensitive (86% CpG methylation in control, decreased to 63% in exposed), but not -tolerant (84% control, 86% exposed), embryos. We observed similar patterns at CYP1B, CYP1C, and the aryl hydrocarbon receptor repressor, AHRR, which show similarly blunted expression. This genic methylation loss was correlated with increased levels of natural anti-sense RNA transcripts in cis (cis-NATs). Because cis-NATs can amplify gene induction signals, loss of these transcripts may cause blunting of these genes' expression. Genome-wide, PAH-tolerant embryos showed reduced plasticity in DNA methylation response to PAH (N=1212 differentially methylated regions (DMRs) vs. PAH sensitive embryos (N=3074 DMRs) that was not due to mutational loss of CpG sites. Notably, we observed population differences in gene methylation in pathways linked to the PAH tolerance phenotype, including AHR, RXR, estrogen and voltage-gated potassium channel signaling, as well as developmental processes and energy metabolism. Observed DMRs are functionally relevant; 35% of genic DMRs in PAH-sensitive embryos and 54% of genic DMRs in PAH-tolerant embryos were correlated with altered gene expression. Our data support the existence of stable epigenetic responses to chronic environmental stressors in a natural experimental setting, with broad implications for natural or directed adaptation strategies for other populations. **Disclaimer/Disclosure:** This research was funded by a seed grant from the Triangle Center for Evolutionary Medicine (TriCEM) (to C.W.), by a Diversity Supplement to an Outstanding New Environmental Scientist (ONES) R01 from NIEHS (R01ES034836-03S1 to C.W. and M.C.) and by the Oregon Institute of Occupational Health Sciences at Oregon Health & Science University via funds from the Division of Consumer and Business Services of the State of Oregon (ORS 656.630) (to C.W.).

1.08.T-05 Physiological and behavioral responses to selection for copper resistance in *Drosophila melanogaster*

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Heavy metal pollution has pervasive environmental, health, and evolutionary impacts. In humans, health risks range from permanent neurological disease to increased morbidity of degenerative syndromes. My previous work has demonstrated that variation in susceptibility to metal toxicity is influenced by a combination of genetic variation, environmental exposure, and their interaction. My lab focuses on copper given the important role this metal plays as an environmental contaminant and as a critical micronutrient for normal physiological development and maintenance in most organisms. Through my work with natural and laboratory *Drosophila* populations, it has become clear that copper resistance has a complex genetic basis and is influenced by the physiological status of the individual as well as the ability to perceive and behaviorally respond to stressors in the environment. I collected flies from two potentially copper contaminated environments and am currently evolving copper resistance in replicated experimental populations using an evolve and resequence approach to assess changes in copper, lead, cadmium, and starvation resistance. By measuring these traits throughout selection, I am tracking and characterizing the genetic control of evolved responses to copper stress. I am examining the effects of adult selection on developmental resistance to understand the ontological consequences of selection, and I am examining variation in copper avoidance throughout selection to identify possible behavioral contributions to evolved resistance. Using an integrative approach that leverages whole genome and RNA sequencing and experimental evolution, we will track the dynamic shifts in allele frequency and gene expression in response to copper stress in diverse naturally derived genetic backgrounds. By focusing on multiple physiological and behavioral traits, these approaches will provide critical insight into the interconnectedness of multiple response traits, while also illuminating genetic factors that influence behavioral and learning disabilities linked to metal poisoning. **Disclaimer/Disclosure:** This work is supported by NIEHS 4R00ES033257-03.

1.08.T-06 Assisted gene flow prevents extinction when adaptation alone fails under chronic insecticide stress

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Rapid environmental change is driving vulnerable species toward extinction, raising urgent questions about whether populations can adapt fast enough to persist under chronic pollutant exposure. Although some populations evolve resistance, the mechanisms and limits of evolutionary rescue remain poorly understood in natural settings. Here, we tested whether adaptation alone is sufficient to prevent extinction under insecticide stress, and whether assisted gene flow can improve persistence outcomes. Using outdoor mesocosms, we exposed replicate *Drosophila melanogaster* populations to a lethal dose of the insecticide spinosad. Populations were introduced into outdoor enclosures and evolved over nine generations either with or without prior exposure to the insecticide. Control populations - naïve to insecticide - were matched with "resistance-enriched" populations that received assisted gene flow via prior laboratory selection. We tracked demographic trajectories, six life-history traits, and genome-wide allele frequency change under common garden rearing to isolate genetic responses. Populations without assisted gene flow exhibited rapid initial adaptation but high extinction rates, with 60% collapsing early in the experiment. In contrast, resistance-enriched populations showed no extinctions, sustained demographic recovery, and more consistent gains in survival, fecundity, and stress tolerance. Temporal genomic data revealed parallel shifts in allele frequencies at known resistance loci and signatures of directional selection across the genome. Multivariate trait analysis suggested potential trade-offs between early survival and long-term persistence, indicating that rapid adaptation may carry hidden costs. These results show that while rapid adaptation can occur, it is not always sufficient for persistence. Assisted gene flow provided a consistent demographic advantage, demonstrating its potential as a strategy to support population resilience under pollution stress. Our findings highlight the importance of integrating evolutionary processes,

genetic connectivity, and resistance management into ecological risk assessment frameworks.

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1.09.P-Tu Immunotoxicological Investigations: From Molecules to Model Organisms, Wildlife, and the Environment

1.09.P-Tu-005 Investigating potential B cell immunotoxicity induced by 2-[difluoro(trifluoromethoxy)methoxy]-2,2-difluoroacetic acid (PFO2HxA)

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Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic compounds with myriad applications across consumer goods and industrial products. Two well-studied members of this class, perfluorooctanoic acid and perfluorooctane sulfonic acid, have been classified as presumed immune hazards to humans; this raises questions of the ability of other PFAS to induce immunotoxicity, especially those that are lacking in toxicological data. Many understudied PFAS can be found across the country in drinking and surface waters. One of these compounds, found in surface waters of North Carolina, is 2-[difluoro(trifluoromethoxy)methoxy]-2,2-difluoroacetic acid (PFO2HxA; CAS 39492-88-1). Despite this compound being detectable in North Carolina surface waters, its toxicity in living organisms has only been studied minimally. Based on previous studies from our laboratory, indicating exposure to individual PFAS can elicit immune system alterations, this study aimed to investigate potential immunotoxicity of PFO2HxA in an experimental mouse model. Adult male and female C57BL/6 mice (6-8 weeks old) were exposed by gavage once/day for 30 days with PFO2HxA at 0, 0.05, 0.5, or 5 mg/kg. Immunophenotyping of various markers for B cell maturation and differentiation was performed through flow cytometry and the T cell-dependent antibody response (TDAR) also was evaluated. The TDAR did not vary statistically by dose. However, analysis of B cells demonstrated statistically significant differences in marginal B cell numbers in females between the control (0 mg/kg PFO2HxA) and the 0.5 mg/kg PFO2HxA dose groups, as well as between the control and the 5 mg/kg PFO2HxA dose groups. There were also statistically significant differences in memory B cell numbers in females between the control and all other dose groups (0.05, 0.5, and 5 mg/kg PFO2HxA). Differences between numbers of plasma cells, follicular B cells, and naïve B cells in females between the control and PFO2HxA dose groups were also observed, but were not statistically significant. Oral exposure to PFO2HxA might affect different markers of immunotoxicity, particularly in relation to B cells. Concerningly, PFO2HxA is one of many understudied compounds found in North Carolina surface waters; additionally, PFAS mixtures could possibly lead to additive effects. Further investigation is necessary to confirm these findings and investigate effects on additional immunotoxicity markers.

1.09.P-Tu-006 Developmental effects of hexafluoropropylene oxide dimer acid (HFPO-DA) or GenX *Shyla Streeter and Jamie DeWitt, Oregon State University*

Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent contaminants with bioaccumulation potential and long half-lives in organisms, raising concerns for human and wildlife health. Increased PFAS exposure has been associated with developmental toxicity, specifically adverse effects on immune function, which may heighten susceptibility to disease. This study investigated whether gestational exposure to the PFAS known as hexafluoropropylene oxide dimer acid (HFPO-DA or “GenX”) disrupts developmental endpoints relevant to the immune system in mice offspring. Pregnant C57BL/6 dams were exposed to GenX from gestational day (GD) 1 through GD17 at 0, 1, 5, or 10 mg/kg. Litters were culled to six offspring at PND1 and offspring stayed with dams until weaning at PND21. Offspring received no additional exposures. At PND51

offspring were injected via the tail vein with sheep red blood cells (SRBCs) to induce the T cell-dependent antibody response (TDAR). Five days later, at the peak of IgM antibody production, offspring were humanely euthanized, and blood, spleen, thymus, heart, liver, and kidney were collected and stored for later analysis. Preliminary data indicate that on average, relative spleen and thymus weights of both male and female offspring slightly after weaning and at PND56 were smaller for treated offspring compared to control offspring, with female offspring appearing slightly more sensitive than male offspring. On average, relative liver weights of treated offspring were 10-17% greater compared to control offspring in PND56 offspring and in PND21 offspring, were ~20% greater for female and only ~0.4% greater for male offspring. On average, liver peroxisomal enzyme activity of treated groups measured at PND21 increased by nearly 40% in male offspring and nearly 50% in female offspring compared to control offspring. The increase persisted to PND56, particularly for female offspring that had an over 200% increase. These data indicate that the gestational effects of GenX exposure appear to persist in the liver. Additional analyses will include T cell-dependent antibody responses and immunophenotype of splenic and thymic lymphocytes to better understand effects of gestational exposure on the adaptive immune system. However, these preliminary data suggest that gestational exposure to GenX produces persistent effects on well-studied liver outcomes in rodents.

1.09.T Immunotoxicological Investigations: From Molecules to Model Organisms, Wildlife, and the Environment

1.09.T-01 Mitochondrial dysfunction in B cells following PFAS exposure: Implications for antibody response suppression

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Per- and polyfluoroalkyl substances (PFAS) are synthetic, highly persistent environmental contaminants commonly detected in drinking water. Despite widespread industrial and consumer use, PFAS exposure has been linked to adverse health effects, including reduced vaccine-induced antibody responses. In animal models, PFAS impair the T cell-dependent antibody response (TDAR), a key measure of adaptive immune function. While mechanisms remain unclear, PFAS may disrupt B cell development and metabolic function, ultimately impairing antibody production. Because naïve B cells undergo high metabolic stress when differentiating into plasma cells, we hypothesized that PFAS-induced TDAR suppression stems from disrupted mitochondrial metabolism. To test this, we examined mitochondrial bioenergetics and ATP production in activated B cells from PFAS-exposed mice. Our study focused on four emerging PFAS detected in North Carolina surface waters - PFMOAA, PFO4DA, PFO2HxA, NBP1, and NBP1, alongside legacy PFOA. Adult male and female C57BL/6 mice were orally gavaged with either 0 or 50 mg/kg/day (7.5 mg/kg/day for PFOA) of each compound for 30 days. After exposure, splenic B cells were isolated and stimulated *ex vivo* with anti-CD40 and IL-4 antibodies to assess mitochondrial respiration and real-time ATP production. *Ex-vivo* stimulation significantly increased mitochondrial activity in both control and exposed cells, validating the assay. However, PFAS exposure consistently reduced mitochondrial reserve capacity and ATP production. For example, PFMOAA-exposed cells showed a 36% non-significant reduction in ATP production versus controls despite similar stimulation-driven increases (156% control vs. 143% exposed, $p < 0.05$). PFOA exposure led to a 17% reduction in spare capacity in males. Female B cells showed greater increases after PFOA exposure, indicating sex-specific responses. PFO4DA and PFO2HxA exposures resulted in diminished mitochondrial function post-stimulation, particularly reductions in spare capacity and ATP output. NBP1 exposure negatively impacted mitochondrial respiration in unstimulated cells but not in stimulated cells. These findings suggest that PFAS exposure impairs mitochondrial flexibility in activated B cells, potentially contributing to reduced antibody responses. Our study highlights mitochondrial dysfunction as a mechanism of PFAS immunotoxicity and underscores the need to assess metabolic impacts of both legacy and emerging PFAS compounds. **Disclaimer/Disclosure:** This study was funded by a Pilot Project Program at North Carolina State University's Center for Human Health and the Environment.

1.09.T-02 Per- and polyfluoroalkyl substances (PFAS) toxicity and DNA repair in human melanocytes from diverse racial groups

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Per- and polyfluoroalkyl substances (PFAS) are persistent pollutants linked to endocrine disruption, immunomodulation, and genotoxicity, potentially increasing disease susceptibility. While systemic effects are well-documented, emerging evidence links PFAS to increased melanoma incidence in certain populations, yet direct impact on melanocytes remains unknown. This study investigates whether PFAS exposure alters melanin production and induces cytotoxic or genotoxic responses in melanocytes derived from racially diverse backgrounds. Methods: Primary human melanocytes derived from donors of diverse racial backgrounds were exposed to individual PFAS compounds of varying carbon chain lengths including PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, and PFAS mixture. Treatments were conducted at 10nM and 10µM concentrations for 24hr. Following exposure, cells were assessed for melanin content, S100β expression, proliferation, reactive oxygen species (ROS) generation, and DNA repair enzyme, OGG1. PFAS exposure elicited differential responses in melanocytes of varying racial origin, reflected in distinct patterns of cellular proliferation, oxidative stress, and molecular signaling. Caucasian melanocytes exhibited sustained, dose-dependent increases in proliferation and ROS generation. Asian melanocytes showed moderate responses, with reduced proliferation and lower ROS production. In contrast, PFAS exposure remained ineffective at inducing proliferation or ROS formation in melanocytes from Black donors. At the molecular level, OGG1 expression was upregulated at nM PFAS concentrations but downregulated at µM levels, suggesting possible impairment of DNA repair mechanisms. Despite these variable stress responses, PFAS exposure consistently upregulated S100β, a melanoma-associated marker, across all melanocyte populations highlighting a potentially shared oncogenic risk mediated through distinct cellular pathways. The differential responses of melanocytes to PFAS exposure across racial backgrounds suggest that inherent cellular characteristics critically influence susceptibility to environmental toxicants. Asian and Caucasian melanocyte exhibited greater sensitivity to PFAS-induced genotoxicity and upregulation of melanoma-associated markers compared to those from Black donors. These findings suggest race-associated differences in PFAS toxicity and emphasize cellular variability as a key factor in environmental risk assessment. **Disclaimer/Disclosure:** Funding: UL Research Institute

1.09.T-03 Effects of PFOS and MeHg co-exposure on post-transcriptional modifications in zebrafish

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Perfluorooctanesulfonic acid (PFOS) and methyl mercury (MeHg) are legacy bioaccumulative contaminants with potential adverse health outcomes for aquatic wildlife and for humans through the fish consumption pathway. While risks from co-exposure have been managed at many waterbodies by setting chemical-specific ambient water quality criteria and fish consumption advisories, questions remain regarding the protectiveness of assumptions of additivity for mixtures, and the extent to which PFOS and MeHg share a common mechanism of action for outcomes, such as immunotoxicity and neurotoxicity. Here, we performed an eight day exposure to embryonic Zebrafish (*Danio rerio*) to a range of environmentally relevant concentrations of PFOS and MeHg, both separately and as binary mixtures. Dose-response analysis, including visual inspection with isoboles for mixtures, are presented for developmental and survival endpoints. In addition, post-mortem analyses include RNA sequencing and subsequent qPCR for confirmation of targets, and post-translational analyses (Western blotting and immunohistochemistry) to confirm protein activation of gene targets. Noncongruent dose-response curves for PFOS and MeHg for the same endpoint complicate the use of the hazard index approach in ecological and human health risk assessments. In addition, dose-response curves for biomarkers are presented, which may inform mechanisms of action and contribute to the development of adverse outcome pathways for PFOS and MeHg. This study adds to the growing body of science on chemical mixtures of contaminants that are likely to co-occur and become risk management priorities for many watersheds.

1.09.T-04 Does Perfluorooctane sulfonate exposure alter Host–pathogen dynamics in great lakes salmonids?

Adrian Deil Manliclic, Rachel Leads, Katie King, Megan Shavallier, Thomas Loch and Cheryl A. Murphy, Michigan State University

Perfluorooctane sulfonate (PFOS) is among the most widespread per- and polyfluoroalkyl substances, with frequent detection across various environmental media, including water and fish samples from rivers, lakes, and streams throughout North America. Although the environmental and health risks of PFOS are increasingly recognized, its specific effects on wildlife, particularly fish, remain poorly understood. In particular, its impact on disease susceptibility and survival in fish has received limited investigation. Given the high concentrations of PFOS detected in fish populations across the Great Lakes region, evaluating these effects is essential. In this study, juvenile lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), and steelhead trout (*Oncorhynchus mykiss*) were exposed to PFOS via water immersion using a flow-through system for 96 hr. Following exposure, fish underwent species-specific microbial pathogen challenges via static immersion, using established disease challenge protocols. Post-challenge, all fish were transferred to clean flow-through systems for observation and monitoring of disease progression and survival. Tissue samples were collected at defined intervals for histopathology and quantitative PCR to confirm pathogen presence and quantify viral load. This study aims to elucidate the role of PFOS in modulating host-pathogen interactions in fish, with significant implications for aquatic animal health, environmental management, and conservation. If PFOS impairs the immune function of these keystone species, targeted remediation within affected watersheds will be crucial to protect biodiversity and ensure long-term ecosystem sustainability. **Disclaimer/Disclosure:** This project is funded by Great Lakes Fishery Trust

1.09.V Immunotoxicological Investigations: From Molecules to Model Organisms, Wildlife, and the Environment

1.09.V-01 Chemical stress-responsive AGT-N1 counteracts renointestinal distress via autophagic and microbiota reprogramming

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The integrated stress response (ISR) plays a pivotal role in the cellular stress response, primarily through global translational arrest and the upregulation of cellular adaptation-linked molecules. AGT-N1 protein has been predicted as a potent stress-responsive biomarker of clinical inflammatory and metabolic distress in various types of diseases. Herein, we assess whether ISR-driven cellular stress contributes to pathophysiological outcomes by modulating AGT-N1. Clinical transcriptome analysis demonstrates that PKR is positively associated with AGT-N1 expression in patients with renal injury. AGT-N1 expression is dependent on protein kinase R (PKR)-linked ISR during acute renointestinal distress in mice and genetic ablation of AGT-N1 aggravates chemical-induced lesions in renal tissues and the gut barrier. An in-depth evaluation of the gut microbiota indicates that AGT-N1 is associated with the abundance of mucin metabolism-linked bacteria and their enzymes. Moreover, stress-responsive AGT-N1 facilitates mucin production and cellular survival via the reorganization of the autophagy regulatory network. Collectively, ISR-activated AGT-N1 counteracts pathological processes via the protective reprogramming of the autophagic network and microbial community, thereby providing robust predictive biomarkers and interventions against renointestinal distress (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, RS-2023-00240942, and RS-2024-00462450)).

1.09.V-02 Agricultural aminoglycoside exposure compromises uremic toxicity in the gut-kidney axis

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Epidemiological and experimental evidence has implicated a potent link between agricultural antibiotic exposure and susceptibility to various diseases. Clinically, antibiotic treatment during platinum chemotherapy is associated with poor prognosis in patients with malignancy. In the present study, crop-protecting aminoglycoside exposure was assessed for its impact on renal distress as a sequela of platinum-based chemotherapy. Clinical transcriptome dataset-based evaluations demonstrated that levels of dysbiosis-responsive genes were elevated during renal distress, indicating pathological communications between gut and kidney. Experimentally, mucosal exposure to streptomycin aggravated platinum-induced renal tubular lesions in a mouse model. Moreover, antibiotic-induced dysbiosis increased susceptibility to gut mucosal inflammation, epithelial disruption, and bacterial exposure in response to cisplatin treatment. Further investigation of the luminal microbes indicated that antibiotic-induced dysbiosis promoted the dominance of *Bacteroides* species. Moreover, the functional assessment of dysbiotic microbiota predicted tryptophan metabolic pathways. In particular, dysbiosis-responsive *Bacteroides acidifaciens* was associated with the production of the uremic toxin indoxyl sulfate and renal injuries. The results of this study including bacterial community-based evaluations provide new predictive insights into the interorgan communications and interventions against dysbiosis-associated disorders (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, RS-2023-00240942, and RS-2024-00462450)).

1.10.T Innovative and Novel Applications of New Approach Methodologies: From Tool Development to Case Studies

1.10.T-01 Viewpoint on challenges and opportunities in the development and adoption of new approach methods

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Despite the rapid development of New Approach Methodologies (NAMs) to help inform safety assessments, adoption of NAMs in regulatory frameworks has been relatively slow. This slow transition is due to one or more factors such as adapting relevant legislation, the lengthy validation process, limitations of context of use, building scientific confidence in the methodology, and adopting harmonized regulatory test guidelines. At the 2024 Agrochemicals (AGRO) Division of the American Chemistry Society's fall meeting, 476 and in-person symposia brought together international experts in the NAMs field to provide viewpoints on challenges and opportunities to increase the adoption of NAMs in regulatory frameworks. This presentation will highlight many of the opportunities and challenges presented by the speakers in these symposia to improve the development and increase the adoption of NAMs in the regulatory context. Key areas that were discussed during the symposia and will be summarized during this presentation include: improving interdisciplinary and multipartite collaboration, concrete examples of how NAMs have been successfully applied in regulatory safety assessments, the outlook to accelerate NAMs development and adoption for wildlife testing including endocrine activity, approaches to build scientific confidence in NAMs for regulatory safety evaluations, achieving public trust and societal acceptance of NAMs for regulatory safety assessments, and strategies for accelerating transition towards greater adoption of NAMs for safety assessments in regulatory frameworks.

1.10.T-02 Development and readiness evaluation of zebrafish-based testing battery for developmental neurotoxicity assessment

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A large number of chemicals remain unevaluated for developmental neurotoxicity (DNT), prompting the need for more rapid and efficient screening methods. The OECD in vitro DNT battery (DNT-IVB) models key neurodevelopmental processes, with substances affecting at least one considered DNT-positive. However, current DNT-IVB has limitations such as reliance on rat-derived rather than human cells for modeling complex

tissue functions, underdeveloped myelination assays, and inadequate glia-specific assessment. Compared to in vitro systems, zebrafish embryos enable integrated assessment of DNT-relevant processes in a single model. This study aims to adapt OECD DNT–IVB endpoints to zebrafish larvae, developing a complementary approach that addresses limitations of in vitro assays while aligning with established frameworks. We developed zebrafish larval assays evaluating: 1) Proliferation (BrdU incorporation, 24hpf), 2) Migration (OPC movement in spinal cord, 48hpf), 3) Neuronal/glia differentiation (NBP expression for OPC-to-oligodendrocyte transition, 5dpf), 4) Apoptosis (TUNEL, 24hpf), 5) Myelination (myelin number/length, 5dpf), 6) Neurite outgrowth (motor axon length, 3dpf), 7) Synaptogenesis (neuromuscular junction analysis using SV2/α-BTX, 5dpf), 8) Neural network formation neurotransmitter quantification by HPLC, 5 dpf). Test readiness was evaluated using scoring criteria including exposure clarity, endpoint relevance, specificity controls, and data evaluation methods. To validate the assays, we employed a tiered chemical selection strategy: five DNT-positive compounds selected from EPA Drug, NTP Drug, and Withdrawn Drug lists based on evidence in the NeurotoxKb database; ten endpoint-specific positives from the Recommended DNT Reference Chemical Test Set, identified using ToxCast bioactivity data; ten negative controls; and five PFAS compounds representing environmentally relevant chemicals—all tested within sub-lethal, non-malformation concentration ranges. This study is expected to demonstrate that zebrafish larvae can capture key neurodevelopmental endpoints defined in the OECD DNT–IVB. As no single in vitro method covers the full spectrum of DNT-relevant processes, the zebrafish model offers a biologically relevant lower-tier in vivo approach that can enhance the interpretability of in vitro findings and inform decisions regarding the necessity and design of higher-tier studies, including those in rodent models. This research was supported by a grant (RS-2025-00331685) from Ministry of Food and Drug Safety in 2025.

1.10.T-03 Refining mitochondrial neurotoxicity adverse outcome pathways using *Caenorhabditis elegans* and mechanistic analysis of mitotoxic exposures

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Mitochondrial dysfunction is associated with neurotoxicity, in particular dopaminergic cell death. 5-15% of pollutants likely affect mitochondrial function as their primary mechanism of action. However, there are many different mechanisms of mitochondrial toxicity, and it is not clear which drive neuronal cell death. Furthermore, the reason for the unusual sensitivity of neurons and especially dopaminergic neurons to mitochondrial toxicity is unclear. We hypothesized that exposures resulting in redox stress, but not exposures causing bioenergetic deficiency only, would cause dopaminergic cell death. We have been exposing *Caenorhabditis elegans* to mitochondrial toxicants acting by different mechanisms, assessing redox and bioenergetic changes, measuring morphological degeneration and loss of neurons, and testing pharmacological and genetic rescues. We have tested inhibitors of electron transport chain complexes, mitochondrial uncouplers, redox cyclers, and mitochondrial DNA damage. To date, these chemicals have included rotenone, pyridaben, antimycin A, pyraclostrobin, pentachlorophenol, Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, paraquat, benzyl viologen, fluoroacetate, and dicyclohexylcarbodiimide, and indoor and outdoor environmental mixtures. We have also tested how high sugar diets, exercise, and microbiome changes that alter mitochondrial function affect neuronal toxicity. To date, all exposures that cause a significant increase in oxidation state cause neuronal loss. Dopaminergic neurons have been the most sensitive. Exposures that cause energetic depletion only cause neuronal cell death if they also caused intracellular oxidation. We propose that mechanistic neurotoxicity analyses with *C. elegans* are a useful New Approach Methodology to generate data informing mechanism-specific mitochondrial toxicity-initiated Adverse Outcome Pathways. **Disclaimer/Disclosure:** This work was funded by the National Institutes of Environmental Health Sciences Superfund Research Program (P42ES010356) and by the National Institutes of Health (R01ES034270).

1.10.T-04 New faces, same threat: Metabolic toxicity of bisphenol A alternatives in human cell models

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Bisphenol analogs are widely adopted as alternatives to bisphenol A (BPA) in the production of plastics, coatings, and resins. Although often marketed as safer options, several of these compounds have been linked to adverse health outcomes involving oxidative and metabolic stress. This study evaluated the cytotoxicity of seven bisphenol analogs (BPS, BPF, BPAF, BPP, BPAP, BPB, BPE) in eight human cell lines representing key organs. BPE was most toxic in Caco-2 intestinal cells ($EC_{50} = 0.2 \mu M$), while HepaRG (liver) and HEK293 (kidney) cells were highly resistant to BPS ($EC_{50} > 1000 \mu M$). BPAF, BPP, and BPAP had low EC_{50} s across cell types, indicating broad toxicity. Mechanistic assays showed that BPAF caused a >10-fold increase in radical oxygen species (ROS) in Caco-2 cells ($1,270.4 \pm 187.4$ RFU/cell at $10 \mu M$; $p < 0.01$), while HepaRG cells had a milder response (161.6 ± 14.1 RFU/cell). BPAF, BPAP, and BPE disrupted mitochondrial membrane potential in Caco-2 and HepaRG cells at concentrations as low as $0.01 \mu M$. BPP uniquely elevated mitochondrial calcium in HMC3 cells at $10 \mu M$ (320.3 ± 52.7 RFU/cell), suggesting apoptotic signaling. Caco-2 cells were the most sensitive overall, while HMC3 cells were more resistant except to BPP. Four bisphenol analogs (BPP, BPAP, BPAF, and BPE) were selected for further testing in Caco-2, HepaRG, and HMC-3 cells to investigate their effects on ER stress and apoptosis pathways. Ongoing ELISA assays targeting PERK, eIF2 α , ATF4, and cytochrome C aim to clarify the role of the PERK/eIF2 α /ATF4 axis and mitochondrial apoptosis in bisphenol-induced toxicity.

1.11.T Microbial Interactions with Environmental Contaminants: Molecular Mechanisms to Ecosystem Effects

1.11.T-01 Transformation and defluorination of fluorotelomer carboxylates (FTCAs) by *Pseudomonas* sp. strain 273

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Per- and Polyfluoroalkyl substances (PFASs) are widespread and persistent contaminants that have negative effects on human and environmental health. Due to their unique chemical properties, PFASs have been widely used in firefighting foam, food packaging, nonstick cookware, and various other consumer and industrial products. Numerous studies demonstrated that soil, sediment, and sludge microbial communities can transform and defluorinate polyfluoroalkyl substances (aka precursors) and ultimately form more recalcitrant and problematic perfluoroalkyl acids. To date, few defluorinating isolates have been obtained, limiting our ability to elucidate pathways and identify genes and enzymes involved in precursor transformation. The soil isolate *Pseudomonas* sp. strain 273 grows with fluorinated alkanes, and we challenged this bacterium with 12 different fluorotelomer carboxylates (FTCAs) including 1:3, 1:4, 1:5, 2:2, 2:3, 2:4, 5:3, 6:2, 7:3, 8:2, 8:3, and 10:2 FTCA. Time-series sampling over a 3-week incubation period was conducted to monitor bacterial growth, fluoride release, FTCA consumption, and catabolite formation. Fluoride release data indicate that *Pseudomonas* sp. strain 273 can transform FTCAs with even number non-fluorinated carbons (i.e., n:2 and n:4 FTCAs). Biotransformation products were detected including (n-1):3 FTCAs and perfluorocarboxylate with (n-1) perfluorinated carbons. Further, defluorination was dependent on the availability of a primary growth substrate such as sebacic acid, suggesting FTCA defluorination is a co-metabolic process. These results demonstrate that *Pseudomonas* sp. strain 273 is capable of defluorinating n:2 and n:4 FTCAs, and establish this bacterium as a model for elucidating the enzymatic machinery involved in C-F bond cleavage. The findings provide new insight into the transformation pathways and products of FTCA catabolism, contributing to a deeper understanding of PFAS behavior in the environment.

1.11.T-02 Temporal variability and microbial responses to emerging contaminants within the sewer environment

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Urban wastewater contains diverse and persistent bioactive emerging contaminants, including pharmaceuticals, personal care products, pesticides, and other xenobiotics, that resist conventional treatment processes and persist in the environment. These compounds interact with sewer microbial communities to form chemically dynamic

mixtures with poorly understood ecological and toxicological impacts. This study investigates how fine-scale temporal fluctuations in wastewater composition influence microbial community structure, functional potential, and acute toxicity. Hourly composite samples were collected over a 16-hr diurnal cycle from a municipal manhole in Boulder, CO. Each sample underwent bulk chemical analysis (pH, conductivity, dissolved organic carbon), long-read metagenomic sequencing (Nanopore PromethION), and bacterial growth inhibition bioassays using *Escherichia coli*, *Pseudomonas putida*, and *Bacillus subtilis* as sentinel organisms. A custom bioinformatics pipeline translated predicted proteins from assembled contigs and aligned them against a curated UniProt enzyme database to assign Enzyme Commission (EC) numbers, enabling pathway-level inference of microbial metabolic capacity linked to xenobiotic degradation. Results revealed pronounced diel shifts in taxonomic composition: human gut-associated taxa (e.g., *Bacteroides*, *Escherichia*) peaked during high-flow morning periods, while sewer-adapted biofilm taxa (e.g., *Acidovorax*, *Pseudomonas*) dominated during low-flow intervals. Functional profiles showed a temporal tradeoff in oxidoreductase activity – critical for contaminant transformation – that declined during peak human waste input and rebounded later in the day. Bioassays revealed time-specific toxicity patterns and organism-specific sensitivities, including antagonistic and synergistic effects within complex contaminant matrices. These findings demonstrate that traditional composite sampling overlooks key temporal dynamics in microbial-contaminant interactions. Integrating high-resolution temporal sampling with functional metagenomics and bioassays provides an ecologically and regulatory-relevant framework for developing adaptive wastewater monitoring and treatment strategies while aligning with growing regulatory interest in omics-based tools as scalable alternatives to compound-by-compound chemical testing.

1.11.T-03 Microplastic concentration effects on planktonic microbial community composition in a large-scale freshwater mesocosm study

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Microplastics are abundant and ubiquitous throughout aquatic environments, and will only increase in abundance with projected rates of plastic production and mismanagement. Microbial communities are also found throughout these environments, performing the functions essential for the maintenance of life within their ecosystems. We currently know little about the effects microplastics have on aquatic microbes and their functions within natural aquatic environments, especially at increasing microplastic concentrations that reflect current and future trends. Studies in the laboratory have shown that plastics, and the compounds plastics leach into the water, can promote the growth of heterotrophic bacteria and carbon transformation. However, plastic leachates can also inhibit the growth of marine phototrophic bacteria, and thus primary production. To determine whether shifts in microbial community composition and functions occur with increasing microplastic exposure under environmental lake conditions, a series of large-scale mesocosms were established in an oligotrophic boreal lake at the International Institute for Sustainable Development Experimental Lakes Area, with microplastic particle concentrations ranging from no-plastic controls to those projected for future lakes. The planktonic particle-associated and free-living microbial communities were sampled in each mesocosm at five timepoints over 11 weeks. We performed 16S rRNA and ITS1 amplicon sequencing on each community sampled to determine prokaryotic and eukaryotic community composition and putative functions. We hypothesized that, as microplastic particle concentration increases, heterotrophic microbes increase in relative abundance compared to phototrophic microbes, and any effects on phototrophs are strongest in the free-living community. Such shifts in community composition and function would have implications for broader ecosystem functions as microplastic concentrations increase in aquatic environments, as phototrophs are essential to carbon cycling and serve as the base of the food chain in aquatic systems. This study furthers our understanding of how microplastic concentrations affect microbial communities in natural aquatic ecosystems by using a large-scale,

in-lake mesocosm approach, allowing for direct application to future ecosystem risk assessments for microplastics.

1.11.T-04 Effects of BiOX (X=Cl, I) and BiOX-GO nanomaterials on aquatic microorganisms

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Bismuth oxyhalides BiOX (X=Cl, I) and their composites with graphene oxide (BiOX-GO) are innovative adsorbent nanomaterials developed for water treatment. Their advantageous properties include a three-dimensional (3D) porous structure, a high specific surface area, and photocatalytic activity. These materials have been investigated for the adsorption of organic contaminants and heavy metals. However, their environmental impacts on microorganisms remain largely unexplored. In this study, the aqueous suspensions of BiOX and BiOX-GO were characterized, and their biological effects on microorganisms (*Escherichia coli*, marine bacteria *Aliivibrio fischeri*, and protozoa *Tetrahymena thermophila*) were evaluated. In ultrapure water, BiOX and BiOI-GO formed agglomerates with a diameter close to 2 micrometers, while BiOCl-GO agglomerates were smaller, approximately 500 micrometers in diameter. All materials exhibited a negative charge in water. After 24 hr of contact with *E. coli*, the BiOCl-GO water suspension demonstrated antimicrobial effects at 10 mg/L (minimum bactericidal concentration or MBC), BiOI at 100 mg/L, and BiOI-GO at 500 mg/L, while BiOCl showed no antibacterial activity at 500 mg/L under the study conditions. In contrast, none of the materials affected the bioluminescence of *A. fischeri*, an ISO standard marine ecotoxicity organism, nor did they impact the viability of the eukaryotic microorganism *T. thermophila*. In comparison, pure GO reduced the bioluminescence of *A. fischeri* by 50% at 95 mg/L. The results suggest that BiOCl-GO is the most effective antimicrobial material while exhibiting no toxicity to naturally occurring aquatic microorganisms. The incorporation of BiOCl onto thin layers of GO enhances the antibacterial properties of BiOCl while preserving the environmental safety of the adsorbent material. **Disclaimer/Disclosure:** The research was supported by the Estonian Research Council (PRG2188).

1.11.T-05 Assessing the biodegradation potential of PFOS by estuarine microbial communities

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Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals widely used in industrial and consumer products such as firefighting foams, non-stick cookware, and food packaging, due to their strong carbon-fluorine bonds, which impart resistance to heat, water, and oil. Among them, perfluorooctanesulfonic acid (PFOS) has been one of the most widely produced and persists in the environment despite being phased out of US production in the early 2000s. Rationale: PFOS is highly water soluble and can be transported through surface waters before binding to organic particles and accumulating in estuarine sediments, where it may persist for decades. Microbial communities in sediments are involved in organic matter cycling and contaminant transformation under both aerobic and anaerobic conditions, suggesting they may also have the potential to degrade PFOS. Study Question and Hypothesis: Can estuarine sediment microbes transform PFOS into shorter-chained less accumulative metabolites, and if so, which organisms and genes are involved in that process? We hypothesize that exposure to PFOS will lead to enrichment of microbes capable of breaking down the compound and an increase in genes associated with defluorination. Methodology: To explore this, we cultured microbial communities from estuarine sediments collection at two South Carolina sites with different levels of urbanization: the heavily developed Charleston Harbor (CH) and the relatively undeveloped St. Helena Sound (SHS). Cultures were exposed to PFOS under aerobic and anaerobic conditions. Fluorometry is used to detect fluoride ion release, while HPLC-MS tracks PFOS concentrations and any resulting metabolites. Microbial communities will be analyzed by DNA sequencing of the 16S rRNA gene to identify microbial taxa potentially involved with PFOS metabolism or detoxification. Preliminary Results: Initial data support this approach. PFOS

was the most abundant PFAS detected across all sediment and surface water samples, even in the less developed site. This persistence highlights the need to better understand how estuarine microbes interact with these contaminants. This study aims to identify microbial taxa and genetic pathways that could inform future PFAS bioremediation strategies. **Disclaimer/Disclosure:** This project is funded by 1) Biological Sciences Professional Development Course Grant in Aid of Research (BSPDC-GIAR) and 2) Clemson University, Department of Biological Science Start-up Funds.

1.11.T-06 Characterizing the influence of fungicides on plant-surface bacterial community composition, processes and drug-resistance

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The Global Burden of Diseases, Injuries, and Risk Factors Study, estimated that antibiotic resistant (AR) bacterial infections caused 1.14 million deaths in 2021. Fungicide exposure has been shown to alter the composition of soil bacterial communities, horizontal gene transfer rates, and abundance of antibiotic resistance genes and are suspected drivers of AR. Plants are often exposed to fungicides but the leaf surface is a uniquely harsh environment for bacteria, so it is unclear if plant-surface bacterial communities will have similar responses to fungicide as those seen in other niches. Our studies aim to determine if fungicides increase the abundance of AR genes in plant-surface bacterial communities through the alteration of horizontal gene transfer dynamics or bacterial community composition using both field and laboratory techniques. The field portion of this work was conducted on turfgrass in Madison, WI during the summer of 2022. Leaf material was collected prior to fungicide application, as well as 4 hr, 96 hr, and one week post application. qPCR and 16s sequencing results were used to determine abundance of genes known to confer AR and bacterial community composition respectively. Additionally, we are conducting lab studies in tomato plants and in vitro to determine if fungicides impact the transfer frequency or host range of these AR genes. This work will increase our understanding of the impact fungicides have on plant surface bacteria communities and may lead to novel antibiotic stewardship approaches.

1.12.P-Th Nano and Advanced Materials Safety: Research Progress, Applications and Challenges

1.12.P-Th-084 Translocation of nanoplastics from soil to crops impairs pollen viability with potential implications to pollinators

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Soils are nowadays considered among the major storage sites and sources of nanoplastic (size < 1000 nm) entering the natural environment through sewage sludge, abandoned wastes, agricultural activities, and atmospheric deposition. Due to their peculiar chemical and physical properties, nanoplastics can easily interact with plants including crops and potentially translocate up to leaf and flowers entering the food chain, with potential toxic effects to the plants as well as exposing pollinators. In the present study, we assess 1) the uptake, translocation, and effects of nanoplastystyrene (PSNPs, approx. 20nm) in pumpkin *Cucurbita pepo* L as a model crop plant and 2) PSNPs translocation in flowers and effects on pollen. To investigate the uptake and translocation of PSNPs, we used ¹⁴C-radiolabeled PSNPs ([¹⁴C]PSNPs) at concentrations similar to those expected in the environment (1 µg/L) and under worst-case pollution scenarios (1 mg/L). Effect of PSNPs on the crops have been observed from both labeled and unlabelled particles. Effects have been observed during the germination up to plant development and flower production in the pumpkin *Cucurbita pepo* L. . For the first time, our study provides evidence of [¹⁴C]PSNP uptake by plant roots and translocation from roots to flowers, with subsequent effects on pollen. Most notably, PSNP effects were observed on the apical region of secondary roots which reveals a significant increase in the ROS production and in primary leafs with significant reduction in the efficiency of photosystems. [¹⁴C]PSNPs was detected in pumpkin flowers and mostly in the anthers whose pollen showed a significant reduction in the viability associated with abnormalities in morphology and

hydration. Our study provides compelling evidence that nanoplastics are capable of translocating from soil up to the flowers and affecting pollen raising significant food safety concerns and ecological implications on pollinators. These results are particularly alarming given the current multiple challenges faced by pollinators, such as climate change and pesticides.

1.12.T Nano and Advanced Materials Safety: Research Progress, Applications and Challenges

1.12.T-01 Tiny particles, big consequences: Multi-generational molecular toxicity and the future of risk assessment for emerging pollutants

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Environmental pollution is a major contributor to global disease burden, causing over 9-million premature deaths annually. The rapid rise of advanced materials has introduced engineered nanomaterials (NMs), microplastics, and other nano-scale particulates into everyday products, posing new regulatory and health challenges. These emerging pollutants (EPs) are designed for enhanced reactivity or antimicrobial properties, with metallic nanoparticles like silver, gold, and titanium dioxide common in consumer goods. However, they are released into ecosystems where they undergo transformations altering their bio-activity and toxicity. Current regulatory models, based on legacy-data and soluble chemical testing, fail to account for the persistence, reactivity, and particulate nature of EPs. Standard ecotoxicological studies focus on acute-effects using pristine-particles under simplified conditions, neglecting real-world transformations and long-term health risks. Once in the environment, EPs interact with organic matter and co-pollutants, that change their surface chemistry and toxicological profiles. These dynamic interactions challenge existing risk assessment frameworks, particularly in predicting chronic and developmental outcomes. There is mounting evidence that EPs cause transcriptomic and epigenetic disruptions, persisting across generations, especially concerning for neurotoxicants affecting early development. Despite this, current guidelines rarely incorporate multi-generational studies. *Daphnia*, already used in OECD regulatory testing, is a promising model for multi-generational toxicity studies. This research expands on single-generation approaches by integrating chronic exposures across six generations (F0-F5), coupled with transcriptomics, epigenetic profiling, and comparative genomics. Using environmentally transformed vs. pristine NMs, the survival, reproduction, behavior, and gene expression responses are assessed. Omics data will feed into adverse-outcome-pathways and inform machine learning models for predicting toxicity and mode of action. Initial results show persistent gene expression changes and distinct molecular signatures for transformed NMs. This study addresses critical gaps in regulatory science and aligns with initiatives including the UK and EU-REACH Chemicals Strategy, and the UN Sustainable Development Goals, supporting a paradigm shift toward evidence-based, mechanism-informed regulation of particulate pollutants.

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1.12.T-02 Toxicity assessment of hexagonal-boron nitride and PFAS through behavioral and lipid disruption assays towards the model nematode, *Caenorhabditis elegans*

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Per- and polyfluoroalkyl substances (PFAS) are contaminants of emerging concern due to their widespread presence and persistence in soil and water with negative effects in the environment. Two dimensional (2D) nanomaterials (NM), including hexagonal-Boron Nitride (h-BN) have shown promise for the degradation of PFAS. A safe(r) and sustainable by design (SSbD) remains essential in assessing technologies using h-BN for PFAS degradation to ensure minimal environmental impact. It is important to ensure that h-BN released from applications is less toxic than PFAS being treated. *Caenorhabditis elegans* was used as a model organism due to its fully sequenced genome, shared genetic homology with humans, and availability of functional genomic tools. Toxicity screening was conducted across growth, behavioral, and lipid metabolism endpoints. We assessed the impact of h-BN, perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and PFOA/PFOS mixtures. Effects on lipid metabolism in *C. elegans* following PFOA exposures was crucial in discerning PFAS effects separate from h-BN. Changes in lipid accumulation were observed starting at 0.15 mg/L PFOA. Lipidomic analysis was conducted for the characterization in lipid composition. Changes in growth and locomotion occurred at 0.8mg/L. Preliminary data suggest that h-BN affects growth at concentrations greater than 10 mg/L, and behavioral effects at 2 mg/L up to 4 mg/L. Bulk h-BN did not induce significant mortality up to 100 mg/L. Meanwhile, exfoliated h-BN was more toxic at lower concentrations of 50 mg/L. These findings will help guide SSbD development of nanotechnology applications such as h-BN for utilization in environmental remediation of PFAS as well as understand the mechanisms of potential toxicity due to PFAS. **Disclaimer/Disclosure:** NSF-CBET #2228033, USDA NC 1194, and UK-CARES ACCESS.

1.12.T-04 Graphene grey areas in *daphnia* ecotoxicity exposures: Mixtures, movement and monitoring
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Graphene and silicon nanomaterials are widely used as "dry" lubricants for sporting equipment, boats, machines, engines etc. Aquatic ecotoxicity assessments with these materials is challenging as they are often hydrophobic due to the surface properties of the particles, in addition, they can often be applied in combination with mineral oil to act as a carrier for the particles, further increasing the hydrophobicity and changing the surface properties. The ecotoxicity of these advanced materials is important to understand, including any potential adaptation to the standard aquatic testing approach to take into consideration the physico-chemical properties of the materials and how they may change in different environments. *Daphnia magna* have widely been used for ecotoxicity testing of different materials, including chemical, advanced materials (including nanomaterials) and microplastics in isolation and in varying combinations. Due to the robust foundation of endpoints and research to date, *Daphnia* are an excellent model to explore aquatic risk in freshwater environments. A panel of commercially available materials were assessed; Graphene, Graphene + Silicon, Silicon, Graphene + oil, mineral oil. This included the characterisation of the particles using DLS and zeta potential in control medium and also in the testing medium (with the *Daphnia*) to determine how the materials may change during the test period. Acute toxicity testing was also undertaken with *Daphnia magna* (Bham 2) following the OECD 202 TG recommendations, before sublethal testing was undertaken looking at molecular markers such as increase in ROS in addition to changes in behavior such as movement and swimming. Variations with experimental design and exposure order were trialed to determine if this had any significant impact on the outcomes of the tests, for example, adding the toxicant to the test vessel prior to the medium and *Daphnia* vs adding the toxicant last, and subsequently using different ages/development stages of the *Daphnia* for sublethal assays. Advanced materials are challenging to assess due to the surface properties, in this case hydrophobic particles, which prove difficult to evaluate effectively using the current protocols which are optimized for chemicals. Modification, such as exposure order, age of *Daphnia* etc in addition to physico-chemical characterization can increase our understanding of the risk of AdMa in freshwater ecosystems.

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1.12.T-05 Intranasal exposure to aluminum oxide nanoparticles induces neurotoxicity and alzheimer-like pathology in IRC mice

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An increasing environmental and occupational exposure to nanoparticles (NPs) calls for further concerns towards exposure and public health risk assessment. The extensive application of Aluminum nanoparticles (Al-NPs) across industrial and consumer sectors has led to their widespread distribution in air, water, and food matrices. Aluminum (Al) is also a predominant constituent of ultrafine particulate matter (UFPs), which are increasingly associated with adverse health outcomes. Yet, a limited body of research exists on the neurotoxic effects of inhaled Al-NPs in the context of neurodegenerative diseases like Alzheimer's. This study investigates neurotoxic effects associated with nano-sized Al-NPs exposure on hippocampal neuronal cell lines (HT22), and in IRC Mice. Cultured HT22 cells and healthy adult ICR mice (6-weeks old) were exposed to varying doses of 13nm, 10µm, 5µm and 50nm of Al-NPs, 50 nm of Carbon Black (CB-NPs), and regular Aluminum oxide. Following exposure, Al-NPs induces oxidative stress, pro-inflammatory, cytotoxicity, apoptosis activity, oxidative DNA damage, neurobehavioral, and Alzheimer-like effects in a concentration-dependent and particle size-dependent manner. Al-NPs significantly elevated oxidative stress markers in mice in a dose- and size-dependent pattern. The levels of necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), amyloid beta 1–42 (Aβ_{1–42}), and phosphorylated tau (P-Tau) in the serum and cerebrospinal fluid increased significantly after exposure, indicating increased neuroinflammation and Alzheimer-like pathology. Nostril exposure to Al-NPs reduced serum serotonin (5-HT), dopamine (DA), and acetylcholine (ACh) levels, while increasing GABA, with effects intensifying as particle size decreases and dose increases. Learning and memory impairments were significantly increased in mice exposed to Al-NPs, with deficits worsening as particle size and concentration decreased. In conclusion, findings from this study suggest that intranasal exposure to Al-NPs resulted in the accumulation of Al in the hippocampus and cortex regions of the brain leading to molecular and biochemical changes underlying the observed impaired learning and memory in treated mice. These findings highlight a potential environmental risk factor for neurodegenerative diseases, including Alzheimer's disease, underscoring the need to limit human exposure to Al-NPs through public health and regulatory measures.

1.12.T-06 Nanotechnology and sustainable food and agricultural futures: Insights and perspectives from U.S. stakeholders

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The development and use of nanotechnology and nanoscale materials in food and agriculture may improve sustainability. For instance, nanoparticles may be used to create more efficient methods for delivering agrochemicals to plants, as well as provide sustainable coatings for fresh produce to extend their shelf life and reduce food waste. At the same time, experiences with other novel agrifood technologies highlight the importance of understanding and integrating stakeholder perspectives throughout research and innovation cycles. Drawing on research funded by USDA-NIFA grants that engage diverse stakeholders in the U.S. using an online engagement platform, our studies show that stakeholders evaluate nano-enabled food and agriculture products not only in terms of environmental, health, and safety considerations but also based on whether there is a clear societal 'need' for these innovations. Stakeholders tend to view nanomaterials used directly in food products as less responsibly developed than those applied to improve agricultural production practices. Our recent work also explores perceived benefit-risk trade-offs of nano-agrifood applications and how these relate to their sustainability. In conclusion, understanding these perspectives is essential for guiding the sustainable design and responsible deployment of nanotechnology in food and agriculture. Such insights can help ensure that future innovations both mitigate potential risks and align with societal needs and expectations.

1.12.T-07 Unraveling the enigma of silver vs. silver nanoparticles: Which one is the real culprit of toxicity?

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The exciting advancements in nanotechnology, especially with silver nanoparticles (AgNPs), have sparked a growing interest in uncovering their potential effects on the environment and human health. With a wide range of commercially available AgNP products that are often proprietary and not well-characterized, understanding how these nanoparticles behave in different scenarios becomes a challenging task. The rapid surface oxidation and dissolution of silver ions (Ag^+) further complicate matters, leaving us with significant gaps in our knowledge. To bridge this gap, it's essential to identify how factors like shape, size, and surface coating influence nanoparticle stability, their interaction with biological systems, and their overall ecotoxicity. We are particularly keen on discovering what makes AgNPs more toxic than Ag^+ ions and how these characteristics disrupt ecosystems. In our research, we focus on the design of innovative hybrid lipid-coated AgNPs, experimenting with various shapes and sizes that can resist surface oxidation. Using advanced techniques like UV-Vis spectroscopy, transmission electron microscopy, and ICP-MS, we characterize the stability of these unique nanoparticles. Our results reveal that AgNPs fully shielded from oxidation do not release any Ag^+ ions, which is a significant finding in the quest for safer nanomaterials. These oxidant-resistant AgNPs showed minimal ion release (<0.2%) even after seven days in fishwater, and remained structurally stable for over seven months. Toxicity studies using embryonic zebrafish models revealed size- and concentration-dependent effects: smaller AgNPs caused increased mortality and sublethal morphological abnormalities at higher concentrations, even in the absence of Ag^+ ions. Interestingly, while bacterial and *Daphnia* populations were negatively affected by certain shapes and surface chemistries of AgNPs, zebrafish embryos showed resilience under specific conditions, highlighting species-specific responses. Our studies shed light on the complex interaction between AgNPs and their environment, contributing valuable insights into the toxic release of Ag^+ ions and the intrinsic toxicity of nanoparticle design. Ultimately, this work could pave the way for designing safer nanoparticles that minimize environmental and health risks.

1.13.P-Th Pesticides as Multiple Stressors

1.13.P-Th-081 Glyphosate sequestration and acetylcholinesterase response in the invasive bivalve *Corbicula fluminea*

Stephen Fitch, Author

Glyphosate based herbicides (GBH) like Roundup are among the most widely used agrochemicals worldwide, with annual application increasing 15 fold in the past 2 decades due to the introduction of Roundup ready crops. It has high exposure potential to aquatic systems through runoff, overapplication and erosion. This study evaluates whether Asiatic Clams (*Corbicula fluminea*) can remove glyphosate from the water, and the associated physiological impacts via acetylcholinesterase (AChE) inhibition, a biomarker for neurotoxicity. *C. fluminea* is a wide-spread invasive in North America, particularly in degraded areas such as irrigation ditches and impacted streams, making its interaction with GBH ecologically relevant. While some studies have shown glyphosate sequestration by bivalves like Golden Mussels (*Limnoperna fortunei*), no studies have looked at North American species or tested full formulation GBH. Understanding both the clam's filtration capacity and its physiological response may offer insight into bivalve mitigation of glyphosate contamination and ecological risk. To test this, *C. fluminea* were stocked at densities of 0.5, 1, 2, and 4 clams/L in 2-liter jars, and exposed to a concentration of 10 mg/L glyphosate for 7 days. Full formulation Roundup was added to reach a concentration of 10 mg/L in the water. Water samples were collected at day 0,4 and 7 and analyzed using ELISA to quantify glyphosate concentration. Clam tissues were analyzed using Bradford assay and AChE ELISA to determine protein content and AChE activity. It was hypothesized that glyphosate levels would decrease the most in the high-density clam group, and all clams exposed to glyphosate would exhibit AChE inhibition. AChE inhibition is expected to be greater in low-density treatments due to prolonged exposure, because the glyphosate will not

be filtered as quickly. This project is ongoing, but preliminary data suggests glyphosate levels declined in jars with clams. AChE activity appears to be inhibited in clams exposed to Roundup, and further statistical analysis will be done to determine the extent.

1.13.P-Th-082 Efficacy of copper and organic herbicide mixtures to prevent cyanobacterial blooms

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Aquaculture ponds are shallow, warm, nutrient-rich environments designed to culture commercial organisms at high densities and low cost. Using an environmental perspective, aquaculture ponds can be excellent case studies on how eutrophication can influence ecosystem communities. Environmental conditions in aquaculture ponds are ideal for the development of cyanobacterial blooms, which, when in high densities, release off-flavors that can taint commercial species, making them unsuitable for human consumption, and toxins that can have environmental and human health effects. Copper sulfate is often used to mitigate cyanobacterial blooms; however, copper costs are increasing, creating additional financial burdens. Aquaculture ponds are also high in organic material, which decreases copper bioavailability and treatment efficacy. Organic herbicides have been proposed as an alternative; however, they are neither as effective nor as economical as copper. It has been suggested that mixtures of copper and organic herbicides may be more effective than copper alone. The present study aimed to assess the efficacy of different herbicide mixtures towards cyanobacteria. Two cyanobacterial species (*Anabaena* spp. and *Microcystis aeruginosa*), which commonly bloom in aquaculture ponds, were exposed to binary mixtures of herbicides: bispyribac sodium, copper, diuron, diquat, hydrogen peroxide, and penoxsulam using dose-response assays. Binary mixtures were conducted using a radial design with seven different ratios (100:0%, 87.5:12.5%, 75:25%, 50:50%, 25:75%, 12.5:87.5%, and 0:100%) and eight treatments per mixture. Preliminary results suggested that ratios with higher diquat and diuron were more effective against cyanobacteria, and mixtures with higher percentages of copper were less effective. The results indicate that copper-organic herbicide mixtures may reduce the amount of copper needed to treat cyanobacterial blooms and potentially reduce costs. While promising, follow-up research characterizing chemical stability in suspension and effective dosages are required. Furthermore, additional considerations need to be made regarding differences in chemical stability in controlled laboratory media and aquaculture ponds.

1.13.P-Th-083 Assessment of risks to plants and soil invertebrates from lead and arsenic at former orchard lands at the Hanford site, Washington

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Historical use of lead arsenate as a pesticide in orchards at the Hanford Site has resulted in legacy lead and arsenic contamination in surface soils within 100-OL-1 OU. Site-specific bioassays were conducted using both soil invertebrates and terrestrial plants. Fourteen-day earthworm bioassays showed no mortality or sublethal effects on earthworms in any sample; the highest concentrations of lead and arsenic (390 and 128 mg/kg, respectively) were identified as site-specific NOECs for soil invertebrates. Phytotoxicity data consisted of results of bioassays performed by the Washington Department of Ecology and PNNL. The Ecology data consisted of 14-d lettuce bioassays. The PNNL data consisted of 21-d bioassay results for cheatgrass, lettuce, and Sandberg's bluegrass. The Ecology and PNNL datasets are complimentary, and when combined provide a robust representation of arsenic and lead phytotoxicity. Observed phytotoxicity can be attributed to interactive effects of lead and arsenic (in the cases of cheatgrass and Sandberg's Bluegrass) or lead, arsenic, and pH (in the case of lettuce). Growth of lettuce is highly influenced by pH. Soil pH appears to have a dominant effect on phytotoxicity, exceeding that of lead or arsenic. The sensitivity of lettuce to soil pH raises questions concerning the relevance and suitability of lettuce as a test species upon which to base management decisions. Site-specific EC20s for plants for lead and arsenic were 642 mg/kg and 121 mg/kg, respectively. Bioassay results were applied to lead and arsenic data from 133 DUs in the 100-OL-1 OU. One hundred twenty three of 133 DUs (92.5%) are estimated to present no risks to plants or soil invertebrates. Risks to the soil invertebrate

community, based on combined exposure to lead and arsenic, are limited to a single DU. Combined exposure to lead and arsenic is estimated to present a risk to the terrestrial plant community in 4 of 133 DUs or 3% of DUs. This conclusion is based on EC20s derived from site-specific bioassays and is supported by species- and site-specific plant growth estimates developed from multiple regressions using site-specific bioassay data. Overall, risks to the soil invertebrate and terrestrial plant communities at the 100-OL-1 OU are localized and limited.

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1.13.T Pesticides as Multiple Stressors

1.13.T-01 Co-occurrence of pesticides and PFAS from agricultural pesticide applications

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Per- and polyfluoroalkyl substances (PFAS) are frequently found in streams with most research focusing on urban sources of PFAS to surface water (e.g., firefighting foams, industrial releases, and wastewater treatment plant discharges). Pesticide applications are an under-investigated source of PFAS to streams in both agricultural and urban areas. Although many PFAS are not applied as pesticides there are concerns of increased PFAS levels in pesticide containers and as “inert” ingredients not listed on pesticide formulation labels.

Additionally, some pesticide active ingredients can be considered PFAS because their structures contain at least one fully fluorinated methyl or methylene carbon atom, as defined by the Organisation for Economic Co-operation and Development (OECD). To better understand the potential co-occurrence of pesticides and PFAS in predominantly agricultural watersheds, the U.S. Geological Survey selected 10 streams for sampling in northern California, USA. Agricultural inputs included rice, nuts (almonds, walnuts), tomatoes, alfalfa, and various row crops. Water samples were collected in May and July of 2024 and corresponded with peak target pesticide application timings. Water samples were analyzed for more than 183 pesticides and pesticide transformation products and 57 PFAS compounds. To evaluate potential PFAS occurrence associated with agricultural seed treatments, several types of insecticide and fungicide coated seeds (wheat, canola, soybeans, maize) were extracted and analyzed for 52 PFAS. This work will (1) compare the occurrence and concentrations of target pesticides (e.g. the insecticide methoxyfenozide) with commonly detected PFAS (e.g., perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS)), (2) evaluate the contribution of pesticides that qualify as PFAS (e.g., the fungicide fluopyram) to overall PFAS concentrations, and (3) determine if PFAS are present in pesticide-coated seeds.

1.13.T-03 Chronic, low concentration pesticide exposure alters reproduction and behavior in the intertidal sea anemone, *Anthopleura elegantissima*

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Widespread pesticide and herbicide use paired with frequent transport away from application sites has led to pesticide presence in nearly all terrestrial and aquatic environments globally. Pesticides have unintentional toxic effects on non-target organisms by interfering with cellular processes, behavior, feeding, reproduction, and disrupting endocrine processes. The aggregating anemone, *Anthopleura elegantissima* is an important species along the North American Pacific coast due to its symbiotic relationships that contribute to high productivity, and its clonal abundance that structures the rocky intertidal habitat. Commonly used pesticides, atrazine, diuron, and carbendazim were previously detected in coastal waters of Oregon, U.S.A. This study examined potential effects of these pesticides at environmentally relevant concentrations on reproduction, symbiont concentrations, and behavior of *A. elegantissima* over an eight week period. Pesticides individually and in combination significantly decreased male gonad development in atrazine ($p < 0.001$), carbendazim ($p < 0.001$), two-way mixture (diuron and carbendazim) ($p = 0.05$), and a three-way mixture ($p = 0.08$) treatments. All pesticide treatments significantly increased cloning behavior compared to the control, suggesting that cloning could be a stress response. Pesticide exposure also significantly increased tentacle retraction movement suggesting

possible metabolic or energy impairments; our study is the first to document behavioral changes in anemones. All three pesticides significantly impacted a non-target marine invertebrate at environmentally relevant concentrations which underscores the importance of studies that focus on effects to marine invertebrates, paired with comprehensive pesticide monitoring in coastal areas.

1.13.T-04 Behavioral sensitivity of juvenile chinook salmon to realistic body burdens of pesticide mixtures

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Pesticides are commonly used in agriculture, industry, and residential areas, often entering nearby aquatic systems via runoff or spray drift, especially during storms. In California's Sacramento-San Joaquin Delta, pesticide contamination is widespread, with multiple compounds frequently detected in water and sediments. These contaminants pose serious risks to aquatic life, including juvenile Chinook salmon (*Oncorhynchus tshawytscha*), which migrate through these waterways. This study examined how mixtures of three pesticides: bifenthrin, fipronil (current use pesticides), and 4,4'-dichlorodiphenyldichloroethylene (DDE, a dichlorodiphenyltrichloroethane breakdown product), affect Chinook salmon behavior. We hypothesized that sublethal concentrations would disrupt key behaviors like routine locomotion and social interactions. Juvenile salmon were exposed for 9 days to one of four treatments: a Control, a Low treatment (0.125 µg/L bifenthrin + 4.0 µg/L fipronil), a High treatment (0.250 µg/L bifenthrin + 8.0 µg/L fipronil), and a High + DDE treatment (0.250 µg/L bifenthrin + 8.0 µg/L fipronil + 0.45 µg/L DDE). These aqueous doses were selected to reflect environmentally relevant body burdens in wild fish. Behavioral analyses showed that pesticide exposure altered locomotion and group cohesion. Fish in the Low treatment showed mild hyperactivity, with increased movement and speed. In contrast, fish in the High and High + DDE treatments were hypoactive. All pesticide-exposed groups displayed reduced shoaling, as indicated by greater distances between individuals. These sublethal behavioral changes are likely to reduce migratory success and increase predation risk, compounding the physiological and developmental effects already linked to pesticide exposure in Chinook salmon.

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1.13.T-05 Links between behavioral and transcriptomic effects of sublethal exposure to commonly used pesticides across salinities in *Menidia beryllina*, informed with random forest classification

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Global climate change-induced stressors, including sea-level rise and drought, impact estuarine ecosystems and contribute to fluctuations in salinity levels. Salinity modulates chemical behavior in water by altering solubility and lipophilicity, which can influence bioconcentration in aquatic organisms. Pesticides, commonly used for agricultural and household purposes, can enter estuarine ecosystems through surface runoff. In our study, the influence of salinity on pesticide toxicity was studied by exposing *Menidia beryllina* (Inland Silverside) embryos at six days post fertilization (~1-day pre-hatch) to sublethal concentrations of six pesticides (bifenthrin, chlorpyrifos, dicloran, myclobutanil, penconazole, triadimefon) at two salinities (5 PSU and 25 PSU) for 96 h. Behavior and gene expression were evaluated to compare toxicity of pesticides at different salinities and observed effects were linked with machine learning. We found hyperactivity occurred across both salinities in most pesticide exposures in light cycles and in 25 PSU during dark cycles of the behavioral assay. Gene Ontology and KEGG pathways associated with neurotoxicity, altered development, and endocrine disruption were dysregulated, and a greater number of differentially expressed genes were accounted for in 5 PSU pesticide exposures. With a random forest model, PI3K-Akt signaling and cytoskeleton in muscle KEGG pathways were determined to be important predictors of total distanced moved behavior in the light cycles. Our results suggest increased pesticide bioavailability at lower salinities, potentially leading to increased toxicity.

This work highlights the need to incorporate dynamic salinity conditions into pesticide risk assessments, as climate change stressors further impact toxicity of chemicals present in aquatic environments.

1.14.P-Tu Pollution Impacts on Endemic and Threaten Species—A Focus on Metals and Persistent Pollutants

1.14.P-Tu-004 Unveiling persistent organic pollutants in southeast Asia via nontargeted analysis of a sentinel species, Irrawaddy dolphins (*Orcaella brevirostris*), From the Mekong River

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Persistent organic pollutants (POPs) biomagnify and bioaccumulate in aquatic ecosystems, threatening environmental and human health. This project investigates POP exposure in the critically endangered Mekong River Irrawaddy dolphin (*Orcaella brevirostris*), an apex predator and sentinel species. With fewer than 100 individuals left and high calf mortality, this population is at risk of extinction. While the cause of calf deaths is unknown, contaminant exposure may play a role. Blubber samples from nine dead stranded animals collected by the World Wildlife Fund (WWF)–Cambodia research group were analyzed using a nontargeted analysis to identify and characterize contaminant exposure. Cambodia's reliance on farming and agriculture using harmful pesticides and wartime land exposure to defoliants and dioxins increases contamination risks. Analyses revealed a complex suite of halogenated organic compounds, including polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), chlordanes, mirex-related chemicals, chlorinated benzenes, dieldrin-related chemicals, polybrominated diphenyl ethers (PBDEs), methylsulfonyl-PCBs, polychlorinated terphenyls (PCTs), toxaphenes, and polybrominated biphenyls (PBBs)—POPs from industrial and agricultural activities. Natural compounds including methoxy brominated diphenyl ethers (MeO-BDEs), 1,1'-dimethyl-2,2'-bipyrroles (DMBPs), 1'-methyl-1,2'-bipyrroles (MBPs), and methoxy polybrominated biphenyls (MeO-PBBs) were also detected. Additionally, less-studied compounds such as tris(4-chlorophenyl)methane (TCPM) and its metabolite, tris(4-chlorophenyl)methanol (TCPMOH) were detected, similar to California marine mammals. Notably, compounds absent from previous studies were observed, including methoxy chlorinated diphenyl ethers (MeO-CDEs), dioxins, and oxadiazon, a likely carcinogenic herbicide linked to developmental toxicity. This is one of the first documentations of oxadiazon in marine mammal blubber. Another novel detected compound, pentachloroaniline (PCA) is likely pesticide related. Numerous unknown compounds were also found, underscoring the importance of nontargeted analytical approach. These findings highlight regional contaminant differences compared to previous studies and the need for pollution monitoring, research, and policy action. Understanding chemical burdens in Irrawaddy dolphins informs conservation efforts and human health risks, as seafood is a predominant food source for local communities.

1.14.T Pollution Impacts on Endemic and Threaten Species—A Focus on Metals and Persistent Pollutants

1.14.T-01 Sensitivity of juvenile endemic Alabama prosobranch gastropod species to nickel, potassium and chloride exposure

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Alabama is a hotspot for freshwater prosobranch gastropods, but many of these populations are imperiled due to high rates of endemism, narrow distributions, and increasing anthropogenic impact around lotic ecosystems. Three species of juvenile prosobranch gastropods endemic to the Cahaba River and its tributary, Six mile Creek, (*Somatogyrus georgianus*, *Elimia cahawbensis*, and *Elimia* spp.) were exposed to nickel (Ni), potassium (K), and chloride (Cl) individually for 96 hr to assess the chemical acute and the relative sensitivity of the snails to

each chemical. Chemical concentrations ranged from 0 – 4 mg/L Ni, 0 – 1000 mg/L K, and 0 – 4000 mg/L Cl. Daily mortality was measured to determine 96-hr lethal concentrations. Tissue Ni and K concentrations were also measured to determine the metal accumulation and threshold effect concentrations. Results of median lethal effect concentrations (LC50) showed that *S. georgianus* was the most sensitive for Ni and K with a 96-hr LC50 of 0.609 mg/L Ni and 26.572 mg/L K, but it was the least sensitive to chloride with a 96-hr LC50 of 2697 mg/L Cl. Nickel accumulation data showed a positive correlation between the Ni concentration in the water and the concentration in the soft tissue for all three species. The median lethal accumulations (LA) for *S. georgianus*, *E. cahawbensis*, and *Elimia* spp were 0.0068 ug/L Ni, 0.728 ug/L Ni, and 0.223 ug/L Ni respectively. These results suggest that *S. georgianus* is the most sensitive to Ni, aligning with the observed survival data. Further research is needed to determine the mechanism of Ni toxicity to these snail species and to explain their different sensitivities to Ni. Information on the influence of water quality and age on Ni toxicity to these snail species is also important for environmental management to minimize the impacts of pollution on the endemic macroinvertebrate populations of Alabama.

1.14.T-02 The influence of water hardness and pH on copper toxicity to the marsh ramshorn snail (*Planorbella trivolvis*)

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The marsh ramshorn snail (Pt) is an intermediate host of the trematode Bd, a pathogen of channel catfish. To reduce economic losses during channel catfish aquaculture operations, methods are being sought to interrupt the Bd life cycle by reducing marsh ramshorn snail populations in culture ponds. Copper (Cu) is an effective molluscicide and has been used to treat snails. While Cu toxicity is influenced by water quality parameters, such as hardness, pH, alkalinity, and dissolved organic matter, our knowledge about the factors influencing Cu toxicity to *P. trivolvis* is limited. This study seeks to understand the effects of hardness, pH, and dissolved organic matter on Cu toxicity to *P. trivolvis*. The standard 96-h toxicity test was used to determine the toxicity of Cu to the snail. Results showed that hardness and pH strongly influenced Cu toxicity to laboratory-cultured snails. The 96-h LC50 increased from 16 µg/L Cu to 43 µg/L Cu when hardness was increased from 40 to 180 mg/L as CaCO₃. Increasing pH from 6.2 to 8.1 also resulted in an increasing LC50 from 17 µg/L to 42 µg/L Cu. These changes in Cu toxicity are because of pH on Cu speciation in water. The bioavailable Cu (i.e., Cu²⁺) is higher at the lower water pH. With field-collected snails, the effect of hardness was not clearly observed. More research needs to be conducted to understand the different responses of lab-cultured and field-collected snails to Cu and effects of other water quality parameters on Cu toxicity.

1.14.T-03 Lead isotopic compositions suggest ingested fragments as source of toxicity in bald eagles (*Haliaeetus leucocephalus*)

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Lead poisoning remains a major cause of morbidity and mortality in bald eagles (*Haliaeetus leucocephalus*) across North America, with a hypothesized source of exposure due to ingestion of fragments from feeding on carcasses containing spent lead ammunition. However, direct evidence linking bald eagle lead poisoning to ingestion of lead fragments is limited. We measured lead concentrations and stable lead isotopic compositions in blood and fragment samples from bald eagles admitted to The Raptor Center at The University of Minnesota College of Veterinary Medicine that subsequently died of confirmed or suspected lead poisoning (n = 7). First, to validate that clotted cardiac blood collected at necropsy could be used for investigating lead exposure sources, lead isotopic compositions of paired samples of whole blood collected prior to death and clotted cardiac blood collected during necropsy were measured (n = 3). Stomach contents collected during necropsy

were examined for ingested ammunition fragments, which were isolated, leached in 1% HNO₃, and analyzed for lead isotopic composition. All plasticware used in blood processing was acid-cleaned under trace-metal clean conditions. Blood samples were digested in Teflon vials with concentrated HNO₃ acid and diluted in 1% HNO₃. Lead concentrations and isotopic compositions were measured using a Finnigan MAT Element sector field ICP-MS, with ²⁰⁵Tl as an internal standard. Preliminary results suggest that lead ratios (²⁰⁷Pb/²⁰⁶Pb) of whole blood and clotted cardiac blood were not measurably different, validating the use of clotted cardiac blood for investigations of lead exposure sources. Lead ratios (²⁰⁷Pb/²⁰⁶Pb) of ingested lead fragments were not measurably different from the bird's blood, indicating that lead-based ammunition was the likely source of this exposure. Our findings provide evidence linking lead poisoning in bald eagles to ingestion of lead-based fragments, suggesting pathways by which this species is lead poisoned.

1.14.T-04 Mercury, cadmium and polychlorinated biphenyls in New York's blue crab (*Callinectes sapidus*): Current concentrations and trends

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New York's marine and estuarine waters are the native habitat of blue crab (*Callinectes sapidus*), a benthic omnivore serving as a potential reservoir of contaminants within the food web and recreational fishery. The Hudson River and marine district represent an ecosystem with numerous sources of contamination and wide-ranging remedial actions. The last comprehensive assessment of contaminants in blue crab in New York State was completed in 2004–2005. This current study includes a focus on evaluating recent concentrations of mercury (Hg), cadmium (Cd), and polychlorinated biphenyls (PCBs). Hepatopancreas and leg muscle tissues, both commonly eaten by human consumers, were both analyzed due to their different physical properties which affect the accumulation of toxic substances. Sampling was completed at fourteen sites throughout the lower Hudson River, NY Harbor, and Long Island in 2021 and 2022. This study compares current results with historical data, showing how blue crab contaminant levels have changed over time in locations with varying human input and remediation. The data collected will be used to update health consumption advisories, provide a continuing assessment of the effectiveness of remedial measures, and assess spatial differences and temporal changes. Results from this study show site variability and that contaminant levels have not consistently declined despite remedial actions.

1.14.T-05 Persistent organic pollutants disrupt behavior in endangered estuarine fish, delta smelt

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Contaminant exposure is a critical but often underrecognized driver of species decline in estuarine ecosystems worldwide. Many pollutants, particularly hydrophobic organic contaminants (HOCs), such as polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs), enter aquatic systems through urban and agricultural runoff and atmospheric deposition. These compounds tend to accumulate in sediments and suspended solids, posing long-term risks to aquatic organisms, especially during sensitive developmental stages. This study examined the sublethal effects of environmentally relevant mixtures of HOCs on the early life stages of an endangered pelagic fish, Delta Smelt (*Hypomesus transpacificus*). While this species is endemic to the Sacramento-San Joaquin Delta (California, USA), the findings have broader implications for conservation and toxicological risk assessment in estuaries globally. Larvae were exposed for 96 hr to reconstituted mixtures mimicking bioavailable HOC profiles estimated from field-collected sediments and suspended solids. Behavioral assays revealed significant disruptions in swimming activity and thigmotaxis, consistent with sublethal neurotoxic effects. These alterations in behavior may impair survival-critical functions such as

predator avoidance, foraging, and habitat use. The results highlight the ecological relevance of behavioral endpoints in evaluating contaminant risk to estuarine species of conservation concern. More broadly, they underscore the importance of incorporating chemical pollution into species recovery and watershed management frameworks, particularly in estuaries affected by legacy and ongoing contaminant inputs.

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1.14.V Pollution Impacts on Endemic and Threaten Species—A Focus on Metals and Persistent Pollutants

1.14.V-01 Metals in hawksbill turtle (*Eretmochelys imbricata*) embryos and hatchlings from Campeche, Mexico

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Among the chemical pollutants with harmful effects on the environment, wildlife, and human populations are metals, which are characterized by their low degradation and ability to bioaccumulate, as well as their mutagenic and teratogenic effects. Metals enter the oceans through industrial emissions, agricultural runoff, domestic drainage, and mining extraction. The hawksbill sea turtle (*Eretmochelys imbricata*) is exposed to these and other pollutants and is on the verge of extinction, as it is the only species that spends most of its life cycle in coral reefs due to its spongivorous diet. Metals accumulate in marine animals almost exclusively through their diet, making the hawksbill sea turtle susceptible to bioaccumulation of these contaminants. One route of elimination for pollutants in sea turtles is excretion through eggs, which can contribute to malformations and mortality in embryos and hatchlings and interfere with juvenile survival. Therefore, this study analyzed the Pb, As, and Hg metal concentrations in hawksbill embryos, hatchlings, and hatchling livers and determined their potential effects. Fifteen hatchlings that did not survive and 15 embryos that did not reach full development were collected during the 2024 season in Campeche, México. Metals were determined by acid digestion voltammetry. Metal concentrations were As>Pb>Hg for embryos and hatchling livers, while Hg>Pb>As for hatchlings. Concentrations in the analyzed groups were considerably higher compared to other regions and exceeded the maximum permissible limits of Mexican regulations. However, regulations need to be updated to include organisms that do not have commercial interest but are of great importance to the ecosystem. The high concentrations of metals in hawksbill embryos and young are likely causing teratogenic effects such as shell malformations; however, it is necessary to continue implementing this type of analysis to clarify the importance of metals in the presence of malformations.

1.15.P-We Quantitative Structure–Activity Relationship (QSAR) Models and Tools in Environmental Toxicology and Chemistry

1.15.P-We-131 Decision tree logic to support more robust hydrolysis predictions

Lauren Cassidy, Jelena Dacres, Savannah Sierco, Alessandra Fistrovich, Laura Morlacci, Chrissy Coley and Ramsey Hanna, SRC Inc.

Hydrolysis is an important physical chemical process to consider when assessing the persistence of substances in the environment. It's also critical for understanding a substance's transformation in water. The rates of reactions, hydrolysis products, and half-lives of organic chemicals are needed to characterize this property. This work is preliminary study to expand the tool HYDROWIN™ tool, extant in the current EPISuite™ with the use of: decision tree logic for binning and a K -Nearest Neighbor (KNN) approach for data gap filling to improve the estimation of hydrolysis half-lives, and the expected products. The ultimate goal is to expand accuracy and coverage for hydrolysis estimations, especially those which are understood not to be rapidly hydrolysable.

1.15.P-We-132 Development of "KATE2025": An updated QSAR model for ecotoxicity prediction and comparative evaluation with previous versions

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Currently, tens of thousands of chemical substances are in use, but ecotoxicity data necessary for ecological risk assessment are lacking for many of them. It is impractical to conduct tests on all these chemicals and the growing concern for animal welfare exists as well. Therefore, there are growing expectations for the use of alternative methods to testing, such as quantitative structure-activity relationships (QSAR), in the ecological risk assessment of chemicals. The Ministry of the Environment (MOEJ) and the National Institute for Environmental Studies have been developing a QSAR model called KATE (KAshinhou Tool for Ecotoxicity). The latest version, KATE2025, was released in March 2025. KATE predicts acute and chronic toxicity of organic chemicals to aquatic organisms based on structural classification and log-log linear regression using the octanol-water partition coefficient (log P) as a descriptor. It also judges the applicability domain (AD) regarding both chemical structure and log P. In order to build a prediction model with reliable toxicity data, KATE uses data from the MOEJ aquatic toxicity test results and the US EPA's fathead minnow database. The first model was released in 2008 and became a stable version in 2011 as KATE 2011. A major revision led to KATE2017, which introduced a new classification scheme and redefined structural AD. In 2020, KATE2017 was updated to KATE2020 in accordance with the conversion of log P estimation tool from Daylight's ClogP to USEPA's KOWWIN. Although the MOEJ's data are considered reliable, part of them were generated based on earlier versions of OECD testing guidelines (TG) and guidance documents (GD), some of which have been revised since the beginning of the MOEJ tests. To improve the reliability of the training data, a data curation process was initiated in 2020. Some toxicity values were recalculated based on the latest TG and GD as needed. Data curation was completed in 2025, followed by the recalibration of regression models, resulting in the release of KATE2025 in March 2025. Compared to KATE2011, KATE2025 shows improved predictive performance and a broader applicability, as demonstrated through external validation using toxicity data on OECD high production volume chemicals.

1.15.P-We-133 Bio-QSARs: Enhancing ecotoxicity predictions for untested species

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Estimating chemical-induced effects across diverse taxa without species-specific testing poses a significant challenge in the ecological risk assessment of agrochemicals, particularly for species listed under the Endangered Species Act (ESA). To address this, we introduced the Bio-QSAR concept for multispecies toxicity predictions. This approach aims to overcome limitations of traditional machine learning-based quantitative structure-activity relationship (QSAR) approaches by incorporating biological information, such as taxonomic distances and physiological features. While physiology is known to drive species sensitivity, understanding the relative contribution of specific underlying processes remains elusive. Our study aimed to utilize existing knowledge about organismal physiology and phylogeny to both understand and predict differences in species sensitivity. We trained machine learning models to predict chemical- and species-specific endpoints using chemical fingerprints/descriptors and physiological variables, including dynamic energy budget (DEB) properties, life history traits, and taxonomic/phylogenetic relationships. Initially, we used publicly available data from the EnviroTox database, with ToxPrints and DEB parameters as predictor variables for chemistry and physiology, respectively. We then broadened the models' applicability by increasing species diversity, incorporating life history traits and taxonomic/phylogenetic relationships, and supplementing the models with broadened ecotoxicology data from EnviroTox database. Bio-QSAR models demonstrate the capability for flexible cross-chemical and cross-species predictions beyond the levels present in the training data while meeting criteria for consideration in regulatory contexts. This advanced approach holds significant promise for applications in environmental risk assessment and chemical research and development, particularly in the

context of ESA. **Disclaimer/Disclosure:** This work was supported by Syngenta Crop Protection. Nika Galic and Maxime Vaugeois are employees of Syngenta.

1.16.P-Mo General: Environmental (Human and Ecological) Toxicology and Stress Response

1.16.P-Mo-130 Analysis of PFAS in surface water and wastewater samples by automated solid phase extraction and QSight LC-MS/MS Based on USEPA method 1633

Jacob Jalali and Sheng-Suan (Victor) Cai, PERKINELMER

Per- and polyfluoroalkyl substances (PFAS) are a group of human-made fluorinated organic compounds. Due to their unique chemical properties, long-term persistence in environment, and associated risks for human health, PFAS have been classified as persistent organic pollutants and have become the current hot topics around the world. Since surface waters are the primary sources of drinking water in many areas around the world, there have been many studies on PFAS exposure in these water resources and the PFAS concentrations were found in the range from low ng/L to µg/L levels. In this study, seven surface water samples collected in Toronto Lakeshore area (Lake Ontario, Humber Bay River, Mimico Creek, and Grenadier Pond) and eight wastewater samples from Ontario, Canada were analyzed using automated SPE and QSight LC-MS/MS based on USEPA1633 method.

1.16.P-Mo-131 Environmental carcinogens and human health: A review of exposure sources and toxicological mechanisms

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Environmental carcinogens are widespread in air, water, and consumer products, posing significant risks to human health. This review summarizes key environmental toxicants - such as polycyclic aromatic hydrocarbons, heavy metals, asbestos, benzene, and dioxins - and their roles in cancer development through DNA damage, oxidative stress, and epigenetic modifications. Both indoor (e.g., tobacco smoke, cooking stoves, poor ventilation) and outdoor (e.g., vehicle emissions, industrial discharge, biomass burning) environments contribute to exposure. These agents can disrupt cellular functions, initiate abnormal growth, and trigger malignant transformation. Exposure risk varies based on source, geography, and vulnerable populations - especially children. This study highlights common sources, major toxicological pathways, and current research on carcinogenic mechanisms. Understanding these interactions is critical for developing preventive strategies and informing public health policy. This 216 aims to raise awareness of environmental carcinogens and encourage further investigation into their health impacts.

1.16.P-Mo-132 Analyzing developmental toxicity of air pollutant exposure in zebrafish embryos via high-throughput assessment

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Air pollution-associated contaminants can enter aquatic ecosystems through atmospheric deposition, stormwater runoff, and other diffuse transport pathways. Among these, lead acetate (a heavy metal), benzo(a)pyrene (a polycyclic aromatic hydrocarbon), and benzyl butyl phthalate (a plasticizer) are commonly detected in urban water systems. These compounds originate from sources such as vehicular emissions, industrial combustion, plastic manufacturing, and military-related activities. Despite their environmental ubiquity and known toxicity, their developmental impacts during early vertebrate life stages remain incompletely understood. Using zebrafish (*Danio rerio*) embryos as a model, this study assessed the developmental toxicity of each compound individually over the first 96 hr post-fertilization, a critical window for organogenesis and neurodevelopment. Embryos were exposed to both lethal and sublethal concentrations. Morphological endpoints (e.g., pericardial edema, axial malformations, delayed hatching) and behavioral measures (e.g., spontaneous movement and light-dark locomotor response) were evaluated to capture a spectrum of developmental disruptions. Lead acetate

exposure induced embryonic hyperactivity followed by reduced larval locomotion, suggesting a biphasic response with early neural excitation and subsequent neurotoxic suppression. In contrast, benzyl butyl phthalate elicited embryonic hyperactivity and a concentration-dependent increase in larval burst activity during light-dark transitions, indicating persistent neuromotor stimulation or disruption. Data for benzo(a)pyrene are currently under analysis and will be presented. Given the conservation of developmental pathways across vertebrates, these findings may inform understanding of early-life susceptibility to airborne and waterborne contaminants in humans. They emphasize the sensitivity of behavioral endpoints during embryonic development and their utility in detecting sublethal neurodevelopmental disruption. When combined with morphological assessments, behavioral analysis enhances toxicological screening, supporting more comprehensive ecological and human health risk assessments for air pollution-associated compounds in aquatic environments. **Disclaimer/Disclosure:** This project was funded by EPA-G2022-STAR-A1, Development of Innovative Approaches to Assess the Toxicity of Chemical Mixtures, Award #8404550 to AS & HP.

1.16.P-Mo-133 Developmental toxicity of structurally diverse per- and polyfluoroalkyl substances in zebrafish

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Extensive research has characterized the toxicity of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), yet far less is known about the thousands of other per- and polyfluoroalkyl substances (PFAS). Here, we evaluated the developmental impacts of structurally diverse PFAS in zebrafish (*Danio rerio*) by integrating morphological, transcriptomic, behavioral, and analytical approaches. Embryos at 4 hr post-fertilization (hpf) were exposed through 96 hpf to representative short- and long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), and perfluoroalkyl ether carboxylic acids (PFECAs). Across the chemical set, longer-chain PFAS ($\leq C11$) produced the greatest potency and severity of developmental effects when nominal concentrations were compared; perfluoroundecanoic acid (PFUndA) was the most toxic. Hallmark malformations induced by PFUndA and several other PFAS included dorsal body-axis curvature, reduced body length, narrowed somite spacing, diminished otolith size, and hypoplastic pectoral and caudal fins. Cardiovascular toxicity - manifested as pericardial edema and diminished trunk-vessel blood flow - co-occurred with these structural anomalies. RNA-seq of embryos exposed to PFOS or perfluorohexane sulfonic acid (PFHxS) revealed extensive differential gene expression, predominantly gene up-regulation, with enrichment in neural-development and behavior pathways. Consistent with these molecular signatures, larvae at 120 hpf displayed hyperlocomotion following exposure to multiple PFCAs and PFSAs. Although thyroid-hormone genes were not among the differentially expressed transcripts in RNA-seq data, diverse PFAS reduced thyroxine (T4) levels at 96 hpf, indicating impaired thyroid hormone system. Collectively, the data highlight developmental neurotoxicity, cardiotoxicity, and thyroid disruption as recurring hazards across PFAS classes and underscore the heightened vulnerability conferred by extended perfluoroalkyl chain length. These findings advocate for toxicological sub-grouping of PFAS by shared activity profiles and mechanisms, which will refine hazard assessment for this chemically heterogeneous family. **Disclaimer/Disclosure:** This work was supported by 1) Comprehensive Study on PFASs organized by the Ministry of the Environment, and 2) Grant-in-Aid for Scientific Research (B) (19H04275 and 22H03746) and Grant-in-Aid for Scientific Research (A) (20H00634) from the Japan Society for the Promotion of Science. The presenter is responsible for all content of this work, which does not include the views of the Ministry of the Environment, Japan.

1.16.P-Mo-134 Transcriptomic responses to BTEX in *Daphnia pulex*: Individual constituents and mixtures

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Benzene (B), toluene (T), ethylbenzene (E), and xylene (X) are volatile organic compounds (VOCs) derived from petroleum that are commonly found in the environment as mixtures. In recent years, the exposure of the human population to VOCs has been a rising concern. BTEX exposure has been associated with high pre-term birth rates in Detroit. The aim of this project is to develop effective tools to identify biomarkers for BTEX exposure associated with either individual constituents and/or mixtures of constituents. The toxicity of these VOCs in *Daphnia pulex* was measured by examining the transcriptomic responses to exposure in water media. The effects of 24-hr VOC exposure on gene expression was examined using 3' RNA-Seq. Exposure resulted in significant changes in gene expression relative to media controls for all four BTEX constituents and a BTEX mixture at concentrations less than 10ppm. Selection criteria for significantly altered genes of interest were uniqueness of expression for individual BTEX constituents as well as altered expression across multiple concentrations of a particular constituent or across multiple constituents, the function of the gene, counts per million, adjusted P-value (FDR) and Log2fold change. A number of genes of interest were identified as potential biomarkers and will be evaluated for concentration-dependent expression and for potential toxic interactions associated with exposure to BTEX mixtures (e.g., additive or synergistic responses).

1.16.P-Mo-135 Rapid and efficient microbial community viability assessment using marker gene targeting

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Distinguishing viable from non-viable microbes remains a major hurdle in microbiome research, limiting our ability to fully understand microbial community structure, function, transmission, and response to environmental perturbations. Microbial viability is critical for interpreting a microbes biological and ecological roles within a microbiome, as only living microbes actively participate in processes such as metabolism, signaling, and host interactions. Existing methods, such as chemical-based viability assays and 16S rRNA transcript detection have proven sub-optimal in complex communities, while shotgun metagenomics and metatranscriptomics, though informative, are often prohibitively expensive and computationally demanding. To address these limitations, we are developing a novel, high-throughput viability assessment approach based on the sequencing of optimized marker gene transcripts. Our method combines rational marker gene selection, in silico primer design, and amplicon sequencing to enable precise, scalable differentiation of actively transcribing microbes within diverse communities. Preliminary work shows that rationally selected marker genes can effectively distinguish viable microbes and provide accurate taxonomic and functional profiles in human and built environment samples. We have constructed a marker gene database, designed and evaluated primers, and developed amplification protocols based on paired metagenomic and metatranscriptomic datasets. Moving forward, we are refining marker gene amplicon design, optimizing amplification protocols for cost-effectiveness and accuracy, and validating the approach in both synthetic communities and complex natural samples with known viability controls. Once established, this workflow will offer an accessible, culture-independent solution for microbiome viability profiling. This approach has broad implications for advancing microbiome-based diagnostics, therapeutic development, and environmental surveillance by providing a much-needed tool to quantify the active, functional members of microbial communities.

1.16.P-Mo-136 Consequences of sexual stress on aggression, microbiome and immunocompetence in female and male *Drosophila melanogaster*

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Lab-based toxicity testing may not account for the effects of social and physical interactions and related stressors on exposure outcomes, which could confound results and data interpretation. Using model organism *Drosophila melanogaster*, we hypothesized that several non-mating and mating scenarios lead to significant differences in sex-specific gene expression in fly heads, gut microbiome composition, aggressive behavior, and

overall immune competence relative to toxicant exposure challenges. We designed a chamber containing two treatment groups of 10 female or male 3-day old flies separated by a mesh barrier, allowing visual but preventing physical interactions. Sex-specific groups of three conditions were examined: 1. a) single-sex scenario: keeping single sex (either male or female) groups separate in two chambers; b) non-mating: female and male flies were kept in separate chambers, and 3) mating: mixed populations of 6 flies of each sex were kept in each chamber. Flies were sampled and dissected at three developmental time points: three days (onset of sexual maturation), seven days (young adult), and 14 days (middle-aged adult). RNA from fly heads was extracted and sequenced using 3'-end shallow RNA sequencing to quantify and contrast patterns of gene expression in treatment groups. Fly trunks DNA was utilized for 16s rDNA sequencing to assess bacterial content in each group. Aggression assays between two males of each mating status were conducted at the three timepoints. Preliminary results indicate similar microbial compositions at three days, but an increase of Morganellaceae and decrease of Acetobacteraceae and Lactobacillaceae in both non-mating females and males at seven days. We also found a significant decrease in frequency and latency of the aggressive behavior in mating male flies. Significant changes in the gut microbiome composition influenced by mating status may compromise host immunocompetence and ability to withstand the challenge of toxicant exposures, necessitating the inclusion of mating in more comprehensive toxicity bioassays. Future directions include subjecting immunocompromised, sexually stressed lab-reared and natural population of fruit flies to chemical challenges using persistent organic pollutants of concern.

1.17.P-Th Late-Breaking Science: Environmental (Human and Ecological) Toxicology and Stress Response

1.17.P-Th-001 Modeling wildfire smoke-induced functional decline via *Caenorhabditis elegans*

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Wildfire smoke (WFS) is an expanding source of fine particulate matter (PM2.5) with well documented cardiopulmonary risks, yet its impact on organismal aging remains incompletely understood. Here, we use *Caenorhabditis elegans* to model how simulated WFS PM2.5 at various concentrations (0.095-100 ug/ml) affects lifespan and functional decline across generations. Parental (P0) worms exposed to WFS particulates were tracked for lifespan, age-associated locomotion endpoints (e.g., straight line distance, peristaltic speed, and pharyngeal pumping), and collected for RNA-seq at lifespan days 3 and 10 to assess transcriptomic markers of accelerated aging. Unexposed progeny (F1) derived from exposed P0 were subsequently evaluated for identical locomotion endpoints to assess for intergenerational effects. Three sets of replicates were performed for all assays. Here, we find that a single 24-hr exposure to 30 ug/ml WFS PM2.5 is sufficient to decrease maximum lifespan compared to controls (15 versus 18 days), impair readouts of neuromuscular function (as reflected by significant dose-dependent decreases in pharyngeal pumping rate and average velocity), and upregulate transcripts associated with accelerated aging (e.g., increases in phase-I metabolism and decreases in muscle organization and signaling). Interestingly, P0 exposure to WFS decreased straight-line distance at 10 ug/ml and above ($p = 0.0054$), peristaltic speed at 100 ug/ml ($p = 0.0457$), while the effect was not seen in the F1 generation. By pairing high-throughput, low-cost lifespan/locomotion assays with parental transcriptomics, this study establishes *C. elegans* as a valuable platform for quantifying WFS-driven aging phenotypes.

1.17.P-Th-002 Grouping method for ecological threshold of toxicological concern for fragrances

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There is a need to develop ecological threshold of toxicological concern (EcoTTC) for screening-level environmental risk assessment. EcoTTC offers a rapid and predictive method to address environmental hazards for substances lacking certain data endpoints. It's a flexible approach that avoids animal testing and utilizes existing information to establish exposure levels for chemicals below which no appreciable risk to the

environment is expected. A key requirement for predictive approaches is the grouping premise on which a prediction is based, such as biotransformation pathway, structural similarity, chemical use, functional group, mechanism of action, omics, or hazard classification. The objective of the current study was to evaluate the validity of functional and structural grouping approaches for computing EcoTTC for fragrance raw materials using ketones (aromatic, decenone, acyclic aliphatic, or alicyclic), esters (aromatic or aliphatic) and aldehyde (aromatic, alicyclic, or acyclic non aliphatic) as case studies. Toxicity endpoints were collected from USEPA's ECOSAR v2.2 (ECOLOGical Structure Activity Relationship) database for neutral organics. Predicted No Effect Concentration (PNEC) was calculated following GHS guidelines. R (v. 4.5.1) was used for statistical analysis. Kruskal-Wallis rank sum tests were performed to determine differences in means, at $p < 0.05$ with adjustment using Bonferroni method. EcoTTC was computed as PNEC at 5th percentile. Analysis indicated no significant difference among the functional groups ($p > 0.05$). The results suggested that grouping based on functional groups should suffice for EcoTTC computation.

1.17.P-Th-004 1H-NMR metabolomics reveals functional group–Specific toxicity of trisiloxane surfactants in honey bees

Kelsey Bouse, Serhan Mermer, Patrick Reardon, Hannah Lucas, Carolyn Breece, Ellen Topitzhofer, Christopher Schmokel and Ramesh Sagili, Oregon State University

Honey bees (*Apis mellifera*; Hymenoptera: Apidae) are routinely exposed to agricultural adjuvants during crop pollination, including Trisiloxane Surfactants (TSS), which are widely applied to improve pesticide performance. Such exposure is especially significant in Central California, where nearly 60% of U.S. honey bee colonies are transported annually for almond pollination and encounter repeated tank mixes containing TSS. Despite this widespread use, TSS remain classified as unregulated “other ingredients,” even as evidence of their toxicity to pollinators grows. This study examines the toxicity and metabolomic effects of three TSS functional groups (acetyl-, methyl-, and hydroxy-) on honey bees. A preliminary-experiment was conducted with TSS-acetyl, TSS-methyl to find appropriate concentration ranges and validate methods for the larger study. For full experiment, honey bees were housed in acrylic cages and fed ad libitum for eight days with sucrose solutions containing TSS-acetyl, TSS-methyl, and TSS-hydroxy across a range of concentrations. Preliminary 1H-NMR metabolomics data for TSS-acetyl and TSS-methyl indicated changes in metabolites associated with neurotransmission, stress, and energy metabolism. Specifically, reduced tyrosine suggested impacts on dopamine driven foraging behavior, elevated glutamine was consistent with nutritional stress, and increased kynurenate pointed to neurotoxicity via N-Methyl-D-aspartate (NMDA) receptor blockage. Such metabolomics shift support the hypothesis that TSS disrupt multiple physiological pathways beyond mortality alone. Ongoing analyses of a broader concentration range from full experiment, using multivariate approaches (PCA, PLS-DA) aim to elucidate functional group–specific mechanisms. Findings will improve understanding of the risks posed by TSS adjuvants and support science-based recommendations for pollinator health protection.

1.17.P-Th-005 Consumer chemical products containing chemicals with increasing internal concentrations: A NAMs-based priority approach

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Most consumer chemical products are composed of mixtures, and predicting their hazards requires constituent analysis and evaluation based on Adverse Outcome Pathways (AOPs). It is particularly important to prioritize chemicals that may affect the skin, respiratory, endocrine, nervous, reproductive, and developmental systems. In this study, we identified chemicals with increasing internal concentrations across different population groups (children, elementary school students, adolescents, and adults), focusing on substances measured in human biomonitoring samples such as blood and urine from the National Environmental Health Survey. We also examined major consumer chemical products containing these substances. To identify chemicals with increasing internal concentrations, we analyzed biomonitoring results for 39 substances included in the Clinical Test for Environmental Hazardous Substances. Fourteen substances showing increasing trends across

population groups were identified (e.g., t,t-Muconic acid, 2-Naphthol, Bisphenol F, Ethyl Paraben). In addition, by including newly surveyed persistent organic pollutants, a total of 45 substances were examined to determine their presence in major consumer chemical products. Among the chemicals with increasing internal concentrations, four substances—2-Naphthol, Bisphenol A, Ethyl Paraben, and Methyl Paraben—were detected in consumer chemical products. These substances were primarily found in products such as air fresheners and deodorizers.

1.17.P-Th-007 Characteristics of aerosol generated from microcystis blooms

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Harmful Algal Bloom (HAB) risk management has primarily focused on oral exposure via drinking water. Recently, the potential inhalation risks of HAB-derived aerosols (HABA) have been discussed although the information of their dispersal characteristics limited. In this study, we investigated the dispersal characteristics of HABA produced by Microcystis, a major bloom-forming cyanobacterium, and microcystins (MCs) under both laboratory and field conditions. Particle size analysis indicated that HABA mainly consisted of fine droplets (<50 nm). Fluorescence microscopy revealed that single Microcystis cells were dispersed, whereas colonies were not detected in the laboratory experiments. This suggests that Microcystis colonies are too large to become airborne and remain suspended. Considering previous reports that MC production is associated with colony formation in Microcystis, it is possible that the dispersal and diffusion of MCs are physiologically suppressed by the colony-forming characteristics of Microcystis. Factors contributing to HABA occurrence were classified as “generation factor” such as wind speed and “persistence factor” as air temperature. Abundance of toxigenic Microcystis quantitated with qPCR in HABA correlated more strongly with persistence factor ($n = 25$, $R^2 = 0.45$, $p < 0.05$) than with generation factor ($n = 25$, $R^2 = 0.05$, $p < 0.05$). MCs analyzed with LC-MS/MS were not detected in the HABA samples, assuming the maximum potential MC concentrations in HABA which were estimated with the number of toxigenic Microcystis and cell quota of MCs reported by WHO (0.2 pg per cell of toxigenic Microcystis) were under detection limit of this study. These findings suggest that the physiological characteristics of cyanobacteria must be considered when assessing the potential for cyanotoxin presence in HABA. **Disclaimer/Disclosure:** This research was supported by the National Institute of Environmental Research (NIER, R25TA00624706-01) funded by the Ministry of Climate, Energy and Environment (Korea). The authors also acknowledge financial support from the BK21 FOUR Program funded by the Ministry of Education (Korea) and the National Research Foundation of Korea, and from the Waste to Energy-Recycling Human Resource Development Project (YL-WE-23-001) supported by the Ministry of Climate, Energy and Environment (Korea).

1.17.P-Th-008 Per- and polyfluoroalkyl substance (PFAS) contamination in the Tennessee river watershed

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The Tennessee river (TN) watershed supplies drinking water to approximately 5.2 million people. Over the years, the watershed has been heavily contaminated with diverse amounts of pollutants, with Per- and Polyfluoroalkyl Substances (PFAS) being one of them. PFAS, commonly known as forever chemicals, are a synthetic class of compounds characterized by extreme persistence, bioaccumulation potential, resistance to degradation, and associations with chronic health effects. In this study, PFAS levels were analyzed in water and sediment samples collected from the Tennessee River near Camden in Benton County in Tennessee. The collected samples were processed and extracted using a solid-phase extraction (SPE) method to retrieve PFAS

compounds, followed by carbon cleanup to remove particulates. Subsequently, the samples were analyzed using Triple Quadrupole Liquid Chromatography-Mass Spectrometry (LC/MS-MS). Analysis of TN river water and sediment samples revealed the presence of several PFAS compounds at quantifiable levels. Among the detected analytes PFOS (0.246 ng/mL), PFTeDA (0.30 ng/mL), PFNA (0.61 ng/mL), PFHpS (1.06 ng/mL), PFHpA (0.36 ng/mL), PFDA (11 ng/mL), and NMeFOSAA (11.2 ng/mL) were observed. These concentrations exceed the EPA interim health advisory level of 4 ppt for PFOS and 10ppt for PFNA and PFDoA, and overall total concentration in drinking water, raising concern for ecological and human health risks arising out of PFAS exposure. Likewise, these PFAS concentrations were also found elevated in sediment i.e PFOS (0.2 ng/g), PFTeDA (0.23 ng/g), PFNA (0.46 ng/g), PFHpS (0.84 ng/g), PFHpA (0.27 ng/g), PFDA (8.8 ng/g), and NMeFOSAA (8.9 ng/g). The presence of PFAS in both water and sediments highlights their environmental persistence and mobility. Collectively, our study highlights the significant environmental pollution caused by PFAS in the Tennessee river. Exploring effective remediation strategies, such as microbial and thermal degradation strategies deserves consideration to mitigate PFAS contamination.

1.17.P-Th-010 Tributyl phosphate (TBP) disruption of lipid and sex hormone regulation: Exploring underlying toxicological mechanisms using zebrafish and fathead minnows

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Organophosphate esters (OPEs) are increasingly used as alternatives to polybrominated diphenyl ethers (PBDEs) in commercial flame-retardant applications. Among these contaminants of emerging concern, tributyl phosphate (TBP) is widely used. However, the toxicological effects of TBP, particularly on the endocrine and metabolic systems, remain largely unknown. Due to its lipophilicity, TBP can persist in aquatic ecosystems and bioaccumulate; thus, its potential impact on fish reproduction and endocrine regulation is of growing concern. In the present study, we investigated the reproductive toxicity of TBP and its underlying mechanisms in two model fish species, i.e., zebrafish (*Danio rerio*) and fathead minnows (*Pimephales promelas*). Following OECD Test Guideline 229, adult breeding pairs of both zebrafish and fathead minnows were exposed to TBP at nominal concentrations of 0, 0.03, 0.3, and 3 mg/L for 21 days. The cumulative number of eggs produced, mRNA expression level changes related to lipid metabolism, and sex steroid hormone biosynthesis were then measured. Exposure to TBP significantly ($p < 0.05$) reduced fecundity in both zebrafish and fathead minnow across the 21-day exposure period. This reproductive decline was associated with altered gene expressions of major steroidogenic genes (i.e. 3bhsd, cyp19a, and/or cyp17) in both female and male gonads of zebrafish and fathead minnows. In addition, the gene expression of pparg, a gene critical for lipid and glucose metabolism, was disrupted in the gonads in both species, suggesting a metabolic disruption could partly explain the reduced fecundity. Our findings indicate that TBP may impair reproduction by disrupting lipid metabolism and gonadal steroid hormone regulation. Given the environmental persistence and bioavailability of TBP, further studies are needed to understand its long-term and transgenerational effects, along with associated human health and ecological risks.

1.17.P-Th-011 Co-exposure of nanoplastics and pesticides: Impacts on neurotoxicity in primary cortical neurons

Sydney Thrall, Sydney Stradtman, Kathryn DeSantis and Brandon Pearson, Oregon State University, Environmental and Molecular Toxicology

Over the past decade, there has been growing concern about the increasing presence of micro- and nanoplastics (MNPs) in both environmental and biological systems. Nanoplastics (NPs) smaller than 200 nm are of particular interest due to their ability to cross the blood-brain barrier, raising concerns about neurotoxicity and their contributions to neurodegenerative diseases. Importantly, the majority of nanoplastics entering the human body are associated with a complex exposome, some of which adsorbs to the plastic particles. This study examined whether polystyrene nanoplastics enhance the neurotoxic effects of rotenone, a known mitochondrial complex I inhibitor and experimental inducer of Parkinson's disease. We hypothesized that co-exposure to polystyrene

nanoplastics and rotenone would result in synergistic neurotoxicity with nanoplastic-induced alterations in the toxicokinetics of rotenone. Primary cortical neurons were isolated on embryonic day 15.5 and cultured in 96-well plates. At seven days in vitro, neurons were exposed to a nine-point concentration range of rotenone (3 to 0.011718 μ M) and 50-nanometer polystyrene (200 to 0.78 μ g/ml) individually, followed by a co-exposure with a fixed 50-nanometer polystyrene concentration (3.125 μ g/ml) across the same rotenone dilution, for six hr. Cell viability was assessed using a fluorescent live/dead cell assay, and a baseline concentration-response relationship was examined for both compounds. Preliminary results indicate that the combination of rotenone and polystyrene nanoplastics exerted a synergistic effect, increasing cell death in primary cortical neurons and decreasing the half-maximal response for rotenone. These results are being replicated in continued studies to confirm their reliability and further characterize chronic exposures. Ongoing experiments will investigate whether rotenone pre-adsorbed nanoparticles of various polymers and weathering states alter neurotoxicity and other neurological health-related endpoints.

1.17.P-Th-012 Accumulation and internal distribution of PFAS and microplastics in in vivo rat model

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Environmental mixtures of endocrine-disrupting chemicals (EDCs), including per- and polyfluoroalkyl substances (PFASs), micro- and nano-plastics (MNPs), are increasingly linked to adverse neurodevelopmental outcomes. In this study, adult male (n = 15) and female (n = 15) rats, aged 88–100 days, were examined to understand the effects of PFAS as dosed EDC and MNPs as unwanted background exposure in typical in vivo animal studies along with the associated accumulation and internal distribution following dietary exposure to a multi-class EDC mixture. This EDC mixture contains 20 μ g/kg of PFOS (main analyte), and other EDC mixtures (2.5 μ g/kg of BPA and BPS), 20 μ g/kg of DEHP and DBP, and 0.1 mg/kg of CB 153) mimicking environmentally relevant mixture EDC exposure conditions. Experimental groups included PFAS-dosed animals (n = 24; at levels of 0.1X, 1X, and 10X) and controls (n = 6; vehicle - 0.06% dimethyl sulfoxide and 0.0037% ethanol in sesame oil). Biological matrices comprising whole blood (20 μ L), and soft tissues (kidney, liver, and cerebellum; 0.1 g each) were collected and analyzed for PFAS and MNPs. Blood was processed via solid-phase extraction, whereas kidney, liver, and brain tissues underwent microwave-assisted extraction. MNPs were analyzed using pyrolysis-GC/MS, and PFAS was analyzed using LC/MS/MS. Among tissue samples, the average Σ PFAS concentration was higher in liver (0.08 - 4.6 ng/mg) in all groups followed by blood (0.003 - 0.92 ng/mg). PFOS was found in all samples and was the most predominant compound followed by PFHxA in all matrices. PFOS concentration in liver and blood was 3 to 6 times higher in group 1X compared to 0.1X, and similarly, 1.1 to 3 times elevated levels were found in the samples of 10X dosed group compared to those of the 1X dosed group. MNPs concentrations in blood ranged from 57 to 714 ng/ μ l with the average of 350 ng/ μ l with polycarbonate and polystyrene being the most frequently detected polymers (detection frequency >80%). Polycarbonate concentration in blood is the highest in all groups compared to other polymers which shows the potential low-level exposure of MNPs from cages, water bottles and other surrounding environment of rats during the study.

1.17.P-Th-013 Hormesis without stress: Perfluorobutanoic acid (PFBA) rewires hormone rhythmicity in fall armyworm

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

Perfluorobutanoic acid (PFBA), a short-chain per- and polyfluoroalkyl substance (PFAS), is increasingly detected in agricultural environments, yet its biological impacts remain poorly defined. We previously reported that PFBA exposure enhanced growth in *Spodoptera exigua* larvae, but the mechanism driving this hormetic response was not identified. In the present study, we used fall armyworm (*Spodoptera frugiperda*), a species with superior genomic resources, to investigate whether PFBA-induced growth stimulation is mediated by oxidative stress compensation or by endocrine disruption. PFBA elicited a biphasic response, hormetic response i.e., low-dose exposure (1 μ g/g diet) enhanced feeding and accelerated weight gain, whereas high-dose

exposure (20,000 µg/g) suppressed performance. To test between the two hypothesized drivers of hormesis, we measured reactive oxygen species (ROS), antioxidant enzyme activities, molting hormone titers, and expression of regulatory genes. PFBA did not consistently alter ROS, catalase, or superoxide dismutase activity, arguing against an stress-compensatory mechanism. Instead, PFBA disrupted endocrine timing. Control larvae exhibited pulsatile 20-hydroxyecdysone (20E) cycles with anti-phasic expression of its inactivation enzyme, 20-hydroxyecdysone oxidase (20EOx). In contrast, PFBA-exposed larvae showed dampened 20E oscillations, flattened 20EOx expression, and reduced temporal variance. Cosinor analysis confirmed a marked reduction in 20E amplitude and a phase shift in peak timing. Expression of Proline-Rich and Coiled-Coil Containing Protein 2 (PRRC2), a translational regulator, followed a similarly dampened profile. Collectively, these findings suggest that PFBA-induced hormesis arises not from oxidative stress but from disruption of endocrine pulse fidelity and regulatory rhythmicity. Such temporal misalignment provides a novel mechanism of PFAS action with broad implications for growth regulation and insect developmental biology.

1.17.P-Th-014 Effects of nanoplastics on amyloid- β fibrillization and neurotoxicity in primary cortical neurons

Sydney Thrall, Kathryn DeSantis, Brandon Pearson and Sydney Stradtman, Oregon State University, Environmental and Molecular Toxicology

Plastic pollution has emerged as a global concern, with growing attention to the potential health impacts of nanoplastics. While environmental distribution is well documented, their neurotoxic potential and possible role in neurodegenerative processes remain poorly understood. In particular, links between nanoplastic exposure and Alzheimer's disease pathology have yet to be clarified. This work investigates how nanoplastics influence amyloid- β (A β) fibrillization kinetics and neuronal health. A cell-free in vitro assay was utilized to examine polymer-specific effects of 50 nm polystyrene (PS) and poly(methyl methacrylate) (PMMA) nanoparticles on A β aggregation. Increasing concentrations of both polymers shifted the fibrillization profile, altering the time to fibril formation. These findings guided subsequent studies in primary cortical neurons isolated from embryonic day 17 ($\pm$ 1) mice. Cultures were exposed on in vitro day 7 to a nine-point concentration range (0.8–0.003125 µg/µL) of 50 nm PS or PMMA nanoparticles for 24 or 120 hr. Neurotoxicity was assessed via fluorescence-based live/dead cell assays and reactive oxygen species (ROS) detection. Preliminary results indicate that exposure to both PS and PMMA induces changes in ROS at multiple concentrations, suggesting oxidative stress as an early neurotoxic mechanism. No notable changes were detected in cell viability following exposure to both 50 nm PS and PMMA. These studies were conducted using pristine, commercially produced nanoplastics, providing a controlled baseline that has to yet capture the complexity of environmentally weathered particles. Ongoing work will replicate these findings and extend them to assess how particle weathering influences neuronal responses and toxicity.

1.17.P-Th-017 Development of a high-throughput assay for quantifying phagocytosis in neutrophil- and macrophage-like human cell lines

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When exposed to immunosuppressive pharmaceutical and environmental chemicals, innate immune functions, such as phagocytosis, may be inhibited, potentially increasing susceptibility to pathogens. To efficiently quantify the immunosuppressive potential of many chemicals, we developed a highthroughput assay that assesses phagocytic function of neutrophils and macrophages. The assay utilizes the high-content imaging Opera Phenix Plus platform and fluorescent pH-sensitive pHrodo™ BioParticles™ E. coli conjugates (Invitrogen). The pHrodo bioparticles trigger phagocytosis by neutrophillike HL-60 cells or macrophage-like THP-1 cells. DMSO differentiated HL-60 cells were imaged with either immunoglobulin G-opsonized or non-opsonized pHrodo bioparticles to determine if opsonization would stimulate higher levels of phagocytosis, allowing for potentially greater sensitivity for detecting inhibition. We determined that opsonizing pHrodo

bioparticles was advantageous for this assay because it increased their cellular uptake, resulting in 89% more red fluorescence in cells when pHrodo bioparticles were opsonized compared to when they were not opsonized. THP-1 cells can be differentiated into three different types of macrophages: naïve macrophages, pro-inflammatory macrophages, and anti-inflammatory macrophages. THP-1 cells were differentiated into each of these macrophage types and their ability to phagocytose opsonized pHrodo bioparticles under control conditions was quantified. The three different types of macrophages showed varying degrees of phagocytosis. Naïve macrophages demonstrated the greatest phagocytic ability. Anti-inflammatory macrophages were on average 23.92% less fluorescent than naïve macrophages, whereas proinflammatory macrophages were on average 67.52% less fluorescent than naïve macrophages (less fluorescence indicates less phagocytosis). Therefore, further experiments on macrophage phagocytosis will include only naïve and anti-inflammatory macrophages since they are the primary phagocytosing cell types. Decreases in neutrophil or macrophage function may weaken an organism's protection from pathogens. Thus, it is important to identify chemicals that can inhibit phagocytosis. The development of this in vitro, high-throughput assay will facilitate the screening of many environmental chemicals for immunosuppressive potential via neutrophil and macrophage phagocytosis inhibition. **Disclaimer/Disclosure:** This abstract does not reflect the official policy of the USEPA.

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

2.01.P-We-016 Synergistic embryotoxicities of benzo[a]anthracene and pyrene mixture in Japanese medaka at individually no-effect concentrations

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Polycyclic aromatic hydrocarbons (PAHs) such as pyrene (Pyr) and benzo[a]anthracene (BaA) are widespread pollutants in the aquatic environments that can induce toxic effects on early development of fish. While high concentrations of individual PAHs are known to induce morphological malformations, environmental exposures often involve complex mixtures at low concentrations. It remains poorly understood whether such combinations with structurally similar PAHs can produce synergistic developmental toxicities. In this study, we examined the combined effects of BaA and Pyr on early embryogenesis using Japanese medaka (*Oryzias latipes*) as a model species. Embryos were exposed from 0-day post fertilization to 0.25 μ M Pyr and 0.5 μ M BaA (n=3, with each replicate consisting of 10 embryos). These concentrations were previously confirmed to be non-toxic when exposed individually. Morphological endpoints were evaluated under a microscope at hatching. Remarkably, co-exposure of BaA and Pyr at these individually no-effect levels resulted in $100 \pm 0.00\%$ incidence of malformations. The symptoms observed in embryos exhibited cardiac hypertrophy, pericardial edema, craniofacial defect, and spinal curvature, which were consistent with Pyr-induced effects typically observed only at exposure with a higher Pyr concentration (2.5 μ M). This indicates that BaA, although no-effect concentration at 0.5 μ M, can significantly enhance toxic potential of Pyr, mimicking the effects typically observed at higher Pyr concentrations. These results suggest a possible synergistic interaction, rather than an additive effect, between these PAHs. As our previous study implicated cytochrome P450 (CYP)-mediated metabolism in Pyr toxicity and showed strong Cyp1a induction following BaA exposure, we hypothesize that BaA-induced CYP activity may contribute to the enhanced effects, suggesting the importance of considering PAHs mixture effects in environmental toxicology.

2.01.P-We-017 Temporal and spatial variability in the mercury concentrations of Florida bass from a coastal subtropical river

Celine Marie Emanuelle Lajoie¹, Ted Lange² and Gretchen Lescord¹, (1)Florida LAKEWATCH, University of Florida, Canada, (2)Florida Fish and Wildlife Conservation Commission

Subtropical freshwater ecosystems, such as the Suwannee River in Florida (USA), are undergoing unprecedented changes due to anthropogenic effects on land-use, and more frequent and severe precipitation events due to climate change. These changes are likely to impact the cycling and bioaccumulation of mercury (Hg), a potent neurotoxin, largely through indirect effects on water chemistry and water level fluctuations.

Despite this, long-term studies examining changes in fish Hg concentrations ([Hg]) in subtropical ecosystems are lacking. Florida Bass (*Micropterus salmoides*), an important recreational fish species in Florida, has been monitored for [Hg] for fish consumption advisories throughout the Suwannee River's watershed for ~30 years by the Florida Fish and Wildlife Conservation Commission (FWC). This dataset, along with other state-managed long-term datasets on climate, water chemistry, and land-use provide an opportunity to examine how changes to the river's watershed have affected fish [Hg], and subsequently Hg risk to fish consumers. As such, this study examines how [Hg] of bass varied spatially and temporally within the Suwannee River watershed, and which land-use, water chemistry, and climatic variables best explained this variability. While data analysis is on-going, preliminary explorations suggest temporal oscillations in bass [Hg], resulting in differences of over 0.5ppm in 2lb bass over 10-year periods. Further modeling will determine which environmental and landscape variables are driving this trend and may include the influx of marine sulfate into coastal freshwater habitats. Ultimately, this work will lead to a better understanding of long-term and spatial Hg dynamics in subtropical rivers and may assist with the management of these waterbodies.

2.01.P-We-018 Abiotic and biotic identification, distribution and quantification of persistent organic pollutants by novel passive sampling in San Diego Bay

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Aquatic contamination is comprised of chemical, biological, and physical waste leaching into waterways across the world. The regional contamination profile is determined by nearby human activity and waste streams (or lack of), particularly when it comes to chemical pollution. Industrial reactions and processes use persistent chemicals for consumer and commercial products at scale and subsequently appear in downstream waste and byproducts. Specifically, organic pollutants such as pesticides, polyaromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) are persistent chemical pollutants dynamically existing in aquatic environments, whether bound to sediment, re-mobilized with other pollutants, bioaccumulated in the food web, or directly in the active water current. Water quality chemistry can examine the pollutant profile and it is vital to consider sampling methods to maximize chemical capture and fill in the monitoring gaps within local environments. The San Diego Bay is a shallow, crescent-shaped bay surrounded by naval activity, shipyards and other heavy industry, fishing, and a large metropolitan population. The bay has a well documented daily exposure to effluent of sewage and wastewater from Tijuana, Mexico and the Tijuana River south of the bay inlet. San Diego coastal waters harbor diverse and critical ecosystems with designated State Marine Reserves (SMR) and State Marine Conservation Areas (SMCA), but the majority of these designations exist along the north county coast far from the busy San Diego Bay flow. Novel composite integrated passive samplers (CIPs) were deployed focusing on capturing both polar and non-polar organic pollutants, including legacy and current chemicals; collection of nearby mussels and oysters as representative lower trophic ecosystem members, and bottlenose dolphins - higher trophic order marine mammals, complements the CIPS analysis and helps assess temporal flow of organic molecules throughout the bay more comprehensively. Moreover, this work, based on biotic and abiotic sampling methods quantifying organic pollutants distribution and bioaccumulation, also evaluates the effects of recent and nearby 6,000 acre wildfire and military jet crash in the vicinity of dolphins' training facility in the bay.

2.01.T Assessing Contaminant Effects in Ecosystems with Multiple Stressors

2.01.T-01 The design and analysis of monitoring programs to support risk assessment and adaptive management of multiple types of stressors using the San Francisco Bay and estuary as the example

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Environmental managers and stakeholders are challenged in the identification of multiple stressors and in estimating their relative importance in meeting management objectives. This challenge is especially exacerbated with systems at large geographic scales, with stressors ranging from nano and microplastics, water quality

parameters, nutrients, pesticides, nonindigenous species, and pathogens. The numbers of regulated endpoints are also daunting, habitats for endangered species, water quality for agriculture and human consumption, recreation, and protection for culturally significant sites. Reliance on biotic indices, chemical by chemical regulation, and the use of statistical approaches that do not address the multiple interactions in the system to answer the key management questions is challenging. Particularly at issue is the accounting for the inherent nonlinear dynamics of these systems, the outside forcing function of climate change, and the use of inappropriate statistical and modeling tools. Uncertainty in both measurements and in analyses are poorly documented. This presentation will use the San Francisco Bay and Delta as an example to demonstrate the advantages of exposure-response modeling to describe toxicity, the use of multivariate probabilistic models to describe interactions with multiple stressors and endpoints. Finally, a monitoring and management approach using the San Francisco Bay and Estuary is proposed that incorporates this integrated adaptive management framework and its implementation.

2.01.T-02 ECOMIX: Understanding and managing the impacts of mixtures of chemicals and co-stressors on mixtures of species in English rivers

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Rivers in England are in an unhealthy state. Only 16% of rivers meet the European Water Framework Directive for Good Ecological Status. While it is recognized that chemical pollution from wastewater emissions, agriculture, highways and legacy mining activities is contributing to the problem, we still have a poor understanding of the real risks of chemicals and co-stressors in England's rivers. For example, current regulatory monitoring only focuses on a small number of chemicals in use, ecotoxicity testing focuses on only a small number of species that may not reflect sensitivity of the actual species that occur in English rivers, and chemicals assessment takes a single compound approach ignoring the fact that rivers are exposed to chemical mixtures and other co-stressors. The ECOMIX project is therefore developing a new catchment-based approach to chemical assessment and management that addresses these challenges. This approach: 1) assesses impacts of mixtures of chemicals and co-stressors on the structure and functioning of UK-relevant species assemblages at high spatial resolution; 2) considers the current situation and also looks to the future to account for the effects of global megatrends on chemical sources, fate processes, exposure and effects; and 3) allows us to target interventions where they are going to have maximum impact allowing us to benefit from the use of chemicals while protecting biodiversity. Focusing on the Yorkshire region in the North of England, the study, which has been running for over 2 years, is using a combination of continuous environmental monitoring, exposure modelling at high temporal and spatial resolutions, ecotoxicity testing on multiple algal and invertebrate species and hierarchical species sensitivity distribution and food web modelling to characterize the current and future risks of mixtures of over 40 high priority chemicals and co-stressors arising from different sources. This presentation will: provide an overview of the project approach; illustrate the results obtained to date on the occurrence, effects and risks of the case study chemicals in Yorkshire's rivers; and describe our plans for a catchment dashboard to support management efforts to improve the chemical quality of rivers in the region and to protect them into the future. **Disclaimer/Disclosure:** This study is funded by the Natural Environment Research Council

2.01.T-03 Bioassessment evaluations to determine the status of cold-water aquatic life beneficial use attainment are confounded by climate warming

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Projected climate warming impacts to the Northern Rocky Mountain ecoregion in Idaho are increasing air temperatures, reductions of the proportion of precipitation falling as snow, and more frequent droughts. In years

with less than average snowpack, or above average air temperature, or both, summer baseflow stream discharge is reduced and water temperature increases. These conditions adversely impact cold-water aquatic life, including stream macroinvertebrates. Under the Clean Water Act, Idaho determines the status of cold-water aquatic life beneficial uses in a stream assessment unit (AU) with bioassessment monitoring data collected by the Department of Environmental Quality (DEQ) under the Beneficial Use Reconnaissance Program (BURP). One part of DEQ's evaluation applies a Stream Macroinvertebrate Index (SMI) score that is compared to SMI benchmark values derived from the distribution of scores from unimpacted reference areas. The current SMI reference benchmarks are based on BURP data collected from 1998 to 2008. Under climate warming, the attainment of cold-water beneficial uses will become less likely over time for a given AU, including reference areas. In this work, the impacts of climate warming on the stream macroinvertebrate community are illustrated using a unique timeseries of BURP-compliant monitoring data collected from 2007 to 2024 from a South Fork Coeur d'Alene River AU near Mullan, Idaho. The Mullan AU data illustrate the way that the SMI score, and underlying metrics, respond during years with reduced stream discharge and elevated water temperature. To confirm the climate related stressor-response patterns seen in the Mullan AU, a comparison is made to DEQ BURP data from locations that have similar stream order and elevation in the adjacent Upper Coeur d'Alene River and St Joe River watersheds. Annual air temperature, snowpack, and stream discharge information from weather stations and stream gages in these watersheds are used to describe the relative degree of thermal stress experienced by the macroinvertebrate communities each summer. In addition to the evaluations of the SMI metrics, temperature species sensitivity distribution (SSD) information is presented, and the SSDs are used to identify potential indicator species that respond to thermal stress. Based on these findings, updates to the BURP reference dataset and a reevaluation of the metrics underlying the SMI are recommended.

2.01.T-04 Aquatic macroinvertebrate diversity and water quality as indicators of wetland health in natural and constructed wetlands of northeast Arkansas

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The Mississippi River Basin has lost approximately 80% of its naturally occurring wetlands since the 19th century due to urbanization and agriculture. This extensive loss reduced the land's ability to cycle and detoxify nutrients and other contaminants. To address this, the U.S. government established the Wetland Reserve Program, now the Agricultural Conservation Easement -Wetland Reserve Easement Program (ACEP-WRE) in 1990, to incentive landowners to convert portions of their property into wetland habitat. Since then, 11,736 km² of wetland habitat has been restored. Although the program has been in place for 35 years, there is limited research comparing the functional similarity of ACEP-WRE sites to natural wetlands. This project compared biogeochemical and ecological similarity of ACEP-WRE wetlands (n=30) within Arkansas to agricultural control sites (n=5), as well as to natural reference bottomland hardwood forest wetlands within wildlife management areas (n=5). Each site was sampled quarterly in triplicate with points a minimum of 500 m apart. Sample collection occurred from February to November of 2024, and collection included water, soil, and aquatic macroinvertebrates. Water samples were analyzed on site for physicochemical properties and transported to the laboratory on ice to measure turbidity, suspended sediments, and nutrient concentrations. Soil samples were collected for laboratory analysis of metals, organic material, and physicochemical properties. Macroinvertebrate beta diversity and community composition were calculated for each site. Data supports ACEP-WRE wetlands' efficacy in cycling nutrients and supporting macroinvertebrate diversity, comparable to or greater than natural wetlands. This study provides evidence for the ability of wetland soil parameters to predict changes in macroinvertebrate community composition within the orders Diptera and Hemiptera. These results provide a framework for future studies to determine the functional abilities of ACEP-WRE sites and inform wetland restoration practices.

2.02.P-Tu Assessing Toxicity in Water and Aquatic Sediments

2.02.P-Tu-013 Evaluating the toxicity of PFAS through sediment exposure pathways: Perfluorooctane sulfonate (PFOS) and perfluorohexanesulfonic acid (PFHxS) single and mixtures effects on *Chironomus dilutus*

Sarah Lanasa, Michael Chanov and Jamie G. Suski, EA Engineering, Science, and Technology Inc. PBC

Per- and polyfluoroalkyl substances (PFAS) have been detected in drinking, ground, and surface waters, as well as sediment and wild animal tissues. This has prompted a substantial amount of environmental and ecotoxicological research into the impacts of PFAS and the need for ecological risk assessments at contaminated sites. The historic use of PFAS containing products including Aqueous Film-Forming Foams (AFFFs) has led to widespread contamination across the United States. Perfluorooctane sulfonate (PFOS), is the most frequently detected PFAS at contaminated sites. The USEPA and the DOD have made significant efforts to evaluate the risks associated with PFOS and other PFAS, and to develop ecological screening values (ESVs). However, due to limited data, the ESVs for sediment-dwelling organisms are derived from aquatic toxicology studies and are not currently included in ecological risk assessments at PFAS-contaminated sites. Sediment-dwelling organisms are crucial to ecosystem functioning as they serve as a food source for higher trophic levels, are commonly used as water quality indicators in bioassessments, and contribute to key ecosystem services. To address this data gap, we conducted a series of studies to assess the effects of PFOS and perfluorohexanesulfonic acid (PFHxS), another commonly detected PFAS, on *Chironomus dilutus*. PFOS and PFHxS were tested both individually and in combination, through acute and chronic spiked sediment exposure tests. Organisms were exposed to five concentrations of PFOS, PFHxS, and a PFOS:PFHxS mixture at a 5:3 ratio. For acute exposures survival was the most sensitive endpoint; the 10-day survival LC50 values were 223 µg/L PFOS, 30,116 µg/L PFHxS and 55:33 µg/L PFOS:PFHxS mixture. Chronic exposures showed greater sensitivity at biomass endpoint; the 20-day biomass IC25 values were >400 µg/L PFOS, 2,304 µg/L PFHxS and 28:17 µg/L PFOS:PFHxS mixture. The results of both the acute and chronic tests indicated that the PFOS:PFHxS mixture was more toxic than the individual chemicals for both survival and biomass endpoints. Comparing the acute and chronic data, acute exposures resulted in lower survival LC50 values for both chemicals and the mixture, suggesting that acute exposure was more toxic to midge survival than chronic exposure. However, chronic exposure produced the lowest biomass IC25 values, indicating that the PFAS mixture was generally more toxic over the longer exposure period.

2.02.P-Tu-014 The application of biomimetic extraction with solid phase microextraction: Moving towards standardization

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Biomimetic extraction with solid-phase microextraction (BE-SPME) is a non-depletive passive sampling analytical method for measuring total bioavailable hydrocarbon concentrations in environmental media. This BE-SPME derived concentration serves as a surrogate measurement for the bioconcentration of hydrocarbons in biota, making it a reliable indicator of aquatic toxicity for complex petroleum substances. This correlation between BE-SPME concentrations and toxic effects has been validated across various species and test substances, with acute toxicity generally observed at a BE-SPME concentration between 10 - 100 mmol/L fiber. Therefore, this technique provides a valuable screening tool for aquatic toxicity. Consequently, there has been significant adoption of the BE-SPME approach in diverse environmental toxicology and chemistry applications. To date, BE-SPME has been used to estimate total body residues in biota, assess aquatic toxicity to a range of species, evaluate bioavailability of hydrocarbons in sediments, evaluate the efficacy of wastewater treatment technologies, and screen petroleum substances for further testing. These applications underscore the versatility of the BE-SPME method as a tool in risk assessment and evaluation. Recently, a round-robin research program was conducted to explore interlaboratory variability and establish method controls to enhance consistency in BE-SPME results using Canadian oil sands process-affected waters, and defined mixtures of hydrocarbons. The results of this study indicate that BE-SPME concentration measurements were, with RSD of

the fully automated method at only 14% across six laboratories. Additional round robin testing is ongoing. The main objective of this presentation is to showcase the range of applications of BE-SPME, highlight the role of BE-SPME in risk assessment of complex hydrocarbon mixtures, and demonstrate the technical readiness of BE-SPME as we strive towards method standardization. Overall, this work aims to provide confidence to the broader research community to routinely adopt BE-SPME test method as a tool for risk assessment.

2.02.P-Tu-015 Ecotoxicity of antimicrobials in potentially sensitive species such as cyanobacteria, algae and protozoa

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The recent publication on reported the estimation of 4.71 million death associated with bacterial AMR in 2021 while they focused that an estimated 1.91 million deaths attributable to AMR and 8.22 million deaths associated with AMR could occur globally in 2050. Alert to this crisis, World Health Assembly of WHO adopted a global action plan on AMR in 2015 and many countries have made their efforts to survey the current status of AMR/antimicrobials not only in human and live stocks, but also in the environment. In Japan, however, Ministry of Health, Labour and Welfare has continued their action plans in 2016 mostly with Ministry of Agriculture, Forestry and Fisheries only on human and livestock. After the COVID-19 pandemic, The Ministry of the Environment, Japan finally kicked off a survey project under Environmental Restoration and Conservation Agency (ERCA) this year on both AMR and antimicrobials in the environment. Our research team in National Institute for Environmental Studies (NIES) joined this project to conduct literature search/review of existing toxicity data of major antimicrobials used in Japan such as macrolides and sulfonamides in aquatic organisms not only in typical aquatic organisms, fish, daphnia, and algae, but also in potentially sensitive species such as cyanobacteria and protozoa including ciliates. We also started to conduct ecotoxicity testing for some antimicrobials using cyanobacteria, algal species, and ciliates to fill in the data gaps for the ecological risk assessment. This is an interim report of the literature search/review and the ecotoxicity tests using these potentially sensitive species. **Disclaimer/Disclosure:** This research was performed by the Environment Research and Technology Development Fund (JPMEERF25S21230) of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan.

2.02.P-Tu-016 Evaluations for sediment toxicities in Ise Bay, Japan, using embryos of marine fish

Seiichi Uno, Kazuki Imamura, Masatoshi Yamasaki and Emiko Kokushi, Kagoshima University, Japan

Coastal sediments near large cities are often polluted by various kinds of chemicals and are sometimes detected at high concentrations in sediments. Even if an anomaly occurs on the sea-benthic environment and results in some biological impacts, it's difficult to detect unless massive mortality of organisms caused. This is because seawater transparency is generally limited, thereby impeding direct visual observation of the benthic area. We established the evaluation method using embryos of marine medaka, Java medaka (*Oryzias javanicus*), and sediments with minimal pore water and without overlying raring water. Ise Bay is located close to Nagoya city, the third largest city in Japan. The bay is home to Nagoya Port, the largest port in Japan, the Chukyo industrial area, and international airport along its shorelines. Furthermore, several medium to large rivers flow through Nagoya city and its surrounding regions discharging into the bay. As a result, these factors contribute to a persistent potential for the introduction of anthropogenic chemicals into the bay. In the present study, we collected sediment samples from several sites in and near Nagoya city in both 2020 and 2025, and subsequently evaluated the variations in sediment toxicity risk over this 5-year using our evaluation method with embryos of Java medaka. Ten embryos were placed in half of them in each sediment, which was laid at a glass petri dish and were allowed to develop on the sediments for 10 days post-fertilization. In 2020, we found high mortality rates with over 70% of exposed embryos to sediments collected from near the airport. Additionally, sediments

collected from the confluence where the Shonai River and Nikko River, both of which flow through central Nagoya city, converge to form an estuary caused 91% embryo mortality. The toxicities of sediments from this point had not changed at all by 2025. Additionally, sediment samples collected in 2025 from an area on the far side of the reclaimed land, opposite the confluence, also exhibited serious toxicity. Although we have been employing the procedure such as fractionation based on polarity, biological reactions in embryos, and comprehensive GC/MS analysis, we have not yet been able to determine the substances that trigger adverse effects in sediments. In this presentation, we will show the current toxicity risk of sediments in Ise Bay.

2.02.P-Tu-019 Insights and key takeaways from conducting toxicity tests with non-guideline aquatic insects

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Invertebrate toxicity testing is an important component in the registration process for today's modern chemicals. While testing requirements encompass a number of standard laboratory-cultured aquatic invertebrates, there is an increasing interest in expanding the number of species tested to improve environmental risk assessments. The number of invertebrate species being requested for risk assessment studies has steadily increased in recent years in order to develop species sensitivity distributions (SSD) for European risk assessments and for Endangered Species evaluations in the US. In 2016 new USEPA guidelines (OCSPP) were adopted for use in toxicity testing, however no new species specific guidelines were adopted. The current OCSPP and OECD guidelines for invertebrate testing include daphnia, mysids, Gammarus, oligochaetes and chironomids. To perform species sensitivity distributions, non-guideline aquatic insects such as stonefly, caddisfly and mayfly have been used to provide additional toxicity information. SSD testing has been utilized for acute testing in water only exposures based on OECD guidelines 202 and 235, and for chronic testing in sediment/water systems based on OECD guidelines 218 and 219. We have summarized our experiences in conducting GLP testing with these additional freshwater aquatic insect species and discuss the challenges which include test design, organism availability, organism age, shipping, acclimation in the laboratory and dietary considerations, as well as the key takeaways and considerations for future testing.

2.02.P-Tu-020 Assessing fire retardant and wildfire impacts on the ecological health of Arizona wetlands utilizing macroinvertebrate indicators

Kalai M Kollus and Catherine Propper, Northern Arizona University

As wildfires are increasing in size and severity in the Western U.S., so is the amount of fire retardant used to suppress fire. Wildfires and fire retardants can have significant impacts on critical aquatic habitats for wildlife. Despite the importance of wetlands for ecosystem health, both the impact of wildfire runoff and fire retardant exposure in lentic water bodies, as opposed to lotic water systems, is under-investigated. Both wildfire runoff and fire retardants can cause adverse impacts on water quality, including toxicity and eutrophication, which may amplify problems throughout the ecosystem. Most assessments on aquatic species have only evaluated mortality from high-dose, short-term exposure to fire retardants under lab conditions. Research has shown that macroinvertebrates are sensitive indicators for trophic impacts of contamination. We hypothesize that exposure to wildfire ash or fire retardants will lead to changes in macroinvertebrate community structure. We conducted two studies; the first study aimed to understand the impacts of fire retardant in field-derived mesocosms. We conducted a multi-species field mesocosm experiment at the following retardant exposure concentrations: 0 g/L, 0.2 g/L, and 1 g/L. Survivability and species richness were significantly reduced in the 1 g/L treatment, suggesting that fire retardant exposure may affect the community structure of macroinvertebrate taxa at environmentally relevant levels. The second study assessed ash-loading impacts from storm runoff in a 2024 managed fire scar. Preliminary results suggest that low-moderate severity burns may impact macroinvertebrate abundance post-wildfire runoff into pond systems. As the wildfire regime continues to change in the Southwestern U.S., land managers may wish to consider prioritization of forest and wetland restoration tactics

that will help mitigate wildfire contamination impacts in aquatic resources that are important for wildlife that are dependent on these small, regional aquatic resources.

2.02.P-Tu-021 From source to mouth, no chance of self-recovery for the uMgeni River: Ecological risk assessment of metal contamination

Jeffrey Lebepe and Mapurunyane Selala, Sefako Makgatho Health Sciences University, South Africa

Metal pollution has become a cause for concern, particularly in rivers flowing through different land uses. The uMgeni River receives water from tributaries draining urban, industrial, and agricultural areas. Studies reported elevated metal concentrations along the uMgeni River main stem; however, the spatio-temporal dynamics in relation to land uses, and the influence of tributaries on metal enrichment along the river are poorly understood. Therefore, the present study evaluated the spatio-temporal variation of metals and ecological risks along the uMgeni River system. Water and sediment were sampled from 11 points in the river and its tributaries during the dry and wet seasons. Samples were digested with aqua regia, and analysis was carried out using inductively coupled plasma optical emission spectrometry. Most metals were below the detection limit in the water column, whereas significant concentrations were observed in sediment. Copper (Cu), chromium (Cr), and zinc (Zn) in sediment exceeded the guidelines for aquatic ecosystems, whereas the lead (Pb) concentration was below the guideline. No significant seasonal variations were observed within each site, however, most metals in the sediment showed a definite trend in relation to land use along the longitudinal gradient. The contamination factor showed a low to moderate contamination after the first confluence, with Cr, Cu, Pb, and Zn showing moderate to significant enrichment for the enrichment factor (EF). Moreover, the contamination degree (CD) showed a moderate to high degree of contamination for all sites after the first river-tributary confluence, whereas the geoaccumulation index of > 0 was observed for Cr and Zn at the downstream sites. Similarly, the metal pollution index exhibited a slightly polluted category, with the potential ecological risk index (RI) showing a significant increase from the first river-tributary confluence downstream. The RI observed at the downstream sites was below 150, suggesting low ecological risk. Nevertheless, the main tributary, the uMsunduzi River, was found to be the primary driver for moderate to significant contamination categories for EF and CD in this river system. These findings provide an insight into the trend of metal pollution status along the uMgeni River and the potential effect of the poorly managed industrial and domestic wastewater effluents, and urban storm water runoffs on the ecological integrity of freshwater ecosystems. **Disclaimer/Disclosure:** The work was funded by the National Research Foundation of South Africa, Grant No. 121848

2.02.T Assessing Toxicity in Water and Aquatic Sediments

2.02.T-01 Lessons learned from a toxicity testing laboratory on maintaining long-term culturing of *Ceriodaphnia dubia*

Carlita Barton, Los Angeles County Sanitation Districts

Standard toxicological methods established by the Environmental Protection Agency (EPA) are widely utilized for toxicity testing of surface waters under the National Pollution Discharge Elimination System (NPDES) to assess water quality. In-stream risk assessments evaluate water quality conditions to create biological standards and to support watershed management decisions. Toxicity testing serves as a surrogate measure to evaluate the effects of discharges and mixtures of discharges, ensuring environmental protection. For this purpose, *Ceriodaphnia dubia*, a freshwater cladoceran, is commonly used for testing due to its sensitivity. The testing procedure incurs high labor costs, as these organisms must be bred for approximately three weeks before they can be used in evaluations. The Los Angeles County Sanitation Districts' (LACSD) San Jose Creek Water Quality Laboratory (SJCWQL) has successfully cultured *C. dubia* for over twenty-five years, maintaining the same cultures for nearly nine years. This experience has showcased both challenges and successes in rearing these organisms. The preference for *C. dubia* stems from their small size, sensitivity to various toxicants, and capability for clonal reproduction, making them relatively simple and cost-effective to maintain. However,

research conducted by SJCWQL highlights key factors such as holding containers, types of culture water, test chambers, food types, feeding rates, and laboratory environmental conditions, significantly influence the organisms' performance. Because of these variables, it is essential to maintain thorough documentation of their performance for sustaining healthy cultures and minimizing the response variability of *C. dubia*. This presentation will provide examples of proven procedures, data evaluations, and controlled factors that can enhance the effectiveness of long-term culturing of *C. dubia*.

2.02.T-02 Comparing the sensitivities of oysters and standard aquatic species to pesticide active substances

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To support registration of active substances for plant protection products globally, testing on three taxonomic groups of aquatic organisms (fish, daphnia, algae) covering various trophic levels is generally required. However, there are differences in ecotoxicity data requirements based on geography. For example, a marine mollusk (e.g. oyster) test is currently a data requirement for pesticide registration in the United States, but the test guideline and endpoints are not aligned with the data requirements in the European Union. These discordances may lead to questions regarding the coverage of mollusks in the aquatic risk assessment. Therefore, the goal of this project was to compare sensitivities of oysters to standard aquatic regulatory test species to better understand if this taxon is covered in the existing risk assessment framework for pesticide active substances. Oyster acute toxicity data for pesticide active substances were obtained by searching USEPA's ECOTOX Knowledgebase and regulatory documents. Acute toxicity data for fish and invertebrates were then acquired from reliable regulatory sources for each substance to match with the oyster data, and sensitivity ratios (fish or invertebrate endpoint / oyster endpoint) were calculated. Results demonstrate that oysters are similarly or less sensitive (within a factor of 3) compared to the standard fish or invertebrate test species for ~75% of substances in this dataset. For ~25% of the substances, oysters were more sensitive than the standard acute fish and invertebrate test species. When considering pesticide indication, 40%, 18%, and 13% of fungicides, herbicides, and insecticides respectively have substances with sensitivity ratios > 3. Of the substances with a sensitivity ratio > 3, approximately 60% have oyster endpoints that are less than a factor of 10 below the standard test species. These preliminary results suggest that oysters would likely be covered in many global aquatic risk assessment frameworks, particularly in Europe with the standard of assessment factor of 100. Future work will involve investigation of the ~25% of pesticides with a sensitivity ratio >3, including checking reliability of data, additional endpoints, and potential refinements. Overall, this data analysis will help to better inform all regulatory authorities on how to address potential risks to taxa currently not covered in risk assessments.

2.02.T-03 Mechanistic insights into phenanthrene-induced atrioventricular conduction block in fish embryos

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Some of polycyclic aromatic hydrocarbons (PAHs) are common aquatic environmental contaminants that can induce cardiotoxic effects during early vertebrate development. Phenanthrene (Phe), one of PAHs, is commonly detected at relatively high concentrations in the oil-contaminated aquatic environments. While Phe is known to disrupt cardiac electrophysiology, its toxic mechanisms remain unclear yet, especially regarding metabolic activation. In this study, we investigated the symptoms for cardiotoxic effects of Phe, and the relationship with metabolism with cytochrome P450 (CYP) enzymes in Japanese medaka (*Oryzias latipes*) embryos. Embryos were exposed to a few concentrations of Phe from 0-day post fertilization (0 dpf) through early development (n=3, with each replicate consisting of 10 embryos). At 7 dpf, the heartbeat was observed under a microscope. Notably, 12.5 μ M of Phe-exposed embryos exhibited a significant reduction of ventricular beating rate to 0.934 ± 0.07 beats per second (bps), compared to 1.93 ± 0.13 bps in vehicle group (DMSO), which is a characteristic

symptom of 2:1 atrioventricular (AV) conduction block. Importantly, AV block occurred in 100% of embryos in this exposure group. Owing to assessing whether CYP-mediated metabolism contributes to this phenotype, we co-exposed embryos to Phe and piperonyl butoxide (PBO) known as CYP metabolism inhibitor. Co-exposure of Phe (12.5 μ M) + PBO (7.0 μ M) significantly increased ventricular beating rate to 1.43 ± 0.10 bps compared to Phe (12.5 μ M) alone (1.03 ± 0.09 bps) and markedly reduced the incidence of the AV block ($16.7 \pm 4.71\%$). These results suggested that metabolic activation of Phe via CYP enzymes may be responsible for the observed cardiotoxicity. To further investigate the contribution of Phe metabolites to cardiotoxicity, we exposed medaka embryos at 4 dpf for 24 hr to 12.5 μ M of Phe and its representative CYP-mediated metabolites; 1-hydroxyPhe, 3-hydroxyPhe, and 9-hydroxyPhe. Remarkably, only 1-hydroxyPhe exposure induced AV block, highlighting its potential role as a toxic metabolite responsible for disrupting cardiac rhythm. Although the Phe exposure concentration used in this study are higher than those typically found in the environment, our findings show mechanistic insights into how CYP-mediated metabolism of Phe may contribute to Phe-induced cardiac toxicity and provide fundamental data for future environmental risk assessments.

2.02.T-04 Acute and chronic toxicity of ammonia on the endemic freshwater mussel *Cambarunio nebulosus* (Alabama Rainbow)

Ellison Nabi¹, Sean Parham¹, Andrew Barrick¹, Paul Johnson² and Tham C. Hoang¹, (1)Auburn University, (2)Alabama Aquatic Biodiversity Center

Alabama is known for its freshwater species abundance and high levels of endemism. Of the species present in Alabama, $\approx 74\%$ of freshwater mussels are considered imperiled and over 65 species are federally protected, and 138 species are state conservation targets. Freshwater mussels are among the most imperiled aquatic organisms nationally, and understanding sensitivity to anthropogenic perturbations is essential. Agriculture runoff is a major anthropogenic stressor in Alabama, among them ammonia exposure is poorly understood. This study researched the acute and chronic toxicity of ammonia to *Cambarunio nebulosus*, a mussel imperiled and currently under review to determine if it warrants protection under the US Fish and Wildlife Service, Endangered Species Act. Little formal ecotoxicological testing has been completed for *C. nebulosus*. Two acute toxicity tests were conducted using two different age groups consisting of 100-day-old and 220-day-old mussels to determine lethal concentrations (LC). A chronic toxicity test was also performed over 45 days using a continuous flow-through system, as ammonia is transient in suspension. Total lipid, protein, and glycogen concentrations were investigated to determine if ammonia exposure influenced energetic reserves. Results of acute exposure indicated younger mussels were more sensitive to ammonia, with an LC₅₀ of 8.38 mg/L NH₃ (95% CI: 7.38-10.16 mg/L) compared to 12.55 mg/L NH₃ (95% CI: 11.031-14.45 mg/L) for adult mussels. Complete mortality was observed only at the highest concentration of the chronic exposure test. The 45-d LC₅₀ was 0.84 mg/L NH₃ (95% CI: 0.79-0.89 mg/L), which is approximately 15 times more toxic than the acute exposure to the older mussels. Results of sublethal endpoints demonstrated that glycogen, lipids, and protein displayed a bilinear relationship with increased concentrations at low ammonia exposures and decreased concentrations at higher ammonia exposure. Ammonia is a source of nitrogen and is known to promote algal growth, which may explain this observation. The results highlight the nuanced relationship in ammonia exposure, highlighting unauthorized agricultural discharge/runoff can impact Alabama endemic species. Further research is needed to improve our understanding of metabolic costs associated with ammonia and other local stressors that influence *C. nebulosus*. metabolic costs of ammonia and other local stressors.

2.02.T-06 Tracing chemical pollution from inland bays to coral reefs: Investigating distribution and ecotoxicological impacts

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While water quality is one of the major drivers in coral reef health, the effects of complex chemical mixtures on

coral reef community structure and functioning are poorly understood. This knowledge gap limits the ability to identify pollution sources, assess ecotoxicological risks, and prioritize effective, site-specific conservation and restoration efforts. To address this, we tailored state-of-the-art water quality assessment methods to tropical coral reef environments. We investigated the occurrence and dispersal of anthropogenic chemicals and the potential ecotoxicological impacts of environmental mixtures along a bay-to-reef gradient in the Southern Caribbean during the wet and dry seasons. Polar organic chemical integrative samplers (POCIS) were deployed with in situ flow monitoring to obtain time-integrated chemical mixtures from inland bays and adjacent coral reefs. Target analysis of anthropogenic contaminants of emerging concern (CECs), including pesticides, industrial chemicals, and pharmaceuticals, elucidated the concentrations and distribution of pollutants across sites. Most targeted CECs were detected at quantifiable concentrations, with clear spatial and seasonal patterns. Inland bays near urbanized areas showed elevated concentrations of industrial chemicals and pharmaceuticals (e.g., benzotriazole, naproxen), likely reflecting domestic wastewater inputs. Higher concentrations of pesticides (e.g., DEET) were detected during the wet season, consistent with increased runoff and seasonal use. Reef sites located farther from land-based influences had lower chemical loads. Some detected concentrations exceeded environmental benchmarks (e.g., PNECs), highlighting ecological concern. Potential ecotoxicological effects are currently investigated using a battery of in vitro assays for toxic modes of action and in vivo assays with marine fish, invertebrates, and microorganisms to quantify the toxicity of the environmental mixtures to marine organisms. This integrative approach – linking calibrated passive sampling, chemical analysis, and effect-based methods – demonstrated that land-based human activities introduce mixtures of bioactive CECs onto coral reefs. Additionally, it provides a framework for assessing water quality and mixture toxicity in tropical coral reef environments. The findings will help advance investigations into the role of chemical pollution as a co-stressor in coral reef conservation and management strategies.

2.02.V Assessing Toxicity in Water and Aquatic Sediments

2.02.V-01 A Comparative study of copper toxicity in a Eurasian and South American amphibian

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The worldwide distribution of amphibians provides the opportunity to employ the most suitable local species as representative of ecosystem peculiarities in different regions. *Bombina orientalis*, a native species in Turkey, is also broadly distributed in other European and Asian countries, while *Rhinella arenarum* is distributed in several South American countries. This contribution reports, employing a Cu toxicity study, the susceptibility of both species in the embryonic and larval stages. 10 embryos and larvae of *Rhinella arenarum* and *Bombina orientalis* in triplicate were maintained in 40 mL of amphitox solution for acute (96hr) and short-term chronic (7 days) studies. For embryos, the LC50 for 96 hr and 7 days were 0.137 and 0.150; 0.019 and 0.001 mg/L of Cu²⁺, respectively, while for larvae were 0.051 and 0.085; 0.048 and 0.042 mgCu²⁺ respectively. Thus, embryonic stages are a bit more sensitive to copper than larvae. The LC90, 50, and 10 were plotted as Toxicity Profile curves from 24hr of exposure onwards, pointing out that Cu²⁺ toxicity increases sharply after 48 hr of treatment and then gradually, while the distance value between the LC10 and LC90 also became gradually minimal. This fact reveals that in the case of copper, NOEC values for chronic exposures could be very close to the LC10. As sub-lethal effects, Cu²⁺ causes delayed development, reduced growth, shedding of epithelial cells, edema, blisters, axial abnormalities, tail flexures, and wavy tail fins, both in embryos and larvae. TI values for embryo toxicity tests were calculated as 2.06. As the main neurological impairment, the experimental animals exhibited poor swimming ability. It can be concluded that amphibians from both continents exhibit similar sensitivity to Cu²⁺, providing the possibility to report from acute to chronic lethality as well as teratogenesis, neural disorders, among other potential endpoints. AMPHITOX was successfully employed for hazard and risk assessment for different environmental samples from surface water to leachates (Herkovits and Perez Coll, 2003), providing a low cost and scientifically robust method for surface water management, critical

to achieve the United Nations' 17 Sustainable Development Goals (SDGs) specially in highly impacted region by anthropogenic activities in Euro-Asia and South America.

2.03.P-We Ever-Present but Unknown Consequences: Sources, Fate and Transport, and Effects of Microfiber Pollution

2.03.P-We-014 Environmental fate and chemical profile of microfibers from cigarette filters

Alexa Fields, Margaret Stack, George Youssef, Natalie Mladenov and Eunha Hoh, San Diego State University

Cigarette filters are the most common littered item globally. The filters are composed of cellulose acetate, a kind of non biodegradable plastic that breaks down into microfibers when exposed to environmental conditions. Microfibers are considered especially hazardous due to their shape, which allows them to be internalized by organisms more readily than other microplastics. The current research is assessing what chemicals leach from cigarette filters under four different exposure conditions: smoked or unsmoked and light exposed or unexposed to light. The three filter types are from smoked cigarettes, unsmoked cigarettes, and pure cellulose acetate sheets (control). Nontargeted chemical analysis was conducted using two dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC/TOF-MS) to fully compare chemical profiles in the leachate and on microfibers from the exposure conditions. Cigarette filters were photoirradiated in a solar simulator for 6 hr to simulate the full solar spectrum at the elevation and latitude and longitude of San Diego, CA. Smoked cigarette filters were prepared at the University of California, San Francisco using a TE10z smoking machine operated according to ISO Standard 3308:2000. Marlboro Red cigarettes were machine smoked for 10 puffs and extinguished in dry ice before storage at −20 °C until further analysis.

Chromatographic features were compared using hierarchical statistical analysis (e.g., heatmaps) and categorized as persistent, degraded, or newly formed following sunlight exposure. Features were further grouped based on smoked versus unsmoked status. Identified features underwent compound identification through mass spectral similarity within the NIST database. A hazard analysis will be conducted for identified compounds using toxicity data (human and ecological), along with their physical and chemical properties. Results from this study will help understand environmental impacts of cigarette filters as one of most common forms of litter to ecosystems, wildlife, and human health.

2.03.P-We-015 Laundry dryers as a source of microfibers in stormwater runoff

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Microfiber plastics are one of the most abundant types of microplastics in most environmental matrices, including stormwater and surface waters. From a comprehensive regional microplastics investigation in 2019, microfibers were found to make up 39% of the estimated 7 trillion microplastics discharged to San Francisco Bay per year through stormwater. These findings prompted an investigation into the possible sources of this microfiber pollution. Clothing is a known source of microfibers, however, the transfer of microfiber shedding from clothing to the environment is still being investigated. This study evaluated laundry dryers as a source of microfiber discharge in the San Francisco Bay Area. Materials vented from household laundry dryers and a larger laundry facility were captured using a 100 µm nylon mesh and tested for microplastics. Each sample consisted of a single load of laundry run through a wash/dry cycle to completion. The mesh was weighed and subsampled for individual fiber analysis using FTIR spectroscopy. Laundry load and habit data were gathered from the individual household, and a general laundry habits survey was conducted through SFEI's social media network, collecting basic information about individuals' laundry habits and dryer use in the San Francisco Bay Area. From this information, a model of microplastic fiber discharge from household and laundry facilities can be constructed, which provides an estimate of the amount of microfibers discharged from laundry dryers under normal household use. Sample analysis is still ongoing, results will be presented. The San Francisco Bay Area has a population of over 7 million people. A small amount of vented material per dry cycle from an individual household, when aggregated across this population, can add up to several tons of both natural and synthetic

microfibers discharged per year. **Disclaimer/Disclosure:** Awarding agency: California Natural Resources Agency Award Number: C0223003 Subaward Number: 706386

2.03.T Ever-Present but Unknown Consequences: Sources, Fate and Transport, and Effects of Microfiber Pollution

2.03.T-01 Detergent-mediated reduction of fiber fragment emissions during conventional machine laundering of textiles and garments

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Synthetic textile fiber fragments (sFFs) from laundering (washing and drying)—also known as synthetic microfibers—are microplastics contaminating environmental biota, ecosystems, and human food supplies. Reducing sFF emissions is a global concern, but there are few source reduction options. sFF emissions vary by fixed factors such as the type of garment edge treatment, the type of fiber or fabric (e.g. staple vs. filament, or surface treatment such as fleece), washing machine type, water conditions, and drying conditions. However, detergent effects are less studied and, while using any detergent—especially powder—may increase sFF emissions, liquid detergents formulated to reduce sFF emissions remains unexplored. Here, we report a novel “low shed” detergent’s effects on sFF mass emissions, from 2 studies. First, 4 institutions washed each of 4 fabric types using either a conventional detergent or a novel (low shed) detergent, finding that the latter decreased sFF mass emissions despite institutional—operational and methodological—differences. The masses of sFFs per mass of textiles averaged, for each of 4 institutions, 0.08 ± 0.06 , 0.07 ± 0.07 , 0.05 ± 0.04 , and 0.08 ± 0.04 g/kg when using the novel detergent, versus 0.23 ± 0.13 , 0.16 ± 0.11 , 0.14 ± 0.05 , and 0.11 ± 0.05 g/kg for the conventional detergent. Despite multiple fixed differences in washing conditions across the institutions, the sFF shedding amounts significantly differed by detergent. Second, for studies at 1 institution, textile fiber fragment (FF) mass emissions from laundering whole garments comprised of mixed synthetic and cotton fibers were also comparatively decreased with the low shed detergent during washing, wherein the novel detergent resulted in significantly less FF (0.37 g/kg) than the conventional detergent (0.50 g/kg; Wilcoxon test, $p=0.02$, $n=8$). Although whole garment FF masses captured from the machine dryer (lint trap plus dryer exhaust) did not vary by antecedent detergent (0.50 and 0.49 g/kg, using the novel versus conventional detergent, respectively), the overall garment laundering process across washing and drying emitted relatively decreased FF masses with the low shed detergent (0.87 g/kg) compared to the conventional detergent (0.99 g/kg, $p=0.02$). Altogether, the study results demonstrated that detergent type could be an important factor in determining the release of sFFs and FFs during laundering, with a possible way to reduce the release being intentional detergent formulation.

2.03.T-02 Investigating suspension-feeding invertebrates as bioindicators of microfiber pollution

*Kayla Mladinich, Bridget Holohan, Sandra Shumway and **J. Evan Ward**, University of Connecticut*

Suspension-feeding animals interact with microplastics of different shapes and sizes suspended in the water column. Bivalve molluscs have been suggested as potential bioindicator species for microplastics as they are known to consume plastic particles in the natural environment, are widely distributed, sessile, and easy to collect. However, these molluscs are selective suspension feeders and, thus, do not consume all particles to which they are exposed. This study investigated how three less discriminate suspension feeders, the Atlantic slipper snail *Crepidula fornicata* (gastropod), the sea grape *Molgula manhattensis* (tunicate), and the rough sea squirt *Styela clava* (tunicate) interact with microfibers of different sizes and polymer types to determine their suitability as bioindicator species. Data from the study were compared to that of previous experiments with oysters (*Crassostrea virginica*) and mussels (*Mytilus edulis*). Animals were offered aged polyester or nylon microfibers of different lengths, or nylon and polyester microfibers of similar lengths during 2-h exposure

periods. Pseudofeces (material rejected prior to ingestion) and feces were collected during and after the exposures and the microfibers within enumerated. Results revealed that slippersnails and sea grapes both exhibited size-based rejection of nylon fibers, rejecting longer fibers in higher proportions. Polymer type did not influence ingestion or egestion of fibers. Sea grapes were more indiscriminate feeders when compared with slippersnails, oysters and mussels, but egested between 45% and 75% of fibers within 3 h. Although sea grapes rejected proportionally fewer microplastics than slippersnails or bivalves, this species would not make a robust bioindicator because it does not ingest all microfibers it encounters and egests a large proportion of the fibers quickly. Preliminary data for the rough sea squirt indicate that this species ingests > 90% of both microfiber types and egests <10% of polyester fibers within 3 h. Rough sea squirts should be investigated further as a candidate for biomonitoring purposes. **Disclaimer/Disclosure:** Funding provided by Connecticut Sea Grant Program and the Long Island Sound Study Research Grant Program

2.03.T-03 Interactive effects of microfibers and elevated temperature on an Alpine *Daphnia* species

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Plastic microfibers (PMFs) are the most prevalent microplastic pollutant in remote ecosystems, with disproportionate deposition in high-elevation regions. At the same time, alpine regions are rapidly warming, rendering ecosystems within them particularly sensitive to global change. Despite this, the interactive effects of PMF pollution and climate-driven warming on resident alpine species remain poorly understood. Therefore, we ask: What are interactive effects of PMFs and warming temperatures to organisms within these vulnerable ecosystems? This study investigates the combined impact of temperature and PMF exposure on *Daphnia pulicaria*, a dominant zooplankton species collected from an alpine lake. *Daphnia* are a keystone genus in freshwater food webs and are model organisms for ecotoxicity testing; however, alpine-adapted populations remain largely unstudied. Notably, *Daphnia*'s asexual reproduction may enhance resilience to stress, including adaptation to low nutrients and broad temperature ranges. However, PMF pollution may act as an additional stressor that impacts the performance of adaptive and resilient behavior. We conducted a 21-day chronic reproductive toxicity assay using neonate *D. pulicaria*. Organisms were exposed to a PMF mixture at concentrations of 0, 10, 50, 100, and 1000 particles/mL under three summer lake temperatures based on long-term data: median lake temperature, highest observed lake temperature, and a projected extreme. The PMF mixture reflects environmentally relevant polymer type, size range, and morphology. A factorial experimental design was used to examine interactive effects on survival, reproduction, development, and behavioral endpoints across treatments. Heat Shock Protein expression was measured at the end of the experiment as a biomarker of sublethal stress. We hypothesize that elevated temperatures will exacerbate PMF toxicity, such that doses non-toxic at ambient temperatures become harmful under warming scenarios. These results will help elucidate the compound effects of climate and pollutant stressors on alpine *Daphnia*. By incorporating realistic environmental exposures and regionally adapted organisms, this study addresses the importance of context-specific ecotoxicological research. Our findings aim to improve forecasts of ecological impacts under global change, and to highlight how species' life-history strategies shape their capacity to withstand interacting stressors in rapidly changing environments.

2.03.T-04 Unraveling the combined effect of acidification and microfibers from textiles on vital prey species

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Microfibers are the most common microplastic morphology found in environmental samples. Often originating from textiles, microfibers include synthetic polymers such as polyester, however, natural fibers (i.e. cotton) are just as pervasive. Microfiber toxicity is complicated by fiber type, chemical additives (e.g. dyes and softeners) used during textile production, and subsequent weathering in the environment. These factors may interact in complex ways to affect organisms, especially in marine systems which are already impacted by climate change

stressors such as ocean acidification. Understanding microfiber toxicity, especially in conjunction with climate change, is essential for assessing ecological risks, and informing sustainable textile production practices. To address these variables, we are investigating microfiber toxicity in both field-collected (*Holmesmysis sculpta*) and laboratory (*Americamysis bahia*) mysid shrimp. *H. sculpta* are vulnerable to climate change with decreasing populations and densities having implications for prey quantity in the food webs they support, such as gray whales. We hypothesize that *H. sculpta* will have increased sensitivity to microfiber exposures, effects will be most evident in the most processed textile treatments, and effects may be exacerbated by ocean acidification conditions. We exposed *H. sculpta* to weathered polyester treated with commercial softener for 48 hr, and they exhibited hypoactivity. To examine the different complexities of microfiber toxicity, we exposed juvenile laboratory estuarine model mysid shrimp, *A. bahia*, to polyester, cotton, or dyed cotton that was either untreated or treated with a commercial softener or eco-softener. After a 7-day exposure, mysids underwent an acute extreme ocean acidification (low pH) trial before they were analyzed for changes in growth, behavior, and physiology. Initial findings from the polyester exposure indicate in non-weathered treatments, toxicity may be driven by chemical additives while weathered treatments may be driven by morphology evidenced by hypoactivity, similar to the hypoactivity observed in the marine mysid, *H. sculpta*, in these treatments. To connect our laboratory model, we are conducting 48h microfiber exposures using *A. bahia*. Together, these results will allow us to assess how microfibers and climate change may impact the quality of a vital prey species and unravel the variables associated with microfiber toxicity and textile production.

2.03.T-05 Inhalable flame retardant polyester fibers as a potential driver of immunomodulation in asthma

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Dust is a major source of human exposure to plastic polymers, which mainly contain polyester fiber. The human nasal cavity is a potential deposition site for microplastics, particularly polymer fibers. Laboratory studies indicate that micro- and nano-sized fibers can be released due to mechanical stress and UV light, with ongoing research showing that polyester fibers can degrade from larger to smaller sizes under UV exposure. It is essential to understand the vulnerability of the respiratory system to polymer fiber, particularly among vulnerable groups such as young children and people living with chronic respiratory conditions such as asthma. The objective of this study is to evaluate the vulnerability of the respiratory system and the alteration of allergic inflammation in response to intranasal exposure to polyester fibers by studying an asthmatic house dust mites (HDM) in vivo model. In our analysis of flame-retardant polyester baby clothing, we identified polyester fibers measuring less than 100 nanometers in diameter. These fibers were studied alongside exposure to HDM in a murine model. The co-exposure resulted in significant changes in the allergic immune response, particularly regarding eosinophil counts in bronchoalveolar lavage fluid. Proteomic analyses showed a notable increase in specific proteins in the lungs of mice co-exposed to polyester fibers and HDM compared to the control group and those exposed only to HDM. The distribution of differentially expressed proteins indicated significant activation related to the negative regulation of endopeptidase and peptidase activity, which are crucial for the maturation of granule proteins and the survival of eosinophils. Our understanding of nanoplastic exposure from secondary sources and its environmental cycle is still in its early stages. The outcomes of this project illuminate the often-underestimated environmental determinants that can alter asthma pathophysiology, especially among vulnerable populations like young children. **Disclaimer/Disclosure:** The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Funding for this research was provided by the Center for Metals in Biology and Medicine P20 GM130422, the Center for

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

2.04.A.T-01 How do different plastic polymer types impact the periphyton dynamics of coastal stormwater ponds?

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Stormwater ponds are common best management practices (BMPs) used in residential developments to protect water quality. These ponds trap stormwater runoff and reduce the load of environmental pollutants, such as plastics and microplastics (< 5 mm), from reaching local waterways. While periphyton has been widely used by aquatic scientists as a biological indicator for water quality, little is known about how plastic pollution impacts periphyton dynamics. To fill this research gap, we measured the impacts of three types of plastics (tire wear particles, polystyrene foam, and polypropylene geotextile) on the periphyton dynamics in residential stormwater ponds throughout coastal South Carolina. Matlock passive diffusion periphytometers housing bottles containing different treatments of plastics were deployed in low- and high-density residential stormwater ponds in the summer of 2025 to measure the periphyton community response through chlorophyll-a concentration. The goal of this study was to evaluate how plastics impact periphyton communities to evaluate periphyton's capacity to be used as a biological indicator for plastic pollution. The findings from this study will be used to create recommendations for stormwater pond management practices and improving water quality analysis.

2.04.A.T-02 Discovery of microplastics in the Alabama River: Distributions, transport and loads, sources and relative comparison with world rivers

Safeerul Islam Hashmi, Andrew Barrick and Tham C. Hoang, Auburn University

Rapid increase in plastic pollution has received increased attention in the present century. Plastics enter aquatic systems through multiple routes and remain there for hundreds of years. Overtime, plastics are converted into microplastics (MP), particles less than 5 mm in three dimensions, due to the microbial activity and environmental weathering. The scope of MP contamination in the biosphere is poorly understood. The aim of this study was to investigate MP contamination in the Alabama River. Results showed that MP concentrations in the Alabama River are comparable with MP concentrations in world polluted rivers. The study revealed that MPs in the Alabama River likely did not travel long distances and sedimentation on the riverbed may be a major sink for MPs. Polyethylene terephthalate (PET) was the most abundant polymer (21.7%) found in samples whereas polymethyl pentene (PMP) was 16.1%, polypropylene (PP) (13.8%), polyvinyl chloride (PVC) (12.4%), polybutylene terephthalate (PBT) (12%), acrylonitrile butadiene styrene (ABS) (6.7%), and polyethylene (PE) (5.3%) on average. It was estimated that hundreds of billions to trillions of MPs enter Mobile Bay per day. This poses potential exposures and risks to aquatic life in the Bay and the Gulf of Mexico. Microplastic concentrations were quantitatively correlated with the watershed drainage into the river and human pollution suggesting contributions of human activities to MP contamination. The present study provides a first look at MP contamination in the Alabama River. Continuous monitoring of MP and ecotoxicity studies are needed to investigate ecosystem consequences of MPs in the Alabama River.

2.04.A.T-03 Characterization and distribution of microplastics in Biscayne Bay: Abundance, composition and tidal influence

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Microplastic pollution in coastal areas is attracting increasing global concern due to the far-reaching effects it poses on humans and the biota. Biscayne Bay, a subtropical estuary serving as a buffer between canals and the

ocean, is potentially a sink for microplastics. This study attempts to comprehensively determine the identity and abundance of microplastics in Biscayne Bay, combining optical microscopy and FTIR/Raman spectroscopy. Surface water samples were collected using a plankton net from 17 sites within the Bay. Samples were processed using commonly accepted microplastic techniques of filtration, digestion using hydrogen peroxide, and density separation using potassium formate. Particle counting was done using bright field microscopy, while identification was done using a Shimadzu AIRsight combined Infrared/Raman microscope. The effect of tidal movement on the abundance of microplastics will also be investigated by sampling during the high and low tides and making a comparative analysis. Our preliminary results revealed the presence of microplastics with a density below 1.4 g/cm³, with abundances ranging from 2.99-29.4 pcs/m³. Polyacetylene (25%), polyethylene (24%), and polypropylene (12%) were predominantly identified across the Bay's samples, and the predominant particle sizes ranged between 0.3-1.0 mm. The relative abundances generally decrease between sites from the northern portions of Biscayne Bay to the southern portions of it, consistent with the reduction of urbanization. The findings of this study provide valuable information about the composition and properties of microplastic fluxes into Biscayne Bay from inland anthropogenic sources while highlighting areas of elevated microplastic accumulation within the Bay. Finally, the findings of this study will contribute to the efforts of policymakers, government, and regulatory agencies to conduct environmental risk assessments and implement management strategies within Biscayne Bay.

2.04.A.T-04 Microplastics assessment in the digestive tract of white sharks

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Microplastics are one of the most common forms of pollution found in marine environments, and their small size (<5 mm in diameter) allows them to be easily ingested by marine biota. Sharks are sentinel species in the ocean, yet studies characterizing microplastic ingestion in sharks are scarce. The objectives of this study were to describe and quantify microplastics in the feces of white sharks (*Carcharodon carcharias*). Samples were obtained from a total of 35 white sharks, including 7 young-of-the-year, 17 juveniles, 6 subadults and 5 adults inhabiting the Western North Atlantic. Microplastics were isolated, analyzed with a stereomicroscope to determine size, shape, and color, and characterized by polymer type using Fourier transform infrared (FTIR) spectroscopy. Microplastics were detected in all fecal samples, indicating that ingestion is occurring in these sharks. There was an average of 7.7 ± 4.4 microparticles and 2.0 ± 1.1 microplastics in each sample. No significant differences in the number of microplastics were detected among age class, sex, or collection location. Rayon and cellulose were the most abundant microparticle polymers and polyester, polypropylene and nylon were the most abundant microplastic polymers. White, black, and blue were the most abundant colors of microplastics. This research contributes much needed data from a top-level predator in marine ecosystems. Assessing microplastic content in the feces of white sharks can be indicative of environmental contamination and provide insight into the potential risk to these organisms and other consumers.

2.04.A.T-05 Screening-level risk assessment of microplastics from offshore infrastructure coatings

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Microplastics (MPs) generated from the degradation of coatings on submerged offshore infrastructure represent a novel and understudied exposure pathway in marine environments. This study evaluated potential environmental risks associated with MPs released from Fusion Bonded Epoxy (FBE) coatings in a temperate continental shelf setting. A site-specific conceptual model was developed to describe MP generation, transport, settling, and fate in both water and sediment compartments. MP release was linked to hydrodynamic energy and sand transport. Simulations were conducted under representative nearshore and mid-depth conditions using a first-order generation model developed by Curtin University. Transport and deposition were evaluated using sediment transport frameworks incorporating current and wave data. Due to their relatively high density, FBE-

derived MPs exhibited appreciable settling potential. Even under conservative assumptions, predicted concentrations remained well below derived screening levels: 16 µg/L for water (based on species sensitivity distributions and chronic NOECs) and 1,100 mg/kg for sediment (based on EC10s for sensitive benthic invertebrates). Potential removal or redistribution of MPs through biofiltration by epibenthic filter feeders was also evaluated. Although localized uptake and sediment delivery may occur, overall impacts on modeled concentrations were minor and did not alter risk conclusions. These results indicate that MPs released from FBE coatings on offshore infrastructure do not pose an appreciable risk of exceeding conservative screening benchmarks in water or sediment. While episodic high-energy events may drive short-term increases in MP release, predicted exposures remain below levels of concern. Ongoing refinement of toxicity thresholds and improved characterization of long-term coating degradation will support future risk assessments.

2.04.A.T-06 Microplastics aquatic life criteria review and development for regulatory decision making

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Each year there are hundreds of papers on the potential effects of microplastics (MP) to aquatic life, and there is a critical need to understand how these studies will inform on aquatic life criteria that could be in development by US Federal, State, and International regulatory organizations. The Southern Coastal California Water Research Project developed the Toxicity of Microplastics Explorer (ToMEx, ToMEx 2.0), an open-source living database on MP effects on humans and aquatic organisms (Hampton et al., 2022; Hampton et al., in progress), and this tool has been used to develop aquatic life criteria now being applied to evaluate surface water in California and the Great Lakes. The goal of this review is to evaluate the information currently available within ToMEx 2.0 with regards to the quality and abundance of data typically used for regulatory aquatic life criteria for toxic chemicals. The first task evaluated if there is sufficient high-quality data available to derive thresholds; quality criteria included studies with standard criteria endpoints relevant to growth, survival, and/or reproduction, studies that pass ToMEx reliability quality criteria, and reported effect sizes relevant to a population (i.e. $\geq 20\%$ reduced growth, survival, reproduction). We identified 36 papers meeting criteria and conducted a quality review on these. The second task curated data and extracted benchmarks (NOEC, LOEC, EC/LC50) and calculated additional benchmarks (EC/LC10, EC/LC20, etc). We identified 61 NOECs and LOECs and 40 unbounded NOECs from freshwater and marine studies and 28 unbounded NOECs and 4 LOECs for sediment studies. The third task is to develop freshwater and marine water criteria. Initial results indicate that criteria developed using typical regulatory criteria derivation methods are orders of magnitude higher than values that can be developed using the species sensitivity tools embedded with ToMEx. This is because much of the underlying data is not suited (or intended) to be used for criteria for regulatory decision making. At this time, we recommend that regulatory criteria derivation be completed in a detailed review process rather than using the embedded species sensitivity tools. ToMEx should not be used as a shortcut for rigorous regulatory criteria derivation; however, its best uses are as a helpful starting point for compiling resources for criteria review and a resource for academic researchers planning their own ecotoxicological research. **Disclaimer/Disclosure:** Funding for this project was provided by the Foundation for Chemistry Research and Initiatives.

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

2.04.B.T-01 Dissecting microplastic toxicity: Biomarker and cellular responses in freshwater fish models

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Consumer-derived microplastics (MPs) contain complex mixtures of polymers and chemical additives that are more environmentally representative and potentially more biologically active than pristine MPs typically used in laboratory studies. As plastics break down in freshwater ecosystems, MPs may become more biologically available, increasing the overall risk to aquatic organisms. This study assessed the ecotoxicological effects of

cryomilled red and green plastic cup fragments (<120 µm) on fathead minnow (*Pimephales promelas*) larvae and PLHC-1 hepatic cells (*Poeciliopsis lucida*) and focused on sublethal responses associated with oxidative stress, endocrine disruption, and cellular energetics. Larvae (<48 h old) were exposed to 10 µg/L–10 mg/L MPs for 48 h. Accumulation of MPs in fish increased with test concentration with no differences observed due to color. All MP concentrations significantly reduced catalase (CAT) activity (0.5 ± 0.1 to 1.1 ± 0.4 vs. 5.7 ± 0.86 nmol/min/mg in controls), impairing hydrogen peroxide breakdown. Glutathione (GSH), a key antioxidant, declined at ≥ 5 mg/L, while glutathione peroxidase (GPX) and TBARS increased, indicating oxidative damage and lipid peroxidation. Red MPs caused more pronounced oxidative stress responses. Green MPs elevated superoxide dismutase (SOD), GPX, and TBARS, with decreased CAT and GSH, suggesting composition-dependent mechanisms. Thyroid hormones (T3, T4) increased at 5–10 mg/L in both treatments; green MPs also elevated TSH, indicating endocrine disruption. No changes in ATP nor calcium concentrations were observed. PLHC-1 cells mirrored these oxidative trends and showed MP aggregation in live-cell imaging. These findings underscore the toxic potential of real-world MPs. **Disclaimer/Disclosure:** The authors declare no conflict of interests. This work was supported in part by funds from the ONE-URC University Research Committee Awards from the Vice Provost for Research and the C. Gus Glasscock, Jr. Endowed Fund of Excellence in Environmental Sciences at Baylor University.

2.04.B.T-03 Chronic effects of environmentally realistic microplastics on *Daphnia magna*

Safeerul Islam Hashmi, Andrew Barrick and Tham C. Hoang, Auburn University

Daphnia magna is a cladoceran species found in the freshwater systems. As a primary consumer, *D. magna* plays a significant role in ecosystem function and has been historically used to characterize the hazards in a regulatory context, especially with contaminants of emerging concern such as microplastics. Much of microplastic ecotoxicity has focused on standardized microspheres which are only a small subset of microplastics found in the environment. This study emphasized identifying the effects of three different environmentally representative microplastics: nylon fibers, Kevlar fibers, and heterogenous polystyrene fragments. The aim of the study was to determine ecotoxicity using *D. magna* through 21 days long chronic exposure test. Five test concentrations were used along with a control (with no MP added). All three microplastic types did not influence mortality. The reproduction rates (in higher treatments, i.e., T5) for nylon exposure were significantly different from control whereas no such effect was observed when *D. magna* were exposed to kevlar and polystyrene fragments. Dry weights were found to be significantly less than control for nylon experiments whereas for cup particles, the dry weights were significantly higher than the control showing a potential for the accumulation of cup particles inside the organisms' bodies. Ingestion patterns followed an exponential trend with higher concentrations resulting in higher intake/consumption. This study highlighted the possible effects of MPs on *D. magna* and emphasize on the need of further investigating the effects caused by the exposure of different MP types.

2.04.B.T-04 UV Weathering of a USA-based micro and nanosized tire particle mixture: Ingestion and growth inhibition in larval fish and juvenile mysid shrimp

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Tire erosion during driving is the second major contributor to secondary microplastics entering our oceans. Tire particles (TPs) consist of a diverse array of components that vary depending on the tire type, which can influence their potential toxicity. However, most toxicological studies have predominantly focused on a single tire type. In this study, we used a cryo-milled tire tread (CMTT) composite provided by the U.S. Tire Manufacturers Association. The composite was a representative mixture of what may be in our environment and contained three types of tires in proportions that are estimated for the U.S. (41% passenger car tire, 14% light truck tire, 45% truck/bus tire). We studied how both pristine and weathered tire particles and leachates, affected

behavior and growth in two model estuarine species: the Inland Silverside (*Menidia beryllina*) and the mysid shrimp (*Americamysis bahia*). We exposed 5-day post-fertilization fish embryos and 7-day-old mysid shrimp to micro (1–20 μm) and nano (<1 μm) TPs at four concentrations (10, 100, 1000, and 10,000 particles/mL), as well as to 100% leachate. Behavioral assays were conducted using a DanioVision Observation Chamber (Noldus, Wageningen, Netherlands), where each organism was exposed to a dark-light cycle stimulus. Growth was measured using the index $W/L\Delta d$, where W is the organism's width, L is its length, and d is the number of exposure days. Organisms were cleared using clearing reagents to allow visualization and counting of internalized particles. Our findings confirm the internalization of TPs by both species and demonstrate a positive correlation between tire particle concentration and ingestion rate. Both species exhibited a significantly higher ingestion rate of weathered TPs. Effects on growth were species-specific, with weathered TPs having the strongest impact. Behavioral alterations also varied by species, with weathering amplifying the toxicity of TPs in *A. bahia*, but not in *M. beryllina*. Overall, this study highlights the broader environmental impact of mixed tire particles compared to particles from a single tire type. It emphasizes the need to move beyond testing only individual tire types and to include multiple species in toxicity assessments to better capture species-specific responses. **Disclaimer/Disclosure:** The authors declare that financial support was received for the research, authorship, and/or publication of this article. This research was funded by the National Science Foundation Growing Convergence Research Big Idea (to SH, SMB), grant No.1935028. Funding from University of Milano-Bicocca (ML), and the Exchange Extra-UE mobility Programme (CR) also contributed to this work.

2.04.B.T-05 Role of gastrointestinal digestion on MNP morphology and surface reactivity using an in vitro approach

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Microplastics (MPs, 1 μm -5mm) and nano-plastics (NPs, <1 μm) (MNP) are a rising concern for environmental and human health. Ingestion is a critical route of uptake in humans and fish, which are significantly affected by MNPs. Moreover, MNPs can act as carriers of environmental pollutants. Because ingestion is the main route of MNP uptake, gastrointestinal digestion's role in MNP chemical and physical transformations and their toxicity should be investigated. This research aims to use an in vitro digestion system (IDS) to mimic the digestion process of rainbow trout (*Oncorhynchus mykiss*) to analyze what effects gastrointestinal digestion has on MNPs. We hypothesize that the gastrointestinal process will affect the MNPs fragmentation and surface reactivity, which will increase the release of plasticizers and of pollutants sorbed to the particles. To test this hypothesis, we ran the IDS with and without food, with MNPs (polyethylene and polyvinyl chloride). A protocol was developed to extract the MNPs from the undigested fecal matter to characterize the MNPs post IDS. The extraction consisted of ultracentrifuging the samples at 200,000 x g for 4 h, aspirating the remaining liquid and adding 10% KOH. The KOH was left to incubate for 48 h at 60°C. Any remaining matter that was not digested was removed using a 100 μm sieve. The sieve was rinsed with ultra-pure water and the samples were ultracentrifuged again. A tip sonicator was then used to break up the remaining pellet into a powder, and the samples were left to dry. The MNP morphology and size were analyzed using a scanning electron microscope (SEM). A code was developed using Python to automatically size the images. Pristine polyethylene (PE) had an average size of 4.96 μm (SD = 3.38), while the UV-aged PE had an average size of 5.52 μm (SD = 3.74). Pristine polyvinyl chloride (PVC) had an average size of 5.64 μm (SD = 4.05), and the UV-treated PVC had an average size of 13.28 μm (SD = 7.60). The UV treatment resulted in a slight increase in particle size and a moderate increase in surface roughness was shown by the SEM. Currently in progress is the SEM analysis of the digested particles to observe any changes in size and surface morphology. Future plans include quantification of plasticizer and environmental pollutant concentration to see what is leaching out of the plastics during digestion and to test the toxicity and bioaccumulation of the MNPs and plasticizers using rainbow trout gut cells (RTgutGC).

2.04.B.T-06 Impacts of nano- and low-micrometer microplastics (NLMMPs) on freshwater organisms: A comprehensive meta-analysis

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Freshwater organisms play a crucial role as both critical food sources and ecologically significant species. Many freshwater organisms are negatively impacted by persistent environmental pollutants, such as nano- low-micrometer microplastics (NLMMPs, <100 mm diameter). Despite the widespread pollution of these NLMMPs, there is limited literature on the impacts of these particles on freshwater organisms that inhabit freshwater ecosystems environments. Therefore, we completed a meta-analysis that analyzed the current literature surrounding NLMMP studies to identify known impacts and knowledge gaps on freshwater aquatic organisms. The search terms used were "Nanoplastics Fish", "Nanoplastics Mussels", "Nanoplastics Amphibians", and "Nanoplastics Invertebrates". Approximately 163 individual studies were identified as meeting our inclusion criteria. Data used from the studies included organism used, organism age, plastic type, size, and concentration used, exposure duration, and any lethal or sublethal effects reported. We found that the majority of studies were conducted on fish (91%), majority of studies were conducted using polystyrene NLMMP (92%), and the most common exposure duration was acute (<7 days) (66%). Key knowledge gaps identified by this meta-analysis include the lack of investigation on different types of NLMMPs, chronic exposure studies, species diversity across studies, and larval to juvenile NLMMP exposure studies. These preliminary results suggest that while knowledge of NLMMP effects on freshwater organisms has grown in the last decade, there remains a great need for further investigation. The outcome of this meta-analysis aims to offer a comprehensive understanding of the current state of knowledge and subsequent knowledge gaps surrounding NLMMP exposure effects, to identify the known impacts of NLMMP on non-target organisms and what impacts are missing. **Disclaimer/Disclosure:** Funding from NOAA Grant #122 AAN9352 349610-4 Travel Award funding from Midwest Regional SETAC 2025 Best Poster Presentation Award provided by SC Johnson, Racine WI

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

2.04.P-Mo-009 Effects of erythromycin on gene transfer of microplastic-associated bacteria across different salinity levels

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Microplastics (MPs) and antibiotics exhibit widespread co-occurrence in estuarine ecosystems, where dynamic salinity fluctuations create hotspots for their synergistic exposure risks to microbial communities. This study evaluates the impact of erythromycin on conjugation transfer rates between planktonic bacteria and biofilm-associated bacteria colonizing polyvinyl chloride (PVC) MPs across a gradient of salinity conditions, employing *Escherichia coli* as a model organism and 1–2 mm PVC particles as representative MPs. Biofilm-embedded bacteria exhibited higher conjugation transfer rates compared to their planktonic counterparts, with elevated salinity significantly enhancing this horizontal gene transfer process. While erythromycin concentration variations exert negligible effects on *E. coli* conjugation frequency under low and optimal salinity, a pronounced suppression of transfer rates occurs under high-salinity stress. To elucidate the underlying mechanisms, we quantified intracellular reactive oxygen species (ROS) accumulation, ATP production dynamics, and cell membrane permeability alterations. These analyses provide mechanistic insights into how salinity-modulated erythromycin exposure disrupts bacterial conjugation efficiency.

2.04.P-Mo-010 Ecological risk assessment of microplastics in Japanese rivers through inverse modeling of field data

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The ecological risk assessment of microplastics (MPs) in surface water is a pressing environmental concern because of the wide variability in sizes, resin types, and shapes of microplastics, all of which might influence

their environmental behavior and ecological toxicity. Smaller MP particles have been reported to exhibit higher toxicity. While the concentration of large microplastics (LMPs), with a size range of 300-5,000 μm , have been frequently reported, data on small microplastics (SMPs), with a size range of 10-300 μm , remain limited. Several types of MPs with a lower density than water have been observed in river sediments, suggesting that MPs may aggregate with suspended solids (SS) in river water and behave similarly in aquatic environments. In this study, the distribution of LMPs in rivers was modeled using the G-CIEMS multimedia environmental fate model, originally developed to simulate the behavior of organic pollutants. We focused on the dominant resin and shape combinations found in field data from the Tamagawa River in Japan: polyethylene (PE) and polypropylene (PP), in both fiber (Fb) and fragment (Fr) forms. The sedimentation properties of the four MP types (PP-Fb, PP-Fr, PE-Fb, and PE-Fr) were determined based on field observations. Per capita unit loads of these MPs were also estimated. The model's reliability was validated by comparing simulated MP concentrations, based on estimated discharges, with total MP concentrations obtained nationwide monitoring data across Japan. Using the modeled LMP concentration, we also estimated SMP distributions in rivers. Finally, an ecological risk assessment was conducted by comparing the modeled SMP concentrations with ecotoxicological thresholds, specifically the HC5 (5% hazardous concentration) values. **Disclaimer/Disclosure:** This research was performed by the Environment Research and Technology Development Fund (JPMEERF21S11904) of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan.

2.04.P-Mo-011 Occurrence of microplastics and tire and road wear particles in field-collected eastern oysters (*Crassostrea virginica*) and grass shrimp (*Palaemon pugio*) from the Charleston Harbor Estuary, SC, USA

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Microplastics are ubiquitous in aquatic environments and comprise a heterogeneous mixture of synthetic particles measuring less than 5 mm in size. Microplastics can be ingested intentionally or accidentally by aquatic organisms occupying a variety of life stages, habitats, and feeding guilds and can induce adverse effects such as gut blockage, internal abrasion, inflammation, and mortality. Much of the environmental accumulation of microplastics has occurred in estuaries where winds, rivers, tides, and currents transport plastic waste to the coast. Because estuaries are vital to marine biodiversity, fisheries, and the global economy, it is important to determine the potential impact of microplastics on ecologically, commercially, and recreationally important estuarine species. The present study quantified microplastic accumulation in two economically and ecologically important estuarine species, Eastern oysters (*Crassostrea virginica*) and grass shrimp (*Palaemon pugio*), collected from the Charleston Harbor estuary in Charleston, SC, USA. Oysters and grass shrimp were collected from five sites within the estuary (n=20 individuals of each species per site). Tissues were digested using 10% potassium hydroxide and were vacuum filtered onto glass microfiber filters. Microplastics were visually identified, enumerated, and imaged using a stereomicroscope equipped with a camera. Microplastic size, shape, and color were determined using ImageJ. Polymer composition was determined on a subset of microplastics using Raman spectroscopy and attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy. Overall, 83% of oysters and 75% of grass shrimp contained microplastics. In oysters, the number of microplastics ranged from 0 to 28 per individual (0-12.7 microplastics/g wet weight). In grass shrimp, the number of microplastics ranged from 0 to 8 per individual (0-22.5 microplastics/g wet weight). The majority of microplastics identified in each species were fibers. The largest proportion (21%) of these fibers were comprised of polypropylene. Several tire and road wear particles (TRWPs) were also identified in both species and were confirmed to be styrene butadiene rubber. These results provide important information about the accumulation of microplastics in estuarine organisms, the potential for trophic transfer, and human dietary exposure.

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

2.04.V-01 Microplastic pollution in *Pelecanus occidentalis* chicks from Bona Island, Panama: Indicators of early-life exposure in a coastal urban context

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Plastic pollution is an escalating threat to marine biodiversity, especially in coastal urban centers. Seabirds, such as pelicans, are considered reliable bioindicators of environmental contamination, including the ingestion and inhalation of microplastics (MPs). In Panamá, where urban development and marine ecosystems intersect sharply, understanding how plastic pollution affects early life stages of marine fauna is critical. This study investigates the presence of MPs in the stomach and lungs in chicks of the brown pelican *Pelecanus occidentalis* on Bona Island, a breeding colony located near Panamá City. The proximity of this site to one of the busiest shipping routes and densely populated urban areas offers a unique opportunity to assess pollution pathways from anthropogenic sources into marine food webs. The objective of the study was to assess the extent of early-life exposure to both ingested and inhaled plastic particles, which may pose significant physiological and developmental risks to chicks and provide insights into broader environmental contamination in coastal ecosystems. During expeditions in August and September 2024, 18 dying pelican chicks were opportunistically collected. Field necropsies were performed, prioritizing stomach contents and lung tissue for MP analysis. Microplastics were identified and quantified using stereomicroscopy under clean-lab conditions, following standardized protocols to prevent contamination. Widespread MPs contamination was found in both flying and non-flying fledglings. Fibers were the dominant type, followed by fragments and films. Transparent and blue MPs were the most common, although black, red, and other colors were also present. Non-flying fledglings showed a higher abundance of MPs, suggesting a reduction in exposure or accumulation once fledglings begin to forage at sea. These findings provide critical baseline data on the exposure of seabird chicks to MPs during their earliest life stages. Detecting MPs in both digestive and respiratory systems supports concerns regarding multiple exposure routes, including parental feeding and environmental contamination. This study contributes to a growing body of literature on the vulnerability of coastal marine species to plastic pollution and highlights the urgent need for mitigation efforts in urban-adjacent ecosystems. The results will inform regional conservation strategies and support broader efforts to track plastic pollution impacts across the trophic web.

2.04.V-02 Molecular-level insights into nanoplastic inhalation & ingestion effects on biomembrane interfaces

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The smaller size and larger surface area-to-volume ratio of nanoplastics (NPs) enhance their ability to traverse cell membranes with increased propensity for bioaccumulation. In vitro and in silico studies also confirm their efficient membrane translocation, and animal models demonstrate the potential of NPs to breach the blood-brain barrier, inducing neurotoxicity. Once internalized, these particles can cross biological barriers such as the gastrointestinal and respiratory epithelia, where their accumulation has been linked to localized inflammation. The findings raise serious concerns about the molecular mechanisms driving nanoplastic toxicity, particularly their interactions with membrane lipids essential for maintaining cellular homeostasis. Our study focuses on the interfacial behavior of environmentally simulated polystyrene (PS) NPs - a widely used polymer - at model membranes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and palmitic acid (PA). DPPC, the dominant phospholipid in pulmonary surfactants and a representative of phospholipids forming cellular membranes, regulates membrane fluidity and reduces lung surface tension. PA, while a minor lung surfactant component, is important in therapeutic formulations and constitutes a major portion of human fatty acid pools. Notably, the styrene monomer used in PS production is classified as a possible human carcinogen, underlining the need to assess PS NPs' effects at membrane interfaces. Using Langmuir isotherms, Brewster angle microscopy, and infrared reflection-absorption spectroscopy, we investigated how PS NPs perturb lipid organization at aqueous interfaces mimicking cellular environments. The data reveal significant structural disruption, including domain fragmentation and pore formation, with DPPC films showing more pronounced effects than PA. Spectroscopic analyses further confirmed molecular-level interactions between charged PS NPs

and lipid headgroups, validating complex formation previously suggested in vitro. The findings provide critical mechanistic insight into how nanoplastics may impair cellular membrane structure, contributing to adverse physiological outcomes such as impaired surfactant performance, inflammation, and barrier dysfunction. This work reinforces the importance of fundamental lipid–nanoplastic interfacial studies in understanding the toxicological impacts of environmentally relevant nanoplastic exposure through routes such as ingestion and inhalation. **Disclaimer/Disclosure:** The full research is published in the Journal of Environmental Science & Technology (Vol. 59, Issue 21). The article is titled "Nanoplastic-Induced Disruption of DPPC and Palmitic Acid Films: Implications for Membrane Integrity". I am the first author and have obtained the necessary permissions. This research was supported by the Herbert W. Hoover foundation through grant no. GR 137883. The spectroscopic characterization of the environmentally simulated nanoplastics through O-PTIR (optical photothermal infrared spectroscopy) was supported by the NSF MRI award CHE-2117225 and the Center for Chemical and Biophysical Dynamics (CCBD) at The Ohio State University.

2.04.V-03 Microplastics presence in seagrass meadows of NE Quintana Roo, Mexico

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Since the mid-20th century, plastic has been an essential part of humanity and our modern life. Being petroleum-derived materials, plastics have a significant environmental impact. When exposed to changing temperatures, ultraviolet radiation, salinity, and the mechanical force of tides, they fragment into macroplastics and microplastics (MP), which can settle in ecosystems such as seagrass meadows, turning them into critical hotspots for MP accumulation. Previous studies have documented the retention of MPs in seagrass ecosystems. Therefore, this study aimed to assess the presence and identification of MPs in the water and sediments of seagrass areas of northeast Quintana Roo, Mexico. At each of the six sites, one liter of water was sampled in triplicate using a Van Dorn bottle and filtered with a Millipore instrument and 696 glass fiber filters. In addition, three sediment cores were taken using a 30 cm stainless steel corer. All samples were labeled, stored, and transported in a cooler. In the laboratory, six 250 g sediment replicates per site were dried in an oven at 60°C for 48 hr. Later, MPs were separated by density difference using a saline solution of NaCl (146 g NaCl in 400 ml of deionized water). The supernatant was filtered using the same system as for the water samples. The particles were counted and categorized by shape (fibers, fragments, and films), color, and size using a stereoscopic microscope. Identification was performed by Fourier-transform infrared spectroscopy (FTIR) and Gas Chromatography. Water samples MP ranged from 4 MP/L (site 5) to 56 MP/L (site 2). Sediments ranged from 40 MP/Kg (sites 2 and 3) to 136 MP/Kg (site 4). These numbers are lower than those reported for Belize, England, and South Africa seagrasses. Fibers were the most predominant shape in water and sediments, with 52.27% and 76.52%, respectively. As in other studies, blue was the most frequent color in sediments (41.67%) and black (43.58%) in water. MP sizes in sediments ranged from 0.4 mm to 35 mm and in water from 0.1 mm to 38.7 mm. Polymer identification is in process.

2.04.V-04 Toxicity assessment of cigarette butts leachate in co-exposure with microplastics evaluated in *Artemia salina*

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Microplastics (MPs) and cigarette butts represent a growing threat to aquatic ecosystems. Research have demonstrated not only increase of both contaminant in aquatic environment but also adverse effect in aquatic biota. Nevertheless, there is not enough information regarding combination of both contaminants on aquatic invertebrates and the effect of then at different stages of their lifespan. This study aimed to evaluate the toxicity from the joint exposure of cigarette butt leachates and MPs on *Artemia salina* (nauplii and juvenile). To reach this aim leachates were prepared by consuming cigarettes using an artificial lung, then 5 cigarettes butts were mixed with 500 ml of artificial seawater and shaken at 300 rpm for a period of 3 days. Then four bioassays

were conducted: (1) bioassay exposing nauplii to different concentrations of cigarette butt leachates (0;0.78; 1.56; 3.13; 6.25; 12.5; 25; 50 and 100%), (2) bioassay performed with Juvenile exposed to cigarette butt leachates (0;0.78; 1.56; 3.13; 6.25; 12.5; 25; 50 and 100%), (3) bioassay with nauplii subjected to a joint exposure of cigarette butt leachates (0;0.78; 1.56; 3.13; 6.25; 12.5; 25; 50 and 100%) and Green fluorescent MPs (size 1-5 μm with a density of 1.3 g/cc) at a concentration of 16,000 ppm, and MP control (only MP), (4) bioassay with juvenile exposed to cigarette butt leachates+MP tested at similar concentration than bioassay n°3. All experiments were performed in triplicates. Mortality was assessed after 24 hr and 48 hr. The results showed significant mortality ($p < 0.05$) in nauplii and juvenile when increasing leachate concentrations compared to control. On the contrary, a significant decrease in mortality ($p > 0.05$) compared to controls was observed in both life stages under leachates in co-exposure to MP. Juvenile *A. salina* were significantly ($p > 0.05$) more sensitive than nauplii during experiments. Further research is needed to understand biological response induced by MPs and other contaminants present in water systems and its effect in different life stages of aquatic biota. **Disclaimer/Disclosure:** VRII- UNAP CONSOLIDA 2024 (SINCPROYEXT-0105) (G. Aguirre-Martínez Principal Investigator) (ANID-Fondecyt) project N°11180466 (Principal Investigator G. Aguirre-Martínez) and project N°1241723

2.04.V-05 Preliminary insights on the distribution and morphological characteristics of microplastics in coastal waters of Ganjan, Eastern India

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Microplastics (MP) have emerged as a global concern due to increasing plastic waste, environmental persistence, and potential health impacts. This study investigates the distribution and morphology of MP in coastal waters along a 60 km stretch of the Ganjam Coast on the east-coast of India. Employing a stratified random sampling strategy, we collected water samples from four distinct 15 km stretches, targeting fishing harbors, tourist sites, and secluded zones. One liter composite samples, collected at a 60 cm depth, were packed in an ice box, transferred to the laboratory and subjected to wet peroxide oxidation for organic matter digestion, followed by filtration and microscopic analysis. Microplastic abundance was quantified, and particles were categorized by morphology (fibers, fragments, films, pellets, foam) and size. Our study reveals significant spatial heterogeneity in MP abundance, with fishing harbors generally showing the highest concentrations, particularly of fibers (e.g., $\sim 147 \pm 38$ MPs/L in Stretch 1). Tourist sites revealed intermediate levels with a mix of fibers and fragments, while secluded zones typically exhibited lower overall microplastic loads, though film abundance was sometimes elevated. One-Way ANOVA test results reveal that MP abundance is statistically significant both across different activity sites ($F=12.86$, $p < 0.05$) and geographic stretches ($F=2.70$, $p < 0.05$). This highlights the influence of localized anthropogenic activities and regional factors on MP pollution. Spatial distribution mapping using Inverse Distance Weighting (IDW) further illustrated distinct contamination patterns, with higher cumulative microplastic concentrations observed in the northern stretches of the study region. Correlation analysis suggested limited direct linear relationships in MP abundance across different environment types within the same stretch, underscoring the site-specific nature of microplastic pollution. This study provides critical baseline data on MP pollution along the Ganjam Coast, emphasizing differential impacts of coastal activities and the complex interplay of sources and environmental processes in shaping microplastic distribution. These preliminary findings necessitate conducting structured and comprehensive investigation of microplastic contamination and flow through the marine food web along the entire stretch of east-coast of India, which will eventually lead to identifying hot-spots of MP contamination in the region. **Disclaimer/Disclosure:** This study is part of ongoing research on microplastic contamination and its impact along the Odisha coastline, funded through a Corporate Social Responsibility initiative.

2.04.V-06 Breaking the size-toxicity paradigm: Cross-species insights into nanoplastic effects

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Plastic pollution, especially from microplastics (MPs) and nanoplastics (NPs), is a growing environmental concern due to their potential toxicity and ability to infiltrate biological systems. NPs are often considered more hazardous than MPs because of their smaller size and increased tissue penetration. However, the size-dependent toxicity of NPs and their mechanisms across species remain poorly understood. This study examines the toxicity of polystyrene nanoplastics (PS-NPs) of 50 nm, 200 nm, and 1000 nm in two model organisms: zebrafish larvae (aquatic) and chicken embryos (terrestrial). A central challenge is the variability in toxic effects across particle sizes and species. We hypothesized that NP toxicity varies by both size and species, with distinct mechanisms even at similar developmental stages. Embryos were exposed to PS-NPs at 0, 0.1, 1, and 10 ppm during comparable developmental windows. Toxicity was assessed through behavioral assays, biochemical markers, RNA sequencing, and 3D imaging. Results showed species- and size-specific responses. Zebrafish exhibited hypoactivity and neurotoxicity, especially with 50 nm particles. In contrast, chicken embryos showed more pronounced morphological effects with larger particles (200 and 1000 nm). Extracellular matrix and immune-related pathways dominated in chickens, while neurodevelopmental pathways were prominent in zebrafish. Common hepato-related toxicity pathways were identified in both species. IPA revealed common disruptions in hepatic and cardiovascular pathways across both species. These findings challenge the assumption that smaller NPs are inherently more toxic and underscore the need for size- and species-specific considerations in environmental risk assessments. This work advances our understanding of NP toxicity across diverse biological systems and highlights the complexity of nanoplastic interactions in ecosystems. **Disclaimer/Disclosure:** This study is funded by Danmark Frie Forskningsfond (0165-00056B).

2.05.P-Mo Fate and Effects of Metals: Aquatic Biological Perspective

2.05.P-Mo-030 Maternal transfer of total mercury in tetragnathid spiders

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Riparian tetragnathid spiders are used as biosentinels of aquatic contamination because they are specialized feeders of emerging aquatic insects and are also prey items for terrestrial predators (e.g., birds). Analysis of both trophic position (e.g., stable nitrogen isotopes) and contaminant concentrations are needed to utilize tetragnathids as biosentinels, which can present challenges when collecting enough biomass to reach analytical detection limits due to their relatively small size. Because of this, female spiders are targeted, and individuals are pooled in an effort to reach necessary analytical biomass. However, little is known about physiological and molecular-biological processes in adult female tetragnathid spiders that could impact tissue contaminant concentrations (For example, egg laying and reproduction). The purpose of this study is to address one of these gaps in knowledge through investigating the maternal transfer of total mercury from adult female tetragnathid spiders to their eggs and offspring.

2.05.P-Mo-031 Water quality criteria development for aluminum and iron

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Our 16-year aquatic research program has resulted in the development of multiple linear regression models (MLR) to predict toxicity for aluminum (Al) and iron (Fe). The MLR models have been used by the USEPA to develop water quality criteria (WQC) for aluminum (USEPA 2018) and by Environment and Climate Change Canada to develop Environmental Quality Guidelines (2024). Unlike other cationic metals, Al and Fe toxicity correlates with total metal in solution and not dissolved metal. Both countries require the measurement of total metal for compliance. This necessitated development of methods for measuring bioavailable metal to avoid reporting metal originated from suspended solids which is generally not bioavailable. The Al method was approved by ASTM in 2024 (D8596-24) and the Fe method is under development (Crespo et al. 2022, ETC, pp 303). We developed MLR models for predicting chronic Al and 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. Chronic toxicity studies have been performed with 8-10 families to meet diversity requirements for deriving WQG. DOC and pH were statistically important, and hardness was not in the Fe MLR model whereas all three modifying factors were all important for the Al MLR model. The MLR models were then applied to all species in the species sensitivity distribution to derive hazardous concentrations to 5% of the species which were derived in 2 ways: 1) fitting a log-normal distribution to species-mean EC10s for all species (Canadian/European Union methodology), and 2) fitting a triangular distribution to genus-mean EC20s for animals only (USEPA methodology). Overall, the MLR models provide a viable approach for deriving water quality guidelines that vary as a function of DOC, pH, and hardness conditions and are a significant improvement over bioavailability corrections based on single parameters. Site-specific example calculations are shown in the presentation.

2.05.P-Mo-032 Measuring bioavailable aluminum in natural waters

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Aluminum (Al) aquatic toxicity correlates with total metal in solution and not dissolved Al. Considering that fact, water quality guidelines in several countries require analytical measurements of total Al using a strong acid digestion ($\text{pH} \leq 1$). Surface water samples contain suspended solids (clays, minerals) with substantial amounts of naturally occurring Al. Thus, effluent water samples may not meet regulatory standards due to the presence of natural suspended solids. To alleviate this problem, we developed an analytical method that measures only the concentration of Al that is bioavailable to aquatic organisms (bioavailable Al) without measuring the Al contained in suspended solids. A standardized method was developed and approved by ASTM in 2024 (D8596). This test method covers the determination of bioavailable Al in freshwater using an extraction procedure at a pH of 4 by addition of an acidic buffer, an aging period of 3 h, filtration, and acid preservation. The analytical measurement of Al follows standard procedures. This test method is applicable to waters containing dissolved, polymeric, and mineral forms of Al and is applicable to concentrations ranging from 0.01 mg/L to 5 mg/L Al. We applied this method to water samples from 10 rivers in the US and compared bioavailable Al to total Al, acid-soluble Al ($\text{pH} = 2.0$), and dissolved Al. The total suspended solids for these rivers ranged from 0.07 to 624 mg/L and total Al ranged from 3.8 to 3740 $\mu\text{g/L}$. Bioavailable Al concentrations were <0.2 to 496 $\mu\text{g/L}$, ranged from 1 to 54% of total Al, and were typically less than 10% of total Al. This analytical method was used to assist in performing Al laboratory toxicity studies and development of an Al multiple linear regression (MLR) model which has been adopted for use in the 2018 USEPA water quality criterion for Al.

2.05.P-Mo-033 Multiple linear regression models for predicting acute and chronic cobalt toxicity to freshwater organisms

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The U.S. Environmental Protection Agency (USEPA) has selected multiple linear regression (MLR) as the bioavailability-based modeling approach for developing updated aquatic life ambient water quality criteria for metals. Acute and chronic cobalt toxicity tests have been conducted with several freshwater species, including algae (*Raphidocelis subcapitata*), invertebrates (*Brachionus calyciflorus*, *Ceriodaphnia dubia*, *Daphnia magna*), and fish (*Pimephales promelas*), over a range of bioavailability conditions (i.e., varying hardness, pH, and dissolved organic carbon [DOC]). In general, for most species tested, hardness has the strongest influence on cobalt bioavailability and toxicity, pH has a lesser influence, and DOC has a limited influence. MLR models for individual species and pooled species were developed for both acute and chronic cobalt toxicity. The resulting MLR models were applied to acute and chronic species sensitivity distributions to develop examples of potential MLR-based water quality criteria.

2.05.P-Mo-034 Neurotoxicity of maternally transferred methylmercury in early life stage fathead minnows (*Pimephales promelas*)

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Mercury is a non-essential metal of global concern. Emitted by natural and anthropogenic sources into the atmosphere, mercury can be deposited in aquatic ecosystems and be transformed into methylmercury. Methylmercury is a neurotoxic compound and accumulation in aquatic biota from dietary exposure can be associated with adverse health outcomes. In addition, methylmercury can be maternally transferred to offspring and provide an early route of exposure for early life stage (ELS) fish. The maternal transfer of methylmercury can negatively impact neuro- development, chemistry, and behavior in ELS fish. Therefore, understanding the neurotoxic effects of maternally transferred methylmercury is crucial for understanding multigenerational methylmercury toxicity. To characterize the impacts of maternally transferred methylmercury in ELS fish, the present study exposed adult fathead minnows (*Pimephales promelas*) to dietary methylmercury concentrations of 0, 1, and 3 mg/kg methylmercury for 35 days. Endpoints measured include embryonic coiling (spontaneous contraction) and neurotransmitter analysis of dopamine, glutamate, serotonin, GABA, and acetylcholine. Results from the present study will provide insight into the neurotoxicity of maternally transferred methylmercury in ELS fish.

2.05.P-Mo-035 Drivers of contaminant load in elasmobranchs: Coastal development and feeding ecology

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Pollution is a key driver of biodiversity loss in marine ecosystems and poses risks to sharks, a subgroup of elasmobranchs, as apex predators and keystone species vulnerable to the bioaccumulation of persistent contaminants. Coastal estuaries serve as critical feeding and nursery habitats for many shark species, often overlapping with urban development and pollutant inputs. Although contaminant bioaccumulation varies by species, age class, and trophic position, few studies have examined contributions of coastal urbanization and feeding strategy on elasmobranch contaminant burdens. This research aims to (1) evaluate how residence in urbanized versus non-urbanized estuaries influences contaminant concentrations in Broadnose Sevengill sharks (*Notorynchus cepedianus*), and (2) assess how feeding strategy, specialist versus generalist, affects contaminant levels in Finetooth (*Carcharhinus isodon*) and Sandbar (*Carcharhinus plumbeus*) sharks. We hypothesize that sharks from urbanized estuaries will have higher contaminant concentrations than those from less-impacted areas, and that generalist feeders will accumulate more contaminants due to consumption of higher-trophic-level prey. To address these questions, water, sediment, and shark blood and muscle samples were collected using catch-and-release methods from San Francisco Bay, CA, and Willapa Bay, WA (Project 1), and Bulls Bay and St. Helena Sound, SC (Project 2). Structural data were used to assess sex and age class differences. Total mercury was measured via thermal decomposition, amalgamation, and atomic absorption spectrophotometry. Targeted per- and polyfluoroalkyl substances (PFAS) are being analyzed using liquid chromatography–tandem mass spectrometry. Stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and prey sampling will support trophic positioning and contaminant transfer assessment. Preliminary Project 1 results show significant differences in muscle mercury levels by age class in Broadnose Sevengill sharks, with subadults having the highest concentrations. In Project 2, blood mercury levels in Sandbar sharks trend upward with size, and PFAS were detected in water and sediment samples. Prey, blood and tissue analyses are ongoing. These studies provide insight into how habitat use and ecological traits shape contaminant exposure in elasmobranchs. Findings may support conservation strategies, improve understanding of food web contaminant transfer, and inform risk assessments for vulnerable marine species. **Disclaimer/Disclosure:** Project 1 is supported by Save Our Seas Founding (SOSF) grant number 669 awarded to KD Rock. Project 2 is supported by grants funds from the Lerner-Gray Fund for Marine

Research of the American Museum of Natural History awarded to M Walker. Both projects are additionally supported by Clemson University, Department of Biological Sciences KD Rock Start-up Funds.

2.05.P-Mo-036 Updating the unified acute and chronic zinc biotic ligand model for protection of freshwater aquatic life and application for site-specific water quality objectives

Adam C Ryan¹, Robert C. Santore² and Kenneth Schiff³, (1)International Zinc Association, (2)Windward Environmental LLC, (3)Southern California Coastal Water Research Project

Biotic ligand models (BLMs) for predicting zinc (Zn) toxicity to freshwater organisms were developed in a manner consistent with the BLM framework. Species-specific BLMs and an overall ‘unified’ pooled acute and chronic BLM were developed using parameter estimation (PEST) software to predict acute Zn toxicity to four invertebrates and two fish, and chronic toxicity to three invertebrates and a fish. The unified Zn BLM performed better than the US Environmental Protection Agency’s (USEPA’s) existing hardness-based model and similarly to recently developed acute and chronic Zn multiple linear regression (MLR) models. The unified Zn BLM was used to develop acute and chronic 5th percentile hazardous concentrations (HC5s) analogous to USEPA’s aquatic life ambient water quality criteria (AWQC). Using reconstituted moderately hard water (hardness = 85 mg/L as CaCO₃) with 0.5 mg/L dissolved organic carbon as a normalization condition, the acute AWQC value was 75.6 ug/L and the chronic value was 17.4 ug/L. Both values are lower than the AWQC using the nationally recommended hardness equation where both the acute and chronic AWQC are 102 ug/L. The unified Zn BLM is more accurate than the hardness equation and the ecotoxicology database developed for this effort is highly comprehensive, providing high quality Zn AWQC that accurately achieve the intended level of protection for freshwater aquatic life. A sensitivity analysis indicated that increases or decreases of >2 standard deviations in estimated BLM inputs generally resulted in <4% change in AWQC. All sensitivity analysis scenarios are considered highly unlikely given the extremely low joint probabilities of over-estimating or under-estimating the magnitude of all missing inputs simultaneously. Therefore, the level of estimation necessary in the comprehensive database was viewed as acceptable, especially when considering that the alternative would be to omit several sensitive species from the HC5 determinations. Additionally, the Zn BLM-based AWQC developed here are protective of threatened and endangered species in California and appear to be protective of chemosensory endpoints for salmonids. Implementation of the Zn BLM for site-specific water quality objectives (SSWQOs) using the fixed monitoring benchmark (FMB) approach indicates that dry weather and wet weather samples should be considered separately to develop SSWQOs for each condition.

2.05.T Fate and Effects of Metals: Aquatic Biological Perspective

2.05.T-01 Assessing lithium toxicity across a salinity gradient in coastal marine fishes

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Industrial and consumer use of lithium has been steadily increasing with the ever-growing number of rechargeable electrical devices worldwide, not to mention the expansive traditional sources including medicinal, oil and gas, and fertilizers. Despite the growing risk of exposure, there has been relatively little effort to assess the effects of environmental lithium on aquatic life, with little to no effort directed toward estuarine environments. For freshwater fishes, lithium is thought to enter the animal through sodium uptake pathways, and consistent with this hypothesis sodium is protective against lithium toxicity in freshwater fish. However, marine fishes drink seawater and actively absorb the majority of imbibed sodium in an effort to defend against water loss. This may result in the loss of sodium protection in hyperosmotic environments. As such, the current study explored lithium toxicity across a salinity gradient in two coastal fish species, the sheepshead minnow (SHM) and red drum, using a combination of developmental and behavioral approaches. Contrary to conventional understanding, the lowest tested salinity had the highest developmental EC50 for both species. Although for both SHM and red drum the lowest EC20 was well in excess of typical environment scenarios (80.5 mg/L and 64.1 mg/L, respectively). Note that 96-h acute larval tests performed for both species at 30ppt

provided NOEC values of 87.3 and 68.4 mg/L, respectively, which validates developmental toxicity as a more sensitive endpoint. We then performed a series of behavioral open field tests following 96-h of exposure in larval SHM (21 dpf), which revealed significant hypoactivity at 58.3 mg/L lithium. Exposed fish had significantly reduced percent active time, distance travelled, and average speed than controls and a no-effect 6.9 mg/L dose. Preliminary data also suggested that exposure of SHM brain homogenates to nominal 75 mg/L lithium resulted in a significant reduction in mitochondrial oxidative phosphorylation. Overall, these data suggest that the assumption of sodium protection against lithium toxicity in fish should not be extended to coastal habitats, and that behavioral toxicity testing may provide a more sensitive indicator of lithium toxicity in fishes than traditional approaches. **Disclaimer/Disclosure:** This work was supported by the Matagorda Bay Mitigation Trust

2.05.T-02 Evaluation of environmental water and effect of zinc using *Daphnia magna*

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Various inorganic chemicals exist in the environment, they come from natural and artificial sources. Inorganic chemicals include metal, metal ion, metal salts, and nano materials. Some inorganic chemicals have toxicity like heavy metals. These chemicals affect aquatic environment. Zinc, mercury, selenium, copper, lead, nickel, arsenic, fluorine, and boron are listed as substances for which water quality targets should be considered. Due to the environmental importance, environmental criteria of zinc have been set 0.03 mg/L or less for river. In this study, we investigated the safety of environmental water with a focus on zinc. For the investigation of chemical safety, *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 hr old) placed each concentration and control, each beaker observed after 24 and 48 hr to obtain a 50% effective concentration (EC50). Zinc chloride (Kanto Chemical, Japan, Guaranteed reagent, 98.0 %, 7646-85-7) and Zinc nitrate hexahydrate (Kanto Chemical Co., Inc., Japan, Guaranteed reagent, 99.0 %, CAS RN 10196-18-6) were used for Zinc exposure. Zinc solutions were prepared as concentrated solutions in M4 medium. The test conducted with control and five concentrations. 48h-EC50 for Zinc nitrate hexahydrate (7.27 mg/L) was lower than 48h-EC50 for Zinc chloride (1.50 mg/L). Water samples were taken from Akagawa River system (Tsuruoka, Yamagata, Japan) and Aidumamegawa River system (Toyota, Aichi, Japan) in March and February of 2025, respectively. Observed Zinc concentrations were N. D. - 0.077 mg/L at Akagawa River system and 0.012 - 0.395 mg/L at Aidumamegawa River system. Each water sample has almost no effect on the daphnia except for one sample (Akagawa River). These results were reasonable at tested concentration level.

2.05.T-03 Bicarbonate as a toxicity modifying factor for metals in freshwaters: Does it exacerbate or ameliorate?

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Ceriodaphnia dubia reproduction tests have historically been important for evaluation of whole effluent toxicity, development of water quality criteria, and they are often a component of routine water quality monitoring programs. For several metals, including zinc, *C. dubia* is often among the most sensitive freshwater species. In metal bioavailability modeling frameworks, bicarbonate is directly or indirectly considered a toxicity modifying factor (TMF) associated with pH, alkalinity, or dissolved inorganic carbon. Its toxicity modifying effects are believed to be mediated through complexation of free metal ions, whereby metal toxicity generally decreases with increasing bicarbonate concentration. However, it has been demonstrated that bicarbonate can also be directly toxic to *C. dubia* when concentrations exceed approximately 5 mmol/L. In this study, we examined the effects of bicarbonate concentration on zinc toxicity to *C. dubia* and fathead minnows (*Pimephales promelas*). A series of identical *C. dubia* reproduction tests were conducted by two separate laboratories to evaluate the effects of bicarbonate concentrations on zinc toxicity while simultaneously providing data to assess test reproducibility. Fathead minnow growth tests were conducted to provide a basis for

comparing bicarbonate effects on zinc toxicity across taxa. Our results demonstrate that bicarbonate acts as a TMF for zinc in the conventional sense for fathead minnows, agreeing with biotic ligand model (BLM) and multiple linear regression (MLR) model predictions. However, results from two separate laboratories demonstrate that bicarbonate acts as an additional stressor to zinc for *C. dubia* with indications that the interactive toxicity of bicarbonate and zinc is potentially more than additive. Additional evaluations may be necessary to elucidate the mechanistic details behind this interaction, but these results highlight the need to consider potential mixture toxicity when interpreting results from *C. dubia* reproduction tests conducted in waters with elevated bicarbonate concentrations.

2.05.T-04 Evolutionary ionomics: Illuminating the systemic context of metal tolerance in sunfish inhabiting the Tar Creek superfund site

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Why do fish populations differ in metal tolerance? This study investigated the adaptive responses of fish populations to heavy metal pollution at the Tar Creek Superfund site within the Tri-State Mining District, historically contaminated by metals. Despite environmental remediation efforts, persistent contamination by metals, notably lead (Pb), cadmium (Cd), and zinc (Zn), continue to impact the aquatic ecosystem. Remarkably, we discovered populations of Bluegill (*Lepomis macrochirus*) inhabiting this polluted ecosystem. To understand how these conditions affect Bluegill populations, we hypothesized that fish in the most contaminated sites would differ in their elemental compositions. Our investigation examined Bluegill from 2 polluted – 1 highly polluted, 1 moderately polluted - and 2 reference sites. We measured concentrations of metals in fish tissues, including gill, liver, intestine, skin, white muscle, excrement, and water samples using inductively coupled plasma optical emissions spectrometry and mass spectrometry (ICP-OES and ICP-MS, respectively) methodologies. The concentrations of 15 elements were measured, including lead, cadmium, zinc, arsenic, bromine, calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, sulfur, and selenium. Metal concentrations measured in the water from the most polluted site were 12.49 µg/L, 12.17 µg/L, and 7445.39 µg/L for Cd, Pb, and Zn, respectively; and were below the limit of detection in the reference site farthest away from the sources of contamination. Preliminary analyses revealed significantly elevated metal concentrations in fish tissues collected from highly contaminated sites compared to reference sites. There was a strong positive correlation between the concentration of metals measured in the fish tissues and those measured in the water. Moreover, the patterns were tissue-specific, with skin and gill ionomes exhibiting the greatest responses to dissolved metal concentration. Our findings indicate that changes in the ionomes might be associated with evolutionary adaptation of sunfish inhabiting Tar Creek waters. Deciphering the biochemical mechanisms underlying observed ionomic patterns can provide key insights for future research. As such, our study not only illuminates on possible physiological mechanisms of metal bioaccumulation but is also relevant to inform policies addressing ongoing contamination from legacy mining operations.

2.05.T-05 Reproductive effects of arsenic-contaminated natural diet in zebrafish (*Danio rerio*) during chronic exposure

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Understanding the reproductive toxicity of arsenic (As) in aquatic organisms remains limited, particularly using chronic dietary exposures that more accurately reflect environmental scenarios. This study investigated the reproductive effects of chronic, environmentally relevant dietary arsenic exposure in adult zebrafish (*Danio rerio*) using an ecologically realistic trophic transfer model via oligochaete worms (*Lumbriculus variegatus*). Worms were pre-exposed to waterborne arsenite (0, 2.5, and 5.0 mg/L) for 14 days, resulting in arsenic body burdens of 0.25 (Control), 35.69 (Low As), and 77.79 (High As) µg/g dry weight. Zebrafish were then fed these worms at 3.5% of their body weight twice daily for 60 days. Reproductive performance was evaluated by measuring reproductive behavior, gonadosomatic index (GSI), hepatosomatic index (HSI) fecundity,

fertilization rate, hatching success, larval survival, and deformity rates. Additionally, brain, liver, gonads, and plasma were collected for analysis of gene expression along the hypothalamic-pituitary-gonadal-liver (HPGL) axis and quantification of circulating sex steroids (17 β -estradiol, 11-keto testosterone) and vitellogenin (VTG). Gonads (testes and ovary) were collected for histopathological analysis to examine the structural integrity of reproductive tissues. Finally, male fish sperms were collected to assess the effect of arsenic on sperm density and motility. Dietary arsenic exposure caused dose-dependent impairments in reproductive behavior, GSI, HSI, fecundity, fertilization rate, hatching success, and larval survival, alongside increased incidence of larval deformities. Significant alterations in HPGL axis gene expression and circulating hormone levels were observed in both sexes, indicating endocrine disruption. Histological analysis revealed reduced gamete frequency in both ovaries and testes, while sperm quality declined in exposed males. Collectively, these findings demonstrate that dietary arsenic exposure functions as an endocrine disruptor in zebrafish, impairing reproduction through HPGL axis disruption. The study emphasizes the necessity of incorporating dietary exposure pathways in ecological risk assessments to better predict reproductive hazards posed by arsenic in aquatic systems.

Disclaimer/Disclosure: The present study was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to S. Niyogi.

2.06.P-We Fate and Effects of Per- and Polyfluoroalkyl Substances (PFAS) in Coastal Ecosystems

2.06.P-We-023 Developmental toxicity and transcriptomic effects of per- and polyfluoroalkyl substances (PFAS) in mummichogs (Atlantic killifish)

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Per- and polyfluoroalkyl substances (PFAS) are a broad chemical class, including constituents that are highly persistent, bioaccumulative, and detected worldwide. Some PFAS are associated with potential adverse health effects in humans and wildlife; however, the hazards to estuarine and marine species and populations are not fully characterized. Furthermore, comprehensive understanding of underlying mechanistic pathways by which PFAS cause toxicity in estuarine and marine species is still lacking. To address these knowledge gaps, we investigated developmental toxicity and transcriptomic response in mummichogs (*Fundulus heteroclitus*) after exposure to a suite of 11 PFAS. Selected PFAS represent several subclasses based on functional head group (i.e., sulfonates, carboxylates, sulfonamides, and fluorotelomer sulfonates), with compounds of varying fluorinated chain lengths. Mummichogs are an ecologically important estuarine fish, amenable to toxicity testing with a well-annotated genome. To evaluate developmental toxicity during a critical early life stage, embryos were exposed to individual PFAS (nominally between a range of 1-100 μ M) in seawater for a 6-day exposure beginning 1 day post-fertilization (dpf) through 7 dpf, then transferred to clean seawater for further assessment through 30 dpf. RNA-sequencing was conducted at 3 and at 10 dpf, and developmental endpoints such as morphology, heart rate, growth, and swim bladder inflation were evaluated between 10-30 dpf. Among the PFAS suite, only the sulfonamide-containing compounds perfluorohexanesulfonamide (FHxSA) and perfluorooctanesulfonamide (FOSA) induced acute mortality and morphological effects during the assays. Despite generally a lack of overt toxicity for most PFAS evaluated, all PFAS elicited transcriptomic responses, with varying magnitudes of effect. Comparative transcriptomic analysis across compounds and timepoints is underway to elucidate biological pathways perturbed by PFAS exposures and underlying modes of action, and results will be shared during the presentation. The present experimental paradigm and data analysis will contribute to knowledge of PFAS toxicity during critical early life stages, understanding of modes of toxic action, efforts to compare and extrapolate across species, characterization of hazard for estuarine and marine species, and will inform predictions of population-level impacts. **Disclaimer/Disclosure:** The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S.

Environmental Protection Agency. This is preliminary information that is subject to revision, and it is not for citation.

2.06.P-We-024 Effects of per- and polyfluoroalkyl substances (PFAS) on early life stage *Cyprinodon variegatus* (sheepshead minnows) and *Menidia beryllina* (inland silversides)

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Forage fish serve an essential role in aquatic ecosystems by facilitating the transfer of energy from plankton to higher trophic levels. *C. variegatus* and *M. beryllina* are common estuarine forage fish in the eastern US, but there is limited research regarding the response of these species to PFAS exposure, particularly at early life stages. Here, the effects of PFAS on fish survival were determined via micro-plate bioassays. One-day-post-fertilization (DPF) *C. variegatus* were exposed to ten individual PFAS and five PFAS mixtures. At 13 DPF, hatch success and overall survival were evaluated via a Fisher Test, while hatch time was assessed with a Kruskal-Wallis Rank Sum Test with Dunn post-hoc. Survival dose-response curves were constructed for compounds with concentrations tested across multiple experiments. Perfluorohexanoic acid (PFHxA) exposure reduced hatch time, while perfluoroalkane sulfonamide (FASA) exposure increased hatch time ($p < 0.05$). FASA exposure also significantly reduced survival, with perfluorohexanesulfonamide (FHxSA) having an estimated LC50 of 6.1 mg/L ($p < 0.05$). FHxSA also significantly reduced hatch success at concentrations as low as 10 mg/L ($p < 0.05$). Perfluorobutane sulfonamide (FBSA) significantly reduced survival at 25 mg/L ($p < 0.05$). Perfluorodecanoic acid (PFDA) and perfluorohexanesulfonic acid (PFHxS) both resulted in significant reductions in overall survival at 10 mg/L, with no effect at 5 mg/L ($p < 0.05$). Perfluorooctane sulfonic acid (PFOS) had an approximate LC50 of 1.6 mg/L ($p < 0.05$). In contrast, 6:2 fluorotelomer sulfonic acid (6:2 FTS) and 8:2 fluorotelomer sulfonic acid (8:2 FTS) up to 50 mg/L and perfluorobutanesulfonic acid (PFBS) and perfluorooctanoic acid (PFOA) up to 200 mg/L did not affect any endpoint ($p > 0.05$ for all comparisons). Mixture compounds generally resulted in similar outcomes as the component compounds at similar concentrations. Perfluoroalkane sulfonates (PFASs) and FASAs appeared to have the greatest toxicity, with FASAs also affecting hatching. For all PFAS, toxicity generally increased with chain length. Similar to embryos, the survival of one-day-post-hatch (DPH) *M. beryllina* and three DPH *C. variegatus* were unaffected by a three-day exposure to PFOA at 250 mg/L ($p > 0.05$). This study provides insight into compound- and age-specific effects of PFAS in forage fish which can further inform ecological risk assessments and environmental regulations. **Disclaimer/Disclosure:** Funding provided from SERDP Grant Er22-4003

2.06.P-We-025 Ecotoxicity and uptake of AFFF-relevant PFAS to pelagic and epibenthic marine species

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Per and polyfluoroalkyl substances (PFAS) have been found worldwide across several environmental matrices. One of the many sources of PFAS to the environment, aqueous film-forming firefighting foams (AFFF), have historically been used at Department of Defense (DoD) sites, resulting in PFAS-impacted installations that need ecotoxicological data to assess ecological risk. In the present study, the toxicity of several PFAS compounds (PFDA, PFOA, PFHxA, PFBA, PFDS, PFOS, PFHxS, PFBS, 8:2 FTS, and 6:2 FTS) were evaluated. Five standard USEPA chronic marine pelagic and epibenthic bioassay tests were conducted: (1) a 48-hr germination and growth assay using giant kelp (*Macrocystis pyrifera*); (2) a 96-hr embryo-larval normal development assay using purple sea urchins (*Strongylocentrotus purpuratus*); (3) a 7-day larval growth and survival assay using topsmelt (*Atherinops affinis*), (4) a 48-hr embryo-larval normal development assay using Mediterranean mussels (*Mytilus galloprovincialis*); and (5) a 7-day growth, survival assay using mysids (*Americamysis bahia*). In addition, water-to-tissue uptake factors were calculated to assess PFAS bioaccumulation in pelagic environments for two of the species, *A. affinis* and *A. bahia*. PFAS toxicity generally increased with

perfluorinated carbon chain length, with PFOS being the most toxic. The *A. bahia* growth endpoint was the most sensitive to exposure to PFAS, with invertebrate species generally being more sensitive than fish or kelp. Median effective concentration (EC50) values for all species were typically above 1 mg/L, excluding PFOS and PFDA, which were in the 0.1 to 1 mg/L range for *A. bahia*. Results suggest that all PFAS aside from PFOS at AFFF-impacted sites are unlikely to result in direct toxicity to these species, as observed environmental concentrations are typically orders of magnitude lower than thresholds observed in this study. Mysid and fish bioconcentration factors (BCF) were similar for each PFAS, and overall, BCF values increased with longer perfluorinated carbon chain length. While direct toxicity is not likely to drive environmental risk assessments at most PFAS sites, the uptake factors derived from this data will inform ecological risk assessments that target higher trophic levels that consume aquatic life. **Disclaimer/Disclosure:** This work was funded by the Strategic Environmental Research and Development Program (SERDP) ER22-3214 and the Navy Environmental Sustainability Development to Integration Program (NESDI) Project #601.

2.06.P-We-026 Hepatotoxicity-associated biological response changes by exposure transition from perfluorooctane sulfonate to its alternatives

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When unexpected toxicities are discovered in legacy compounds, they are replaced by less toxic alternatives, resulting in exposure transition to living organisms. However, the characterization of biological response of this exposure transition on organisms remains elusive. Considering the replacement of perfluorooctane sulfonate (PFOS) with alternatives, we evaluated the biological effects of exposure transition in adult zebrafish models, focusing on hepatotoxicity, a representative toxic effect of PFOS. Four exposure groups were designed: a solvent control, a PFOS-only group, a PFOS-to-perfluorobutanesulfonate (PFBS) transition group, and a PFOS-to-perfluorobutanesulfonamide (FBSA) transition group. The total exposure lasted 21 days, and the transition to alternative substances was applied by performing one week of PFOS exposure followed by two weeks of PFBS or FBSA exposure. Dissected liver samples were analyzed for measuring hepatosomatic index (HSI), liver-specific enzyme activities, and for analyzing histopathology. In addition, the RNA-seq analysis in the liver was conducted to investigate comprehensive biological response. Across all exposure groups, a tendency for decreased activities of alanine aminotransferase and aspartate aminotransferase was observed along with an increase of HSI. Histopathological liver damage was observed in both the PFOS-only and the FBSA-transition groups, with more severe damage in the PFOS-only group. RNA-seq results showed consistent downregulation of lipid metabolism-related genes in all treatment groups, especially within the peroxisome proliferator-activated receptor (PPAR) signaling pathway, indicating that lipid accumulation and metabolic disorders persisted even after the transition from PFOS to its alternatives. This study is the first to decipher biological responses to exposure transitions considered one of real-world-like exposures. Our findings demonstrated that hepatotoxicity, particularly lipid metabolism disorders, caused by PFOS has not been significantly alleviated despite the transition from PFOS to low toxic alternative compounds. Further studies are needed to investigate the specificity of PFOS and exposure duration dependency. **Disclaimer/Disclosure:** This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Korea government (MSIT) (No. 2022R1A2C2091199).

2.06.P-We-027 Effects of chronic PFNA and PFOS mixtures on the growth and reproduction of adult sheepshead minnow (*Cyprinodon variegatus*)

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Per- and polyfluoroalkyl substances (PFAS) are globally distributed, persistent organic pollutants that have led to concerns about potential ecological impacts due to their bioaccumulation, persistence, and potential toxicity.

While the adverse impacts of select individual PFAS on fish health have been extensively studied, comparatively less is known about the effects of environmentally relevant PFAS mixtures, which more accurately reflect real-world exposures. This study investigates the chronic effects of waterborne exposure to environmentally relevant concentrations of a PFAS mixture on adult sheepshead minnow (*Cyprinodon variegatus*), a small-bodied EPA model fish species commonly used for toxicity testing. Adult fish were exposed to a 20 µg/L mixture comprised of 10 µg/L perfluorononanoic acid (PFNA) and 10 µg/L perfluorooctane sulfonate (PFOS) under controlled laboratory conditions for 28 days. Key endpoints assessed included adult survival, somatic growth (length and weight), condition factor (K), gonadosomatic and liversomatic indices (GSI and LSI, respectively), and reproductive success (egg production and fertilization rates). Where possible, these endpoints were monitored throughout the exposure period to capture potential sublethal effects of PFAS on fish health and population-relevant traits. Preliminary findings indicate that exposure to the PFNA+PFOS mixture may negatively influence both somatic and reproductive parameters, even at low, environmentally relevant concentrations. Observed trends suggest potential endocrine or energetic disruption, consistent with known PFAS modes of action. These data contribute to the growing body of evidence demonstrating the risks associated with PFAS mixture exposure and highlight the need for mixture-focused risk assessments in regulatory toxicology.

2.06.P-We-028 Field Demonstration of a passive sampler to monitor diverse per and polyfluoroalkyl substances in an estuary system

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Per- and polyfluoroalkyl substances (PFAS) are a group of compounds of high concern due to their ubiquity, persistence, and adverse health impacts. With a diversity of chemical structures and properties, detection tools are needed to capture as many PFAS as possible. In this study, a microporous polyethylene tube (MPT) passive sampler was calibrated for 25 target compounds, 8 suspect PFAS, and extractable organofluorine (EOF) in groundwater, downstream river surface waters, and downstream estuary surface waters in an aqueous film forming foam (AFFF)-impacted system. Field measured and fluorine-normalized estimated EOF sampling rates (Rs) in groundwater (7.1 vs 8.4 mL day⁻¹ respectively) and river water (55 vs 66 mL day⁻¹ respectively) were within 20% of each other, demonstrating that EOF uptake is predictably dictated by chain length. For suspect PFAS and AFFF degradation products, Rs were similar to targeted PFAS of alike functional group chemistry and perfluorinated carbon chain length. For example, for 6:2 and 8:2 FTSAS-sulfoxide, estimated Rs values were 1.8 and 6.0 mL day⁻¹, respectively, similar to Rs measured for 6:2 and 8:2 FTS of 2.2 and 6.3 mL day⁻¹. These results suggest that targeted and suspect PFAS and EOF are predictably taken up by MPT samplers, expanding passive sampling capabilities. The findings of this study also demonstrate that some precursor PFAS are likely to exhibit similar partitioning and diffusivity in the environment to terminal PFAS of alike chain length and functional groups. With this tool, time weighted average measurements of an expanded list of PFAS compounds can be achieved to support enhanced environmental monitoring efforts and calculation of bioaccumulation factors.

2.06.P-We-029 Multifaceted implications of PFAS in the Chesapeake Bay and Maryland coastal bays

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Per- and polyfluoroalkyl substances (PFAS) are persistent and mobile emerging contaminants extensively used in consumer products, industrial processes, and aqueous film-forming foams (AFFFs). Their widespread presence raises significant concerns regarding environmental and ecological health. Although PFAS concentrations have been investigated in various aquatic environments, their distribution and ecological impacts remain poorly characterized in the Chesapeake Bay—the largest estuary in the United States—and the adjacent Maryland Coastal Bays (MCBs). This study evaluates PFAS burdens across these systems and examines their accumulation in ecologically important components, including macroalgae, surface water, sediment, toxin-

producing cyanobacteria, and the Atlantic blue crab (*Callinectes sapidus*). Sampling was conducted at 15 sites spanning both bays, targeting surface water, sediment, and macroalgae. Among PFAS compounds, PFNA, PFHxA, and PFOA were most prevalent in surface water; macroalgae exhibited elevated PFBA levels; and blue crabs showed heightened PFNA and PFHxA concentrations. These key PFAS compounds were further assessed for their ecological effects on representative species. Ongoing studies are exploring the impacts on saxitoxin-producing cyanobacteria, with results forthcoming. This research provides critical baseline data for monitoring PFAS contamination and offers insights into contaminant exposure across multiple ecological compartments in the Chesapeake Bay and MCBs. Ultimately, these findings will enhance our understanding of PFAS-related ecological risks and support management strategies to protect vulnerable populations and economically valuable species in these estuarine ecosystems.

2.06.P-We-031 Identification of per- and polyfluoroalkyl substances hotspots in surface water collected along the Mississippi coastline

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Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants that can be found at various levels within ecosystems. Due to their hydrophobic structure, PFAS will often congregate at the surface of water. States, like Mississippi, that rely heavily on water systems are more vulnerable to the harmful impacts of PFAS, thus putting their communities at potential risk for higher rates of adverse health effects. To date, there is no comprehensive data tracking the presence of PFAS within the ecosystems surrounding these anthropogenic activities. Surface water samples were collected from various locations along the Mississippi coastline, including Bay St. Louis, Biloxi Bay, and the Grand Bay National Estuarine Research Reserve (GNDNERR). Each sample was quantified using isotope dilution, assessing the presence of 31 PFAS. Solid phase extraction (SPE) was used to extract each sample, followed by nitrogen evaporation. Each batch of samples was analyzed via UHPLC-MS/MS. Water flow carries nutrients from vegetation-rich rivers, but it can also carry pollutants from sources along the river, in addition to run-off. In Pascagoula, Mississippi, there are several water systems that feed into the ocean that are flanked by industrial sites. Our sample sites were chosen by their varying proximities to these industrial sites along the Mississippi riverbanks and gulf coastlines. To contrast these specific points of potential pollution, samples were also collected in water systems in estuarine reserves and around predominantly residential areas. The contrast in site locations is indicative of the current layout of industry and residences along the riverbanks and coastlines of southern Mississippi. This variation in site location also allowed for a comprehensive profile of the current contamination landscape of southern Mississippi where there is currently no baseline PFAS data. With this project, we will present a collection of data on the concentrations and types of PFAS that are prominent in southern Mississippi water systems. This baseline serves the following two purposes: monitor PFAS contamination after major weather events and industrial disasters; and identify areas of concern for further evaluation.

2.06.P-We-032 Per- and polyfluoroalkyl substances in soft-shell clams from urban and remote regions in Nunavut: Understanding sources of PFAS in the Canadian Arctic

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Per- and polyfluoroalkyl substances (PFAS) are a broad class of anthropogenic contaminants that have been ubiquitously detected in the environment since their production began in the mid 20th century. Many PFAS are concerning due to their persistence in the environment and ability to bioaccumulate and have been detected in a variety of environmental matrices such as surface waters, sediments, and biological tissues. Additionally, PFAS are often found in wastewater effluent and sludge because most treatment processes are ineffective at removal. While PFAS have been previously analyzed in wastewater samples, data from remote communities in the Canadian Arctic is sparse, especially in relation to wastewater treatment plants in these communities. Inuit

communities in these regions rely on the local marine ecosystems for food and other uses, and these ecosystems are under-studied in relation to PFAS contamination. The truncate soft-shell clam, *Mya truncata*, are an important local food source and have been identified as potential biomonitors for anthropogenic contamination due to their long lifespan and accumulation of contaminants. The purpose of this research is to assess PFAS contamination in soft-shell clams to inform human exposure and communicate this information to the local communities. In 2024, 68 soft-shell clams were collected from Qikiqtarjuaq, Nunavut, and from Iqaluit, Nunavut, representing both remote and urban locations, respectively. Additionally, the Qikiqtarjuaq samples receive freshwater input from glacial melt, while the Iqaluit samples are more influenced by wastewater treatment plants in the area. The clams were extracted and analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) for a suite of 38 legacy and emerging PFAS. Results were further analyzed to determine PFAS profiles and differences between the two sampling sites. Results from this research regarding concentrations, profiles of PFAS, and spatio-temporal trends will be presented, as well as implications for the communities in these regions.

2.06.P-We-033 Ambient PFAS concentrations in the United States: A literature review

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Due to per- and polyfluoroalkyl substances (PFAS)'s effects on human and environmental health, there has been increased interest in releasing guidance and regulatory values for these substances. Given the ubiquitous presence of PFAS in environmental media throughout the world it is critical to have an understanding of "ambient" concentrations to provide context for risk management decisions. Though many studies have focused on PFAS concentrations associated with contamination, less research has focused on "ambient" PFAS levels in media removed from known sources of contamination for matrices other than drinking water. To begin to inform an understanding of the ambient condition in U.S. environmental media, we conducted a literature review of PFAS ambient concentrations for 6 environmental matrices (rainwater, freshwater, marine water, freshwater sediment, marine sediment and soil) in locations not associated with known sources or releases. From these studies, reported concentrations of PFAS analytes listed in USEPA Method 1633 were entered into a database. Summary statistics and boxplots were generated to visualize data for six PFAS analytes - PFOS, PFOA, PFBA, PFHxA, PFHxS, and 6:2 FTS. The majority of published studies were for freshwater, with fewer studies reporting ambient PFAS concentrations in rainwater or in marine sediment and marine water. Generally, ambient PFAS concentrations were higher in freshwater than in marine and rainwater, with measured surface water concentrations occasionally exceeding the USEPA's PFOA and PFOS maximum concentration levels (MCLs). PFAS concentrations in soil and sediment were frequently below detection limits, with PFOS and other long chain analytes typically being predominant. Future research and review efforts are needed to help address data gaps and discuss important considerations to further the understanding of ambient PFAS levels.

Disclaimer/Disclosure: The views and opinions expressed in the present study are those of the individual authors and not those of the U.S. Army or sponsor organizations.

2.06.P-We-034 Transcriptomic responses of eastern oysters (*Crassostrea virginica*) following a waterborne exposure to the short chain PFAS perfluorohexanoic acid (PFHxA)

Jessica Donaldson, John A Bowden, Lauren Blackman, Shisbeth Tabora Sarmiento and Joseph H Bisesi, University of Florida

Per- and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic chemicals used heavily in commercial goods due to their chemical properties. However, these same properties make them extremely mobile, persistent, and resistant to breakdown in the aquatic environment. Research has largely focused on the "legacy," long-chain PFAS. However, in recent years, these legacy PFAS have been phased out in many countries due to their growing regulations, ultimately leading to alternatives, namely short-chain PFAS, becoming prevalent in manufacturing and the environment. However, there is still a substantial gap in

understanding the toxicity profile of short-chain PFAS in exposed aquatic organisms. In addition to a focus on “legacy” PFAS, research has focused largely on the effects of PFAS in model organisms. Non-model, commercially, and environmentally important aquatic organisms are vital to assess potential PFAS toxicity to best protect these vulnerable populations. To address these data gaps, the current study aims to evaluate the transcriptomic profile in eastern oysters (*Crassostrea virginica*) following exposure to an environmentally relevant concentration and high concentration of Perfluorohexanoic acid (PFHxA). Oysters will be exposed to 200 ng/l, and 2000 ng/L of PFHxA via waterborne exposure for 28 days. Following exposure, oysters will be measured, shucked, and weighed to examine effects on growth, hepatosomatic index, and gonadosomatic index. Hepatopancreas will be processed for mRNA and libraries were prepared for RNA-seq analysis. iPathways (Advaita Corporation, Anne Arbor, Michigan) will be used for Gene Set Enrichment Analysis. The exposure is still underway with sequencing results awaiting to be received and analyzed for differential expression in oysters to examine novel toxicity pathways. However, we anticipate that there will be evident differences in gene expression between control and exposed oysters. While the exact differences are unknown, the overall goal of the current study is to generate hypotheses on the specific effects of PFHxA in a bivalve. This study will be the first to examine sub-chronic waterborne exposures to PFHxA and the resulting impacts on the transcriptome in eastern oysters. We foresee that these results will drive targeted approach methodologies and assays to assess the effects of PFHxA on a phenotypic level in non-model species.

2.06.P-We-036 Seasonal variation in per- and polyfluoroalkyl distribution in an estuarine ecosystem downstream of municipal wastewater outfalls

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Per- and polyfluoroalkyl substances (PFAS) are persistent and mobile contaminants frequently detected in aquatic environments downstream of both point and non-point sources, including municipal, industrial, and military facilities. While regional and environmental factors influence PFAS concentrations, the role of seasonal variation of PFAS profiles and contaminant loads in estuarine systems remains underexplored. To address this data gap, we assessed seasonal dynamics of PFAS in an urbanized Texas estuary receiving effluent from six municipal wastewater treatment plants (WWTPs). At each outfall, three downstream locations were sampled quarterly to quantify seasonal patterns in PFAS occurrence and partitioning behavior. Water and sediment samples were collected alongside key environmental variables to examine how seasonal shifts in water quality influence PFAS profiles and distribution within estuarine systems. The results highlight significant seasonal fluctuations in PFAS concentrations and profiles across sampling sites, underscoring the importance of assessing temporal variability in understanding PFAS behavior in estuarine ecosystems. These data enhance our understanding of how seasonal processes affect PFAS transport, fate, and ecological exposure in wastewater-impacted coastal environments.

2.06.T Fate and Effects of Per- and Polyfluoroalkyl Substances (PFAS) in Coastal Ecosystems

2.06.T-01 From microbes to predators: Exploring PFAS behavior in estuarine ecosystems and food webs *Kylie Rock, Clemson University*

Per- and polyfluoroalkyl substances (PFAS) are persistent, globally widespread contaminants, accumulating in aquatic environments such as estuaries. Urbanized estuaries often show the highest PFAS concentrations, but knowledge gaps remain about their fate, organismal exposure, and ecological impacts. Our work addresses these gaps through two main objectives: (1) investigating PFAS fate and microbial responses across estuaries with varying degrees of urbanization, and (2) evaluating PFAS exposure in sharks, considering estuary type (urbanized vs non-urbanized) and feeding strategies (generalist vs specialist). In Project 1, we hypothesize that microbes present in PFAS contaminated estuaries will experience selective pressures that favor the enrichment of PFAS-degrading species. Water and sediment samples were collected over a year from Charleston Harbor (CH; urbanized) and St. Helena Sound (SHS; non-urbanized) and analyzed for PFAS concentrations and

microbial abundance and diversity. While individual PFAS congeners were not significantly different across sites, total PFAS loads were significantly higher in CH compared to SHS. Interestingly, the genus *Dehalococcoidia* (phylum Chloroflexi), known for dehalogenation, was more abundant and consistently detected in CH, suggesting microbial community adaptation to PFAS contamination. Ongoing studies include culturing these microbes under aerobic and anaerobic conditions to investigate the degradation capacity of marine microorganisms across multiple PFAS compounds. Project 2 aims to test the following hypotheses: A) sharks from urbanized estuaries will have higher PFAS concentrations than those from less-impacted areas, and B) that generalist feeders will accumulate more PFAS due to consumption of higher-trophic-level prey. To test these hypotheses water, sediment, and shark tissue samples (blood and muscle) were collected from San Francisco Bay, CA, and Willapa Bay, WA (urbanized vs non-urbanized), and Bulls Bay and St. Helena Sound, SC (feeding strategies). Preliminary findings reveal significant differences in PFAS concentrations across sharks by sex, age class, site, and feeding strategy. Ongoing analyses include stable isotope measurements ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and analysis of PFAS in prey species. Together, these studies aim to deepen our understanding of PFAS dynamics in estuarine ecosystems—from microbes to top predators—and the implications for ecosystem health and biodiversity.

2.06.T-02 PFAS in San Francisco Bay: Data synthesis and strategy for future Investigations

*Diana Lin, Miguel Alexander Mendez, Adam Wong, Ezra L Miller, Kayli Paterson, Jennifer Dougherty, Martin Duong Trinh and **Rebecca Sutton**, San Francisco Estuary Institute*

Per- and polyfluoroalkyl substances (PFAS) are a complex class of persistent compounds, including several that are bioaccumulative and known or suspected to be toxic. These unique compounds have widespread uses across consumer, commercial, and industrial products, resulting in ubiquitous occurrence in humans, the environment, and wildlife. The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) has monitored PFAS across the Bay ecosystem, beginning in 2004 with various biota and since expanding to surface water, sediment, urban stormwater runoff, and municipal wastewater effluent. These studies found PFAS throughout the Bay, with particular concern for concentrations in sport fish, which indicate a potential risk for human health and led to the classification of PFAS as High Concern for the Bay under the RMP tiered risk-based framework for emerging contaminants. We will present a comprehensive synthesis of PFAS data for San Francisco Bay, providing insight on occurrence, transport and fate, and potential risks to ecological and human health. Sustained, multi-matrix monitoring of this important class of contaminants of emerging concern is a high priority for the RMP. The data synthesis guides an evolving strategy for future PFAS monitoring and science in the Bay to inform management actions, including possible state regulatory and legislative bans of PFAS in key classes of consumer products.

2.06.T-03 Investigating bioaccumulation of per- and polyfluoroalkyl substances across life stages in migratory estuarine fish

Shannon Jones, Ashley Pavia, Aaron Carlisle and Mi-Ling Li, University of Delaware

Estuaries are both vital fish nursery habitats and potential hotspots of contaminant exposure, including per- and polyfluoroalkyl substances (PFAS). These persistent compounds can bioaccumulate in fish, disrupting development and reproduction. Understanding how PFAS accumulate across fish lifetime is challenging due to changes in habitat, diet, and metabolism as fish grow. Here, we examine how physiological and environmental factors influence PFAS accumulation in migratory estuarine fish using Weakfish (*Cynoscion regalis*) as a model species. We collect water, Weakfish ranging from 45 to 200 mm standard length (estimated 50 days post-hatch to adult) and their known prey organisms from Delaware Bay, which has widespread PFAS contamination. We find that total measured PFAS burden in Weakfish increases with size during early development, peaks in medium-sized juveniles, then declines in larger juveniles and adults. We hypothesize that the initial increase in PFAS burden is related to dietary changes, and we will assess life stage specific dietary exposure via analyzing the prey items. Weakfish migrate from upper bay nursery areas to lower bay and then offshore habitats as they mature. We find that PFAS concentrations in water generally decrease along a spatial gradient from the upper

bay to the lower bay and then to the open ocean, which could explain the decreased PFAS burden observed in late-life Weakfish. These findings highlight how fish growth and migration shape PFAS exposure in estuarine fish and stress the need to consider life history and habitat use when assessing ecological risk.

2.06.T-04 Tracking PFAS fate and transport in a Texas estuary: Partitioning behavior and biological uptake downstream of municipal wastewater outfalls

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous contaminants that can accumulate in aquatic systems downstream of municipal, industrial, and military facilities. PFAS contamination can vary depending on discharge source, meaning environmentally relevant concentrations can significantly vary by region and environmental parameters. PFAS are extremely mobile and persistent in aquatic environments and are ubiquitously detected in sediment and surface waters globally – though data on PFAS fate and transport in estuarine environments are limited. To fill this data gap, we investigated PFAS profiles and environmental behavior downstream of six municipal wastewater treatment plant (WWTP) outfalls discharging into an urbanized Texas estuary. Three downstream locations were sampled at each outfall on a quarterly basis, to evaluate spatial and temporal variations in PFAS occurrence, relative abundance, and partitioning behavior. Paired water and sediment samples were collected along with a suite of environmental variables at each sampling location to assess the effects of water quality parameters (e.g., salinity, dissolved organic carbon) on fate and partitioning. We also analyzed PFAS concentrations in tissues of recreationally important fish species collected from the same areas to evaluate PFAS profiles in high trophic level biota, relative to those present in abiotic media. Our results reveal distinct PFAS signatures across sites, significant seasonal trends in PFAS concentrations, and detectable PFAS loads in fish. These findings contribute to our understanding of PFAS transport, environmental fate, and exposure pathways in estuarine systems receiving treated wastewater effluent.

2.06.T-05 Source apportionment and distribution of PFAS in an estuary: Multi-year monitoring in Casco Bay, Maine

Christoph Aeppli¹, Hannah Sterling¹, Heather Kenyon², Mike Doan² and Ivy Frignoca², (1)Bigelow Laboratory for Ocean Sciences, (2)Friends of Casco Bay

Per- and polyfluoroalkyl substances (PFAS) are persistent, bioaccumulative contaminants of growing concern for both environmental and human health. Although many studies have focused on PFAS occurrence in terrestrial and freshwater systems, their transport, transformation, and fate in estuarine and coastal environments remain poorly understood. These transitional zones provide vital ecosystem services and receive cumulative inputs from diverse sources, yet PFAS data in such settings are limited. To address this gap, we conducted three comprehensive PFAS sampling campaigns across more than 90 sites within the Casco Bay Estuary in Maine, targeting surface waters and, in one campaign, sediments. Additionally, we implemented a year-long, monthly monitoring program in a sub-bay impacted by a recent aqueous film-forming foam (AFFF) spill. Samples were analyzed using EPA Method 1633, allowing quantification of a broad suite of legacy and emerging PFAS at low detection limits. This combined effort aimed to characterize spatial and temporal PFAS patterns, identify dominant sources, and assess their relative contributions to the estuary. PFAS were detected at low background levels throughout the estuary, but distinct source-specific patterns emerged near river mouths and the AFFF spill site. Compositional signatures and concentration gradients pointed to rivers as major contributors, particularly for legacy PFAS such as PFOS and PFOA. The spill site exhibited elevated levels of AFFF-associated compounds, including PFOS, 6:2 FTS, and PFHxA, for several months, with tidally driven advection and dilution serving as the primary mechanisms of attenuation. Surprisingly, PFAS concentrations were not notably higher near urban centers compared to more rural areas, and input via wastewater treatment plants appeared minimal. Instead, non-point sources such as agricultural runoff—likely linked to biosolid

applications—and other unidentified upstream sources seem to dominate PFAS inputs to the estuary. This study provides new insights into the distribution and dynamics of PFAS sources in a temperate coastal system. The findings highlight the value of spatially intensive and temporally resolved monitoring for informing PFAS exposure assessment, source attribution, and coastal contaminant management. **Disclaimer/Disclosure:** Funding was provided from the National Estuary Program / EPA.

2.06.T-06 Connecting land use and PFAS Profiles in human-impacted coastal watersheds

Natalie A. O'Hern, Shannon Jones, Holly Michael and Mi-Ling Li, University of Delaware

Per- and polyfluoroalkyl substances (PFAS) are a group of nearly 15,000 synthetic organic compounds widely used in commercial and industrial applications. Coastal watersheds, which often feature dense populations and intensive land usage, are particularly vulnerable to PFAS pollution from multiple sources. The connection between pollution sources, land use, and PFAS profiles is understudied and has not been evaluated in coastal watersheds. Here, we conduct a field-based research project to characterize the sources of PFAS along the land-to-sea transition within three adjacent coastal watersheds of Delaware. Water, suspended sediments, and bottom sediment samples were collected from 26 sites within these watersheds. PFAS were quantified using targeted analysis in all samples, and water samples were also analyzed using non-targeted methods. We evaluated several geospatial characteristics at each site utilizing ArcGIS and the USGS StreamStats tool, such as drainage area, land use, and river distance from suspected sources. Using our targeted PFAS dataset and conventional statistical analyses (i.e., principal component analysis), we identified three distinct PFAS clusters: military, urban, and agricultural. Ongoing work leverages non-targeted PFAS data to identify more specific sources contributing to each cluster. We account for hydrologic connectivity to separate local land use effects from upstream PFAS contributions. This study will shed light on land use metrics and indicator chemicals that can serve as markers of conventional and emerging PFAS pollution, informing more strategic monitoring and management in coastal watersheds.

2.07.P-We Innovative Toxicity Endpoints: Behavior and Beyond

2.07.P-We-019 Effects of BP-3 exposure in zebrafish reproduction and behavior

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Organic UV filters like Benzophenone-3 (BP-3) enter the aquatic environment during recreational activities, wastewater discharge, and manufacturing. BP3 is the most common UV filter found and one with the highest concentration. BP3 continuous release into marine and freshwater environments, combined with the challenges of wastewater treatment, leads to sustained exposure of aquatic biota. Exposure to BP3 has been shown to alter motor and social behaviors, like reduced shoaling, impaired memory, increased anxiety, and decreased aggression, but little is known about its impact on reproductive behaviors. This study investigates the effects of chronic BP3 exposure on fish reproductive ability. Adult zebrafish were exposed to 0 µg/l, 150 µg/l, and 500 µg/l of BP3 for 36 days. Sexual behavior and egg output were recorded every 9 days. At the end of the exposure, blood was collected to test plasma for vitellogenin (VTG) and testosterone (T). The forebrain and midbrain were collected, RNA extracted, and sent for RNA sequencing. Results showed that females exposed to 150 µg/l had increased egg output, while 500 µg/l exposure reduced it. Plasma vitellogenin levels were higher in females exposed to both concentrations. Males exposed to 500 µg/l exhibited more freezing and less mating behavior, with similar trends at 150 µg/l. Females exposed to both concentrations showed increased aggression. RNA-seq analysis revealed significant gene changes associated with behavior, stress response, and DNA methylation. These findings suggest BP3 negatively affects fish reproductive performance, and further research on BP3's brain mechanisms could enhance understanding of its effects on both sexual and non-sexual behaviors.

2.07.P-We-020 The impact of heavy metal pollution and salinization on the olfactory system and associated behavior on freshwater amphipods

Jaskirat Kaur, Jamie Cochran, Wendy Sanchez Garcia and Andrea Durant, University of Washington

Increasing salinization of freshwater systems exacerbates multiple other ecological stressors by changing water pH, salinity, and the leaching of other chemical mixtures, including heavy metals (e.g., copper, Cu^{2+}).

Amphipod crustaceans are ubiquitous in freshwater systems and are a primary model for sediment toxicity and water quality testing due to their ecological relevance and sensitivity to contaminants. The amphipod species *Hyalella azteca* has high tolerance to elevated heavy metals and road salts compared to other freshwater crustacean model animals. However, recent studies in other aquatic crustaceans show that chronic exposure can impair olfactory systems, potentially disrupting essential behaviors such as foraging and mate detection. We aimed to determine the effects of environmentally relevant stressors—copper (Cu^{2+}) and road salt (NaCl)—and their interactive effects on the olfactory system and associated behaviors of *H. azteca*. Amphipods were acutely exposed for 96 hr, and daily survival rates and foraging behavioral responses were quantitatively assessed using a Y-maze assay, measuring the ability of individuals to detect and locate relevant chemical cues. Our preliminary results show that while survival is not significantly affected by ecologically relevant levels of road salt and copper, significant differences in foraging behaviors manifest differently between these individual stressors and the combination of these stressors. We also demonstrate that key olfactory receptor genes in the antennules are differently expressed between copper and road salt exposures, highlighting critical impacts on the olfactory system of amphipods. These findings indicate that current water quality standards, focused on acute toxicity and broad endpoints of individual stressors to survival and reproduction, do not consider more subtle and chronic sensory effects. Our work highlights the need to integrate behavioral endpoints and underlying physiological changes as early predictors of contamination to better protect aquatic populations.

2.07.P-We-021 Daphnia feeding bioassay and molecular biomarker expression as an early indication for chronic effect

Jingjing Xu, Luis Cubero, Steve Hicks, Chris Hensel and Tamara Dawn Lunsman, Corteva Agriscience

Environmental risk assessment of crop protection products requires toxicity information; *Daphnia magna* is a model organism used for evaluating aquatic invertebrate toxicity. In this study, we investigated the effects of pesticidal compounds (insecticides, herbicides, and fungicides) on *D. magna* feeding behavior and explored molecular biomarkers expression to assess organism stress. First, we measured the reduction of food intake by *D. magna* over a 48-hr exposure period; a reduction in food ingestion (i.e. a change in feeding behavior) could serve as an early indicator of long-term effects because adequate energy is necessary for reproduction and growth. For *D. magna*, acute toxicity is typically evaluated in 48-hr study, whereas, a 21-day exposure is typically used for characterizing chronic effects. When chronic toxicity information is either limited or absent, acute data to estimate the chronic toxicity by an ACR (acute-to-chronic ratio). As noted in numerous previous works, the ACR can vary between species and can be influenced by specific chemical properties. For *D. magna*, out of 532 compounds' data collected from PPDB database, about 85% have an $\text{ACR} \leq 100$. For molecules with higher ACRs, feeding reduction could be a potential reason for lower chronic endpoints. So quantifying *D. magna* food intake in a 48-hr exposure can support predictions and more accurately estimate chronic endpoints. We measured feeding quantity of *D. magna* after acute exposures to 17 pesticidal compounds (insecticides, herbicides, and fungicides) with a relatively larger ACRs. We concluded that not all compounds that impact growth and reproduction affect feeding quantity. However, a reduction in food ingestion was found to be mode of action dependent. For some key modes of action, a feeding assessment could serve as an indication of expected chronic effects. Further, we explored molecular approaches (i.e., real time PCR) to indicate organism stress upon acute exposure. Data suggests that it is promising to use biomarkers' expression to have an early indication of toxicity; however, the use of gene expression, specifically for the prediction of chronic toxicity is still under investigation.

2.07.T Innovative Toxicity Endpoints: Behavior and Beyond

2.07.T-01 The development of a novel shelter-seeking assay for the assessment of predator avoidance behaviors of fathead minnows (*Pimephales promelas*) across multiple life stages

Catherine Anne Wise¹, Lillianna Gonzales², Justin Hunt², Zoie Munoz², Marisa Ross² and Marlo Jeffries², (1)Biology, Texas Christian University, (2)Texas Christian University

Within ecotoxicology, there is a growing recognition of the value of assessing fish behaviors as sublethal endpoints in toxicity evaluations. As such, there is a need to develop assays capable of assessing ecologically-relevant behaviors in fish across their lifespan. The present study focused on predator avoidance behaviors, which impact predator-prey dynamics and influence population sizes, in fathead minnows (FHM). These behaviors, induced by chemical alarm cue, have been documented in adult FHMs. However, they are challenging to quantify and remain poorly understood in larvae. Thus, the overall objective of this study was to quantify and characterize the predator avoidance responses of FHMs across multiple life stages. To that end, the behavioral responses of adult male, adult female, and 14 day post hatch (dph) FHM larvae to alarm cue produced from pond-reared donors were quantified via the animal tracking software, ToxTrac. Exposure of FHMs to pond-reared alarm cue, regardless of life stage, induced significant changes in predator avoidance behaviors, verifying not only the use of alarm cue as a predator stimulus across all life stages but also the use of animal tracking software for quantifying predator avoidance behaviors. Notable stage-dependent differences in predator avoidance were observed, indicating that adult and larval FHMs may employ different strategies for evading predators. For example, adults tended to engage in 'hiding' behaviors (e.g., freezing, shelter seeking, etc.), whereas larvae displayed a preference for escape behaviors (e.g., increasing swim speed, etc.). The results of this study highlight the importance of considering life stage when designing behavioral assays and outline a methodology for assessing predator avoidance behaviors in FHMs across life stages. Future work may standardize the methodology of this experiment through replication and validation of its sensitivity to chemical contaminants, including those that disrupt olfaction or act as anxiogenic/anxiolytic agents.

2.07.T-02 Multigenerational effects of embryonic bisphenol S exposure in zebrafish (*Danio rerio*) neurobehavior

A K M Munzurul Hasan, Som Niyogi and Douglas Chivers, University of Saskatchewan, Canada

Bisphenol S (BPS), a widespread replacement for Bisphenol A, is frequently detected in aquatic environments and has raised concern due to its potential neurotoxicity. This study evaluated the long-term behavioural consequences of embryonic BPS exposure in adult zebrafish (*Danio rerio*) and their offspring. Embryos (F0 generation) were exposed to either 0.01% DMSO or 30 µg/L BPS from 4 to 120 hr post-fertilization (hpf) and reared in clean water until 6 months of age. To evaluate multigenerational impacts, F1 larvae were generated from adults exposed maternally, paternally, or parentally. Behavioral tests assessed social preference, locomotion, and anxiety-like behavior in adults, while thigmotaxis and reflexive movement (dark/light preference test) behavior were evaluated in 120 hpf larvae. Gene expression was measured in both generations using qPCR, focusing on serotonergic signaling and oxidative stress pathways. In F0 adults, BPS exposure significantly reduced social preference without affecting shoaling or anxiety-related behaviors. Although there was no significant change in whole-brain serotonin level (5-HT) levels, a slight upward trend was observed in the BPS group. At the molecular level, genes associated with oxidative stress (*gpx1a*, *tnf-α*) and serotonergic signaling (*slc6a4b*, *htr1d*, *htr2b*) were dysregulated. In F1 larvae, no significant behavioral alterations were observed; however, maternal and parental BPS exposure resulted in significantly elevated 5-HT levels compared to the DMSO group. These increases in serotonin were accompanied by altered expression of serotonergic genes (*slc6a4a*, *htr1aa*, *htr1b*) and antioxidant markers (*gpx1a*, *creb1a*, *mn-sod*), depending on the exposure type. These findings indicate that embryonic BPS exposure leads to lasting neurobehavioral impairments in adults and latent molecular effects in offspring, even in the absence of early behavioral symptoms. This work underscores the importance of assessing both behavioral and molecular endpoints to understand the full scope of transgenerational risks associated with emerging environmental contaminants like BPS.

2.07.T-04 The impacts of dietary PFOA on the reproductive behavior of male zebrafish (*Danio rerio*)

Alexis Khursigara, Samantha Gomez, Catty Geyer, Teika Brown, Nat Hazzard, Marina Gerollari, Rylee Allen and Shelby Zuromski, Montclair State University

Nicknamed “forever chemicals”, per- and polyfluoroalkyl substances (PFAS) are manmade chemicals that are ubiquitous in the aquatic environment. Despite its production and use being heavily phased out, one of the most prevalent PFAS in the environment is perfluorooctanoic acid (PFOA). Recent research has highlighted the suite of injuries that PFOA can induce in aquatic organisms like fish, including changes in behavior, growth, development, reproductive success, and endocrine function. Despite the growing body of research, the effects of chronic dietary exposure of adult fishes have been understudied. To address this knowledge gap, the effects of three dietary concentrations of PFOA were assessed on male zebrafish (*Danio rerio*). After 1, 2, and 4 weeks of exposure, length and mass were taken prior to males entering a mating trial that assessed distance traveled, time spent moving, average velocity, average distance to the female, and time in contact with the female. Preliminary results indicate an effect of PFOA on growth, distance traveled, and velocity. Since males must be active during mating and often chase females to get them to release eggs, changes in these parameters can have significant implications for reproductive success for male fish.

2.07.T-05 Determining how space use affects exposure and toxicity of three common use insecticides

Sam Nutile, Kody Klein, Adam M. Simpson and Lynne Beaty, Penn State Behrend

Insecticides play a crucial role in advancing agriculture, yet their runoff poses environmental risks to non-target areas. Evidence from laboratory and field toxicity assessments confirms the harmful effects of insecticides on diverse aquatic species. However, existing evaluations of insecticide toxicity often assume uniform exposure, an assumption that may not be accurate under field conditions. It is increasingly recognized that behavioral patterns can influence toxicity in the context of insecticide contamination, an aspect that remains understudied. Therefore, the objective of this study was to explore how space use influences toxicity of three widely used insecticides, bifenthrin, permethrin, and chlorpyrifos, on sediment toxicity to *Hyalella azteca*. Glass beakers were modified with steel rings to create exposure arenas with contaminated sediment placed either inside, outside, or both inside and outside the ring, resulting in three unique conditions for 48 h toxicity tests with *H. azteca*. For all insecticides, contaminant placement influenced exposure and resulting toxicity to *H. azteca*. Placing pesticides within the steel ring resulted in higher lethal concentration 50 (LC50) values, indicating reduced mortality rates. Conversely, lower LC50s were recorded when contaminated sediment was placed outside or both inside and outside the ring. This suggests that *H. azteca* preferentially spend time in contact with the outside edge of the beaker, leading to higher exposure and increased toxicity when insecticides were placed outside the steel ring. As such, our results suggest space use is a major factor in predicting insecticide exposure and toxicity and should be considered in the development of laboratory toxicity tests.

2.07.V Innovative Toxicity Endpoints: Behavior and Beyond

2.07.V-01 Intergenerational effects of environmentally relevant concentrations of dietary selenium on zebrafish, *Danio rerio*

Md Uddin, Jinnath Ritu, Som Niyogi and Douglas Chivers, Department of Biology, University of Saskatchewan, Canada

Abstract Selenium (Se) is an essential trace element for fishes; however, it becomes extremely toxic at concentrations slightly above physiological requirements. While the toxicological effects of Se on adult fishes are well documented, its intergenerational and neurobehavioral impacts remain poorly understood. Selenomethionine (SeMet), the predominant organic form of Se in aquatic food webs, is primarily acquired through diet, yet most existing studies have relied on artificial SeMet-spiked diets that do not accurately reflect environmental exposure routes. To address this limitation, we conducted a trophic transfer study by exposing blackworms (*Lumbriculus variegatus*) to waterborne SeMet (50 and 200 µg/L) for 14 days, resulting in Se accumulation of 17 and 43 µg/g dry weight, respectively. These Se-enriched blackworms were subsequently

used as a dietary source for adult zebrafish (*Danio rerio*) over 60 days. Following exposure, adults were bred and the F1 larvae were reared in clean water until 5 days post-fertilization (dpf) to assess intergenerational effects. Our results demonstrated a significant increase in larval mortality and deformities in both Se-exposed groups compared to control. Behavioral assays revealed impaired thigmotactic and reflexive responses, indicating neurobehavioral dysfunction. Moreover, Se exposure led to dose-dependent increases in reactive oxygen species (ROS) production and apoptosis. Molecular analyses showed dysregulation of genes and proteins associated with dopaminergic, serotonergic, and cholinergic pathways, as well as disruptions in neurogenesis. These findings provide novel evidence that dietary exposure to environmentally relevant concentrations of SeMet can exert significant intergenerational neurodevelopmental toxicity in zebrafish. This study underscores the ecological relevance of trophic transfer in Se toxicity research and highlights the need for improved regulatory guidelines to mitigate Se contamination in aquatic ecosystems. **Disclaimer/Disclosure:** MD Helal Uddin was supported by the Dean's Doctoral Scholarship of the University of Saskatchewan and Fish and Wildlife Development Fund, Ministry of Environment, Canada. This work was supported by the discovery grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to S. Niyogi and D. P. Chivers.

2.08.P-Mo Investigating the Sources and Toxicity of Salt and Multi-Ion Mixtures to Support Resource Management

2.08.P-Mo-095 Effect of road salt formulation on *Lemna minor* toxicity

Trevor Alan Grandy¹, Alexis Sanchez², Myah Shier³ and Amanda D Harwood¹, (1)Alma College, (2)North Central College, (3)Wartburg College

Research suggests that freshwater salinization elicits toxicity to a variety of freshwater aquatic organisms. Many studies focus on the effects of salinization on aquatic invertebrate species testing and neglect the impacts on aquatic plants. Additionally, while formulation has been shown to impact toxicity to aquatic invertebrates, less is known about plants. The objective of the current study was to evaluate the effects of different road salt formulations on *Lemna minor* survival and growth. Standard 7- day toxicity assays were performed at environmentally relevant concentrations of NaCl, Beet Deicer, and three other commercially available formulations to determine survival, frond number, and dry weights post exposure. Preliminary data suggests that NaCl may have an impact on survival and growth of *L. minor*. If freshwater salinization from various road salt formulations impacts *L. minor* survival and growth, it may not only impact the health of this species, but pose a risk to other freshwater species in aquatic ecosystems that rely on aquatic plants.

2.08.P-Mo-096 Impacts of salinization on leaf litter decomposition by *Hyaella azteca*

Trevor Alan Grandy¹, Myah Shier², Alexis Sanchez³ and Amanda D Harwood¹, (1)Alma College, (2)Wartburg College, (3)North Central College

Sodium chloride (NaCl) has emerged as a significant environmental contaminant, primarily due to the widespread application of road deicing salts in response to increasing frequency and intensity of winter storms. Extensive research has demonstrated that freshwater salinization from road salt runoff can cause toxicity to aquatic organisms and negatively impact ecosystem function. However, less is known about how indirect effects of salinization impact the quality of diet and leaf litter decomposition for freshwater invertebrates. Leaf discs from two tree species, *Acer saccharum* and *Acer platanoides*, were conditioned in various concentrations of NaCl and provided as food to *Hyaella azteca*. Survival, changes in *H. azteca* mass, and leaf disc consumption were measured post exposure. Preliminary data suggests that NaCl may influence the feeding behavior and survival of *H. azteca*, potentially reducing ingestion rates and causing mortality. If freshwater salinization impacts the survival and shredding of *H. azteca*, it could have ecosystem level consequences.

2.08.P-Mo-097 Evaluating treated produced water for potential beneficial uses outside the oil field

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Mobil

Desalination of treated produced water (PW) from oil and gas operations can fill a key role in the larger management of PW. It is important to understand the quality of the treated PW with respect to the potential for toxicity to aquatic and terrestrial organisms as well as the chemical composition of the treated water relative to established toxicity thresholds. The present work will utilize existing risk assessment strategies to systematically identify likely chemical stressors and use the data to interpret available whole effluent toxicity test data. The workflow will be applied to several available bench and field scale pilot systems to look for commonalities in types of constituents present in the treated PW, and the likely stressors. Potential implications of these findings on various potential discharge scenarios (e.g., to land, or to surface water) will be discussed.

2.08.P-Mo-098 Investigating the acute and chronic effects of low ionic conditions on cladocerans

Jordana Van Geest¹, Connor Pettem¹, Chelsea Grimard¹, Melanie Gallant² and Derek Green³, (1)WSP Canada Inc., Canada, (2)Nautilus Environmental Company, Canada, (3)Elk Valley Resources Operations, Canada

A North American mining company operating in the Elk Valley of British Columbia has recently been exploring water quality treatments to manage the environmental effects of elevated concentrations of surface water constituents. While most toxicological evaluations surround thresholds of elevated concentrations eliciting toxicity, the current investigation investigated the potential role of low ionic conditions causing harmful ion dysregulation in aquatic organisms. Through this investigation, a desktop literature review was undertaken to identify minimum water quality thresholds for freshwater aquatic receptors exposed to low ionic conditions (i.e., identify minimum concentrations of ions recommended to prevent adverse responses in organism culturing and testing). Limited information was found on the effects of low ionic strength waters on freshwater receptors. Therefore, additional acute (48-h *Daphnia magna*) and chronic (3-brood *Ceriodaphnia dubia*) toxicity testing was conducted using Environment Canada test protocols to confirm that the hypothetical discharge was not acutely toxic to species used for compliance, and that major ion concentrations measured at the edge of the mixing zone would not be at levels that could cause chronic toxicity to sensitive representative aquatic species. The specific purpose of this testing was to address uncertainties surrounding the potential for low alkalinity to cause acute effects to *D. magna* survival and chronic effects to *C. dubia* survival and reproduction. The approach was intended to isolate alkalinity as a source of stress; thus, a wide range of alkalinity concentrations were tested while, while maintaining an overall mixture of other major ion constituents within the range of plausible discharge conditions under varying water quality treatment conditions. Test waters were formulated to provide a target range of alkalinity concentrations (20-100 mg/L as CaCO₃), with minimum concentrations of TDS (≥ 150 mg/L) and hardness (≥ 100 mg/L). Both acute and chronic tests met all control and water quality acceptability criteria. No adverse effects were observed on acute survival or mobility of *D. magna* or chronic survival and reproduction of *C. dubia* in any of the test water treatments (20-100 mg/L as CaCO₃ alkalinity), suggesting that these species are tolerant of acute (*D. magna*) and chronic (*C. dubia*) exposures to >5-fold lower alkalinity than Environment Canada culturing protocols under conditions of moderate hardness.

Disclaimer/Disclosure: This work was funded by Elk Valley Resources Operations.

2.08.T Investigating the Sources and Toxicity of Salt and Multi-Ion Mixtures to Support Resource Management

2.08.T-01 Development of a site-specific effluent discharge benchmark for total dissolved solids and the role of major ion toxicity

Connor Pettem and Gary Lawrence, WSP Canada Inc., Canada

There are currently no federal water quality guidelines for total dissolved solids (TDS), despite rapidly developing science in the evaluation of major ion toxicity, and site-specific testing has been recommended to develop benchmarks for environmental management of TDS. Starting in 2019, a three-phased approach was undertaken for a North American mine that included developing interim discharge and edge of mixing zone targets for TDS, designing and completing validation studies for the discharge and receiving environment, and

finalizing the TDS benchmarks for the purpose of a Water License Amendment for authorization to discharge. In the validation phase, acute and chronic toxicity testing was conducted to confirm that the discharge was not acutely toxic to species used by Environment Canada for compliance, and that TDS concentrations measured at the edge of the mixing zone would not be at levels that could cause chronic toxicity to sensitive representative aquatic species. Through this evaluation, it became readily apparent that TDS composition was an important determinant of toxicity. In baseline tests, site-specific testing displayed no acute or chronic lethality to *Daphnia magna* and *Ceriodaphnia dubia* for a chloride-dominant (50%) TDS mixture >3,000 mg/L, and sublethal reproductive effects to *C. dubia* were not apparent with TDS concentrations >2,500 mg/L. However, modification of the water composition confirmed the findings of others (e.g., Mount and Erickson) that toxicity of major ion mixtures is sensitive to composition. Supplemental chronic testing using *C. dubia* was undertaken where the synthetic discharge was manipulated to test increases and decreases in chloride composition (range of 24% to 60% Cl) while maintaining the overall TDS concentrations constant (4,250 mg/L). The results indicated meaningful changes to the concentration-response profile, with increased toxicity seen (LC50 of 3,250 mg/L TDS) in the high chloride treatments, and no toxicity in the low chloride treatments where sulphate salts were increased (LC50 of >4,250 mg/L TDS). These findings, although limited to a subset of key anions within TDS, provide additional evidence that overall mixture composition is an important factor governing toxic potency, and confirmed the work of others that chloride is more toxic than sulphate in these environmental realistic mixtures.

2.08.T-02 Perspectives on salt toxicity and remediation for aquatic habitats impacted by multiple and distinct salinity sources

Anthony Knafla, Greg Huber, Vicky Winter, Jonathan Donahue, Kush Patel and Sebastine Amaechina, Equilibrium Environmental, Inc., Canada

Various sources of salt contamination are present in the environment. Anthropogenic use of land for oil and gas production, municipal infrastructure (roads, storage yards), residential acreages, salt mining, and agriculture (farming, ranching), has resulted in increasing load of salts onto aquatic habitats. In some regions, anthropogenic salt load is cumulative with naturally salinity. In most jurisdictions, the general rule is, polluter pays. Apportioning environmental impact-related liability to responsible parties in situations where an aquatic habitat has been impacted by multiple sources is challenging. The use of isotopes as well as bromide:chloride ratios has assisted with resolving apportionments. Distinct salinity sources have unique anion/cation combinations that contribute towards net salt loading onto aquatic habitats. Toxicity can result from total osmotic stress in addition to specific ion interactions with biological tissue. Furthermore, interactions between ions at the biochemical level can alter physiological response from exposure to ion mixtures. Research in the lab with sensitive species and single organisms (freshwater mussels, water flea, microalgae, mayfly, amphibian) determined that hardness was an ameliorating factor in chloride toxicity, and physiological response varied with unique cation/anion compositions. Published data suggests lab-based toxic response can be greater than in the field. Studies conducted on various wetland habitats similarly indicated divergence in toxic response from thresholds reported in the literature. Minimal disturbance was identified at the community level between impacted and background wetland habitats. This was postulated to result from elevated concentrations of naturally encountered salts (e.g., sodium sulphate) and adaptation, as well as influence from other toxicity modifying factors. Salinity remediation of aquatic ecosystem is challenged by disturbance to biota and nutrients. For some habitats, natural regeneration may occur with a low disturbance footprint if source loading is controlled. Source control can include managing above ground discharges as well as groundwater remediation preventing discharge into the hyporheic zone. Understanding hydrology and hydrogeology dynamics specific to an ecosystem can be used to gauge whether direct remediation of surface water and sediment is required. Other approaches were tested for facilitating natural regeneration. **Disclaimer/Disclosure:** Funding for portions of this work has been provided by the Petroleum Technology Alliance Canada.

2.08.T-03 Super-stable chloride and sodium mineralization in synergy with halotolerant hydrocarbon biodegradation for new hybrid bioremediation and salinity mitigation for resource-extraction sites

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Natural salts, such as halite (NaCl), are broadly scattered in semi-arid and arid soil environments in western Canada, which is connected to elevated salinity/sodicity in receiving water bodies. The hydrocarbon industry thus often faces the challenge of dealing with hydrocarbons and salinity/sodicity at impacted sites and the transport of excess chloride ions and hydrocarbons to water resources. Nonetheless, technologies that integrate hydrocarbon bioremediation and desalination have not been developed due to the complexity of largely unexplored interactive relationships between hydrocarbons, salts and living salt-tolerant bacteria in contaminated environmental matrices. We developed a mayenite/zeolite composite, which promotes the super-stable mineralization of Cl⁻ and Na⁺, and thus decreases salinity/sodicity. This salinity remediation method can be combined with the biostimulation and/or bioaugmentation of halotolerant hydrocarbon-degrading bacteria for hydrocarbon biodegradation under varying saline/sodic conditions. This combination was applied to co-contaminated groundwater, soils and soil/water slurries from oilfields. Cl⁻ present in soil and water transforms the mayenite as it is incorporated into the positively charged layered framework of hydrocalumite, whereas Na⁺ is adsorbed onto the negatively charged zeolite. Cl-hydrocalumite and Na-zeolite become naturally bonded through cementitious stabilization, greatly decreasing the extractability and leachability of Cl⁻ and Na⁺ in the presence of hydrocarbons. The study suggested the super-stable mineralization of Cl⁻ and Na⁺, resulting in substantially decreased or negligible extractable Cl⁻ and Na⁺ concentrations under acidic to alkaline conditions. Hydrocarbons were also trapped in the mineral matrix, decreasing extractable hydrocarbon concentrations significantly. In suspension, colloidal fine clays settle directly through mayenite-induced electrostatic attraction, without energy-driven mixing. We also identified the beneficial effect of further sodicity reduction and effluent pH control caused by halotolerant bacteria carrying out hydrocarbon biodegradation, which helps to improve water quality and soil health. Thus, hydrocarbon biodegradation and salinity/sodicity mitigation can be synergistically hybridized. The outcomes offer a new direction for developing various passive and semi-passive remediation technologies for hydrocarbon- and NaCl-impacted soils and water at resource-extraction sites.

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2.08.T-04 Water management perspectives for onshore O&G operations

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Oil and gas activities in the Permian basin (TX and NM) generate approximately 20 million barrels of produced water (PW) daily across the industry. The PW is -extracted from the formations where the hydrocarbon resources are located, has a naturally high salinity range 100-250 g/L), and contains ammonia, hydrocarbons, and other constituents. Common management strategies include recycle of PW back into oil and gas operations, injection into saltwater disposal wells, and increasingly include treatment to produce polished desalinated water. This presentation will discuss challenges and opportunities associated with these general strategies. For example, the potential for induced seismicity and the general water scarcity in this region encourages exploration of other potential beneficial reuses of the raw PW as well as reuse of the polished desalinated water. The potential volumes of treated PW could have a beneficial impact on both the quality and quantity of available water in this region. This has prompted recent efforts to characterize treated water for potential discharge into the environment. Other practical considerations are needed for the spatially distributed nature of the operations such as water conveyance systems as part of the management strategies, or even further, the potential implications of mixing very low salinity treated water into the arid, moderately saline environments in

this region. This presentation is intended to provide some general understanding of the issues to support development of practical solutions to these challenges.

2.08.T-05 Field-based evaluation of treated produced water for agricultural irrigation

Huma Malik¹, Emily Bechtold¹, Mike Wilkins¹, James Rosenblum², Ryan Hall³ and Thomas Borch¹, (1)Colorado State University, (2)Colorado School of Mines, (3)NGL

In arid and semi-arid regions, the reuse of treated produced water (PW) from oil and gas operations is being explored as a potential alternative water source for agriculture. However, field-scale studies of PW reuse for agriculture are limited. This field study was conducted in Orla, Texas to evaluate the effects of treated PW irrigation on crop growth, soil chemistry, and the soil microbiome. Sorghum-sudangrass was grown for three months under two irrigation regimes: PW and fresh water (FW). PW was treated using a combination of methods including pretreatment, membrane filtration and ion exchange. Soil chemistry was assessed using standard extraction and elemental analysis techniques, and microbial community composition was evaluated through high-throughput DNA sequencing. Post-harvest analysis showed no significant differences in plant biomass or height between the treatments. However, soil analysis revealed significantly elevated boron concentrations in the PW-irrigated soil. Preliminary findings also indicated modest microbial shifts in bacterial and fungal community composition between FW and PW treatments. These results provide an important foundation for evaluating the viability of treated PW irrigation and can be used to develop regulatory frameworks for PW discharge in the Permian Basin.

2.08.T-06 Data review from a 2024 field pilot treating produced water from the Permian Basin to meet presumed surface discharge standards

Ryan Hall, NGL Energy Partners, New Mexico State University

Oil extracted from the Permian basin in west Texas and southeast New Mexico is accompanied by vast amounts of hypersaline produced water. This 25 million barrels of water per day is presently managed through saltwater injection wells, and to a lesser extent infield recycling within oil and gas. Injection pore space is limited and the region is severely water stressed. This has led industry to consider treatment to enable surface discharge of the treated produced water. There is an overall lack of data demonstrating that Permian basin produced water can be successfully treated to a level enabling surface discharge. Specific concern focuses on unknown organics that may be in the water and what synergistic effects they may have in the environment. This research focuses on third party analytical data obtained from raw and treated produced water samples from a field pilot performed in the summer and fall of 2024 in Orla Texas. The data set includes over 300 target analytes as well as indicators, tentatively identified compounds and whole effluent toxicity testing. Samples of regional municipal drinking water and the receiving body of water were also collected for reference. The data set has been shared with both the Texas and New Mexico Produced Water Consortia. It has been shared with the Texas Commission on Environmental Quality to support pending discharge permits. It has also been shared with the EPA to support their updating of 435 Subpart E.

2.09.P-Th Large River Monitoring; Programs, Design, Methods and Interpretation

2.09.P-Th-110 PCB monitoring in major Puget Sound rivers: High-volume, long-term, statewide

Brandee Era-Miller and Elisa Rauschl, Washington State Department of Ecology

In 2020, Washington State won a settlement against Monsanto, the largest producer of PCBs in the United States. The State Legislature directed some of the settlement funds to support monitoring of PCBs. The Toxics Studies Unit at Washington Department of Ecology created a Statewide PCB Monitoring Program (PCB-MP) to address the long-term problem of PCBs in our waterways. The PCB-MP has 2 components: - Long-term, year-round monitoring on 8 major rivers that feed into the Puget Sound: Nooksack, Skagit, Stillaguamish, Snohomish, Duwamish, Cedar, Puyallup & Nisqually - Exploratory monitoring with a mobile sampler to focus on collaborations with regional partners to further our understanding of how PCBs affect the waters of the State

2.09.T Large River Monitoring; Programs, Design, Methods and Interpretation

2.09.T-01 Bioavailability of metals in slag-contaminated sediments

John Toll, David K. DeForest, Robert C. Santore, Amy Shaw and Kelly E Croteau, Windward Environmental LLC

The sediments of the Upper Columbia River in Washington State have been impacted by slag discharged from a smelter just north of the U.S. boarder with Canada. The slag contains appreciable amounts of entrained metals and as a result, the concentrations of metals in these sediments are elevated. For example, sediment concentrations of both copper and zinc exceed the respective probable effect concentration (PEC) by over an order of magnitude. The bioavailability and toxicity of metals in these sediments were assessed by chemical measures (e.g. Equilibrium partitioning guidelines) and bioassays with *Hyalella azteca*. Despite the high total metal concentrations in these sediments, neither total metal nor slag content are good predictors of toxicity to *H. azteca*. Concentrations of simultaneously extracted metals (excess SEM) are more aligned with measured toxicity, but the best predictor of bioavailability and toxicity is the concentrations of metals in porewater. Equilibrium partitioning theory predicts that the toxic effect of metals in sediments may result from exposure to sediment pore-water in a manner that is similar to effects resulting from exposure to the water column. Porewater metal concentrations were evaluated by comparison with USEPA water quality criteria, and by biotic ligand model simulations that considered the effects of simultaneous mixtures of multiple metals (mBLM). These porewater evaluations allows sediment samples to be ranked to identify which samples would be most likely to cause toxicity. The mBLM can also identify which metals in the mixture of metals are most likely to be responsible for any observed toxicity. The overall good performance of the mBLM suggests that it is a powerful predictive tool for use in assessing risk from metal concentrations in field sediments and could be used as part of a general methodology in the development of sediment guidelines.

2.09.T-02 Monitoring large river systems in the Puget Sound drainage for sources of toxics impacting juvenile Chinook salmon

Alex Gipe¹ and William Hobbs², (1)Department of Ecology, (2)Washington State Department of Ecology

Juvenile Chinook salmon are impacted by toxics throughout their migratory pathways, from their natal rivers to the Puget Sound. In particular, juvenile Chinook from the Snohomish and White-Puyallup watersheds have tissue concentrations of PCBs and PBDEs high enough to increase disease susceptibility and mortality. We will present two case studies that used river-wide monitoring approaches to identify sources of PCBs and PBDEs impacting juvenile Chinook. In each case study, a watershed-wide source assessment was implemented with the goals of (1) identifying and prioritizing contaminant sources and (2) determining the pathways from source to juvenile Chinook. The scale and complexity of large river systems make it challenging to collect system-wide environmental concentrations of low-level toxics, which are needed to assess and prioritize sources. Synoptic surveys utilizing passive samplers deployed for ~30 days during low and high flow events provided system-wide surface water containment concentrations, allowing for the identification of hotspots. Follow-up sampling events were used to identify probable sources associated with each hotspot. In the Snohomish River watershed, areas of high PBDE surface water concentrations were traced to the discharge of treated wastewater from municipal outfalls in two river reaches. Ongoing work in the White-Puyallup watershed has identified several areas within the estuary and river system with high levels of PCBs and PBDEs. Follow-up sampling is currently being conducted to identify the sources of these hotspots. The results of these monitoring programs have been used to introduce best management practices and reduction efforts for PBDEs in municipal sewer and treatment systems with the goal of reducing the impacts of toxics on out-migrating juvenile Chinook in the Puget Sound drainage.

2.09.T-03 Erosion potential and the impact on nutrient concentrations of the Eleven Point River, Arkansas

Karimot Shobowale and Jennifer Bouldin, Arkansas State University

Agricultural land use and water quality effects have co-occurred throughout human history. It is challenging to assess the effects on water quality due to the nonpoint nature of agricultural contaminants. For effective water quality management, it is also important to understand the physical characteristics of a watershed and how it contributes to soil erosion and the transport of contaminants into the receiving waterbodies. The purpose of this study was to understand the interplay between topography and agricultural activities such as hay production & poultry production on the transport of nutrients into the Eleven Point River, Arkansas. Geographical Information System GIS techniques were used to analyze morphometric parameters to categorize sub-watersheds based on erosion potential and the land cover characteristics. The four sub-watersheds sampled were classified into three erosion susceptibility categories based on the summation of the values of morphometric parameters. EAS and DRY were the most susceptible to erosion. THO ranked second, and HUB was ranked the least susceptible. All watersheds were classified as low and medium percent agricultural land cover based on the land cover analysis. A generalized linear mixed model (GLMM) was used to examine how poultry facility density, percent agricultural land cover and erosion potential impact concentration of dissolved nutrients (nitrate, nitrite and orthophosphate) and total nutrients. Results showed that mean nitrate (2.120 – 2.250 mg/L) and nitrite ranged from (0.0224 – 0.0231 mg/L). The GLMM identified erosion category as a significant predictor of nitrate and nitrite. Orthophosphate and total phosphorus ranged from 0.054 – 0.070 mg/L and 0.038 – 0.048 mg/L, respectively. Total phosphorus was significantly influenced by erosion category and percent agriculture land cover, while orthophosphate was influenced by both of these variables and the number of poultry facilities. This study demonstrates that analysis of watershed erosion potential and surrounding land use can help predict nutrient concentrations in surface waters. Further investigation into the geology of the Eleven Point sub-watersheds is recommended to support planning for future land use to minimize potential impacts on water quality.

2.10.P-Tu New Advances in the Ecotoxicology of Coral Reef Organisms

2.10.P-Tu-009 Chemical contaminant inhibition of settlement and survivorship in Caribbean corals

Dorothy-Ellen Abigail Renegar¹, Ellen Skelton² and Carys Louise Mitchelmore³, (1)National Coral Reef Institute, (2)Nova Southeastern University, (3)University of Maryland, Center for Environmental Science

Early life stages of scleractinian corals are generally regarded as more sensitive to contaminants compared to adults. Spawning and larval development of most coral species on Florida's Coral Reef occurs through the summer and fall, which coincides with substantial rain-event driven terrestrial discharges and storm-driven sediment resuspension, associated with risk of exposure to chemical contaminants in the water column and sediment/substrate. Impacts of contaminants on the early life stages of corals can lead to death or sublethal effects resulting in severe structural impacts on populations and communities. The inhibition of larval settlement and/or metamorphosis, which are crucial developmental steps and ultimately determine survival, have been examined for only a very limited range of chemical contaminants. Larvae of *Montastraea cavernosa* were exposed to chemical contaminants as nominal dissolved concentrations or pre-contaminated settlement tiles. Finely ground crustose coralline algae (CCA) was used to induce settlement, and tiles checked for settlement and metamorphosis daily for 96 hr or until no observable swimming larvae are found (i.e. either settled or perished). Target chemicals include metals (copper, arsenic, and zinc), hydrocarbons (chrysene, fluoranthene, and phenanthrene), and common persistent organic pollutants (tributyltin, diuron, and octocrylene) as these chemicals are substrate and sediment-associated contaminants recognized for environmental persistence and potential toxicity. These individual chemical contaminants were identified in a recent metanalysis as contaminants of potential impact as they have been detected at concentrations which exceed both EPA water quality standards and State of Florida threshold and/or probable effects levels in waters and sediments across the southeast coast of Florida. This study is designed to establish preliminary acute and subacute threshold concentrations for inorganic and organic chemical contaminant classes for early life stages in Atlantic corals, and contribute to the establishment of standardized toxicity test methods for early life stages of coral. Standardized test methods are a current need and are essential for determining the environmental risk of

chemical contaminants in coral reef locations. Conducting environmental risk analyses have significant management implications for successful restoration approaches.

2.10.P-Tu-010 Assessment of Caribbean gorgonian's behavioral response to acute exposures of dissolved copper

Brady Estrada¹, *Dorothy-Ellen Abigail Renegar² and Carys Louise Mitchelmore³*, (1)Nova Southeastern University, (2) Nova Southeastern University, (3)University of Maryland, Center for Environmental Science
Caribbean reefs ecosystems have faced notable environmental degradation over the last four decades due to severe heat stress, exposure to aquatic contaminants, and disease. While this has caused drastic reduction in cover for many species of coral, gorgonian populations remain relatively consistent despite chronic exposure to the same stressors. This is partly due to their increased thermotolerance, however gorgonian response to local aquatic contaminants is unknown. To improve our understanding of their tolerance to poor water quality, this experiment will expose a variety of gorgonian species local to the Florida's Coral Reef (FCR) to varying concentrations of dissolved copper as copper is a commonly used reference test chemical. The coast of Florida has several international ports that serve as major inputs of dissolved copper in the water column, with Port Everglades being the closest to this study's target communities. Through a series of acute 96 hr range finding experiments, a diversity of gorgonian species will be exposed to dissolved copper concentrations ranging from 0ug/L-400ug/L, in order to determine LC50s which can be compared to current water quality standards and acute thresholds for other reef organisms, where it is expected that gorgonian tolerance to dissolved copper will be higher than that of hard corals. This would imply that gorgonians resistance to copper is a confounding factor in their persistence in these reef environments. This study will serve as the cornerstone of gorgonian toxicity testing through the development of a new methodology tailored specifically towards gorgonian taxa. The information gained can be applied to our understanding of how local and global environmental stressors impact reef configuration, and how current dominating species such as gorgonians can be used as bioindicators for compromised water quality.

2.10.P-Tu-011 Toward ecological risk assessment of ultraviolet filters in Japanese subtropical waters: Native coral bioassays and field surveys

Rie Tai¹, *Hiroyuki Mano¹ and Wataru Naito²*, (1)National Institute of Advanced Industrial Science and Technology, Japan, (2)Riss, National Institute of Advanced Industrial Science and Technology, Japan

Ultraviolet (UV) filters are indispensable components of sunscreens and other personal-care products because they protect human skin from solar radiation. After use, these compounds enter coastal waters either indirectly via domestic wastewater or directly during recreational activities. The global growth in UV-filter production and consumption has intensified concerns about their ecological effects, particularly on coral reefs. Japanese subtropical waters, linked to the Coral Triangle through the Kuroshio Current, may therefore face regulatory watch comparable to that emerging elsewhere; however, ecotoxicity testing and risk assessment tailored to Japanese reef ecosystems remain limited. This study aims to evaluate the risks that representative UV scattering and absorbing agents pose to coral growth and reef sustainability under environmentally realistic conditions. First, chronic-toxicity protocols will be established for native coral species such as *Acropora digitifera* and used to examine the effects of priority UV filters. Concurrent field sampling will determine ambient concentrations of target compounds in reef waters. In a second phase, exposure models will integrate effect thresholds with predicted discharge scenarios to assess the efficacy of proposed risk-mitigation measures. The presentation will outline the overall study design, describe the newly developed test protocol for *A. digitifera*, and summarize preliminary toxicity testings for UV-filter ingredients. **Disclaimer/Disclosure:** This work was supported by JSPS Grant-in-Aid for Early-Career Scientists Grant Number 23K17056. This work was supported by SHISEIDO Company. The authors would like to thank T. Nakamura, Ryukyu University, for providing larvae and his kind input advice.

2.10.P-Tu-012 The chronic exposure to pharmaceuticals and phthalates results in sub-lethal effects in the zooxanthellate coral *Balanophyllia europaea*

Arianna Bellingeri¹, Chiara Spagnolo¹, Erik Caroselli², Stefano Goffredo² and Ilaria Corsi¹, (1)Department of Physical Sciences, Earth and Environment, University of Siena, Italy, (2)Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy

Corals are fundamental elements of rocky shore ecosystems, which are threatened by wastewater and river discharges, tourism and climate change. Among many threats, the one posed by contaminants of emerging concern (CEC) still needs to be thoroughly investigated. The aim of this study was to test whether a prolonged exposure to low concentrations of three pharmaceuticals (Carbamazepine, CBZ, Ibuprofen, IBU and Valsartan, VAL) and one phthalate (bisphenol A, BPA), individually and as mixtures, might affect the Mediterranean zooxanthellate solitary coral *Balanophyllia europaea*. Both larvae and adults were exposed for 4 weeks. *Balanophyllia europaea* larvae were exposed to CBZ, IBU and VAL at 1-10 µg/L, to BPA at 10-100 µg/L, and to their mixtures, at low (MIX_L: 1 µg/L CB, IB, VS + 10 µg/L BPA) and high (MIX_H: 10 µg/L CB, IB, VS + 100 µg/L BPA) concentrations. Toxicity endpoints were: 1) mortality and 2) settlement/metamorphosis. *B. europaea* adults were exposed to CBZ (10 µg/L), VAL (10 µg/L), MIX_L and MIX_H. The examined endpoints were: 1) mortality, 2) predation capacity, 3) zooxanthellae density, 4) photosynthetic pigment (chlorophyll-a, -c2 and peridinin) content of zooxanthellae, and 6) the oxidative stress biomarkers catalase (CAT) and glutathione S-transferase (GST). Results showed no significant mortality for the larvae but an alteration in their ability to settle and metamorphose which was variably reduced (from 65% to 15%) compared to control upon all exposure conditions, particularly pronounced for 10 µg/L of CBZ. Conversely, the exposure to 100 µg/L of BPA and 10 µg/L of VS caused the induction of larval metamorphosis without prior settlement on 90% of the larvae. Adult exposure resulted in no mortality, but predation ability was increased by CBZ and reduced by MIX_L and MIX_H. Pigments/zooxanthellae ratio was significantly reduced upon exposure to both MIX_L and MIX_H, as well as pigments/cm² of coral surface, while the zooxanthellae/cm² ratio showed a significant increase only for CBZ exposure. The overall results showed that the prolonged exposure to low concentrations of pharmaceuticals and BPA might impact both larvae and adults of *B. europaea*, interfering with the larval settlement-metamorphosis process and pushing the adults towards bleaching.

2.11.A.T One Health: Detection, Fate, Effects, Monitoring and Management of Benthic and Pelagic Harmful Algal Blooms (HABs)

2.11.A.T-02 Targeted survey of cyanotoxins from benthic isolates from around the United States

Avery Tatters¹ and Stuart Oehrle², (1)University of Washington, (2)Waters Lab Northern Kentucky University
Benthic blooms have been the source of many toxic events throughout the world. Typically animal killings are one such common event. Targeted monitoring using Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) is commonly used to monitor for a wide variety of compounds in differing matrices. What is especially attractive about LC/MS/MS is its sensitivity and selectivity. Using a small particle (sub 2µm particle size) column and a simple gradient method and that was developed for the original toxins (6 microcystins at the time, anatoxin and cylindrospermopsin) it has been expanded to include over 30 additional microcystins, variants of anatoxin and cylindrospermopsin as well as several dermatotoxins, various anabenoheptins, euglenophycin, okadaic acid and others. In addition, backward (precursor) monitoring can provide a useful screening tool for the detection of any unusual toxins. This talk will cover several examples of toxin analysis of waters from a variety of locations to show how you can “see” (detect) additional compounds and how you can “search” for others with some basic knowledge. We will show examples of the usefulness of additional toxin screening for benthic sample analysis from a wide variety of lakes, rivers and ponds.

2.11.A.T-03 Investigating microcystin contamination in Alberta's irrigation district: Impacts on soil and crop health

Mia Lahiji¹, Tara Vucurevich¹, Tara Shelton¹, Ilakkiya Thirugnanasambandam¹, Carl Holland¹, Tabitha

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Microcystins (MCs), potent hepatotoxins produced by cyanobacteria, pose significant threats to environmental and human health due to their widespread occurrence in aquatic ecosystems. Concerns regarding harmful algal blooms, cyanobacteria, and their related toxins are rapidly growing as eutrophication and climate change continue to promote their proliferation. These toxins can accumulate in plants and soils, raising concerns about their potential impacts on terrestrial ecosystems and human exposure, including through the consumption of food crops irrigated with MC-contaminated water. Alberta's nutrient-rich lakes and reservoirs are particularly susceptible to harmful algal blooms, though there is a significant lack of information and regulation regarding MC contamination in irrigation water. This study seeks to characterize contamination of three MC variants (MC-LR, MC-RR, and MC-YR) within Alberta's irrigation district, as well as their potential bioaccumulation in crops and soils through MC-contaminated irrigation water. Irrigation water grab samples were collected from sites across Alberta's irrigations districts during the 2024 growing season. MC concentrations were generally low, with only 3% of samples exceeding 1.0 µg/L, and 1% exceeding 10 µg/L. An optimized MC method for plant and soil matrices using HPLC-MS/MS was then applied to barley samples from a controlled greenhouse experiment which were irrigated with MC-amended water at concentrations of 0, 0.1, 1.0, and 10 µg/L over the course of 90 days. No effect on barley plant health nor MC bioaccumulation in the plant tissue was observed. Soil analysis demonstrated a steady decline in MC mass balance over time, suggesting potential MC degradation or irreversible sorption. This data improves our understanding of the risks harmful algal blooms may pose to soil, crop, and consumer health.

2.11.A.T-04 Understanding ciguatera in Hawai'i

Kelsie Kuniyoshi¹, Justin Chan², Eileen Nalley² and Matthew Iacchei³, (1)University of Hawai'i, Manoa, (2)The University of Hawai'i Sea Grant College Program (UH Sea Grant), (3)Hawai'i Pacific University
Ciguatera poisoning (CP) poses a significant challenge to marine biosecurity in Hawai'i, affecting marine biotic and human health. CP is a complex, multifaceted issue caused by ciguatoxins produced by benthic dinoflagellates and has a cascading effect via bioaccumulation and biomagnification on marine ecosystems, fisheries, and food security. CP is the most common non-bacterial foodborne illness globally, and its effects can potentially last a lifetime. Certain areas in Hawai'i, such as the North Shore of Kaua'i, are known as hotspots for CP, highlighting the need for targeted interventions that prioritize ecosystem health and collaborative community resilience. Much of the current local knowledge of CP is colloquial. Our research highlights an integrative approach to understanding the spatiotemporal abundance and distribution of *Gambierdiscus*, the primary dinoflagellate responsible for CP. By generating baseline data on *Gambierdiscus*, we are working towards identifying high-risk areas and ecological drivers associated with ciguatera. Further, this project emphasizes the importance of community collaboration, leveraging local expertise to foster stewardship of our marine resources, and in the next phase of our work, we will be working with local schools to generate data. Our preliminary findings support previous research indicating that algae such as 'Alani (*Dictyota*) and Pākalakala (*Galaxaura*) are associated with higher abundances of *Gambierdiscus*. More broadly, however, our research has revealed that there is an extensive, ongoing need for increased efforts surrounding CP research in Hawai'i combined with community-level management amongst the broader Hawaiian community to develop robust policies that will minimize the risks of CP.

2.11.B.T One Health: Detection, Fate, Effects, Monitoring and Management of Benthic and Pelagic Harmful Algal Blooms (HABs)

2.11.B.T-01 Application of a novel passive sampler for use in the monitoring of harmful algal bloom toxins at the land-sea interface of the United States

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As harmful algal bloom events become more frequent and widespread, reliable and consistent monitoring of associated toxins is a crucial necessity since toxins are incredibly diverse with reactions from skin irritation to paralysis and/or death, affecting people, marine, and terrestrial life. Monitoring programs must account for this variation when implementing policies to track toxin presence and identify emerging toxins of concern. Currently, one type of passive sampler, solid phase adsorption toxin trackers (SPATTs), is used, which has limited use sorbing polar toxins, and in waterbodies with high flow rates, large changes in salinity, and excessive biofouling. We hypothesized a novel passive sampler, Composite Integrated Passive Samplers (CIPS), can overcome these constraints. The mixed-polymer and nanopore structure sorbs chemicals over a wide range of physico-chemical properties (log Kow -1 to 8) in preliminary trials and a reusable, copper cage minimizes biofouling. Two approaches were used to compare the performance of the samplers with a suite of toxins ranging in polarity. First, SPATTs and CIPS were deployed side-by-side for one week at ten sites across the United States. These deployments compared sorption of toxins across different environmental conditions and the ability for CIPS to sorb a wider polar range of toxins. Secondly, the samplers were co-deployed at three sites and triplicates were collected daily for one week to determine the rate of uptake over that period compared to dissolved concentrations in seawater. An extra benefit of CIPS is they come infused with internal standards for the purpose of calculating a time-integrated concentration which is not possible with SPATTs. Samplers were analyzed by mass spectrometry for 30 toxins across multiple classes, and concentrations were compared to determine the performance of the two samplers. Results demonstrate CIPS bind a wider diversity of toxins and retain those toxins once bound with minimal loss back into the water column. For example, microcystins desorb from SPATT within 4 days while CIPS retain them for up to 28 days. CIPS can replace SPATTs within monitoring programs or be used as a supplement to fill in where SPATTs are not as effective. CIPS are incredibly versatile and have the potential to be utilized by various fields and for numerous contaminants beyond algal toxins. Within the field, they will be a necessary tool for purposes of monitoring and protection of human health.

2.11.B.T-02 Development of health-based guideline values for microcystins in fish tissue and associated fish consumption advisories for freshwater fisheries in Oregon

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The Oregon Health Authority (OHA) Healthy Fish Consumption Program manages all aspects of fish consumption advisories in Oregon freshwaters. These management responsibilities include coordinating outreach and education activities for current advisories, sampling freshwater fish tissue throughout Oregon to test for contaminants harmful to human health, and evaluating fish tissue data to determine whether a fish consumption advisory is needed to protect public health. In Spring of 2025, OHA developed a site priority matrix with feedback from Oregon Department of Fish and Wildlife (ODFW) about high use fishing locations, with rank-scoring based on vulnerable population indices, water quality indicators, and history of clean-up actions and/or public health advisories, including those related to Harmful Algal Blooms (HABs), at each site. Based on this risk prioritization matrix, staff with Oregon Department of Environmental Quality (DEQ) collected fish from upper- and lower trophic level, recreationally harvestable species at Walter Wirth Lake, Freeway Lakes, Detroit Reservoir, Fern Ridge Reservoir, Prineville Reservoir, and Henry Hagg Reservoir. In addition to testing for legacy and geogenic contaminants, based on the history of toxigenic HABs at the selected sites, whole-fish, composite tissue samples were analyzed for total microcystins (MMPB method and individual congener analysis), beta-N-Methylamino-L-alanine (BMAA), and 2,4-Diaminobutyric acid (DABA). Here we present on the results of the fish tissue studies, and the development of health-based guideline values for fish consumption related to microcystins, including microcystin-associated consumption advisories for these freshwater fisheries. Where available, comparison between fish tissue concentrations and historic microcystin

concentrations in whole-water samples from the associated waterbodies are made, including duration of HABs and integrated toxin burdens from the 2024 HAB season.

2.11.B.T-03 Copper sulfate treatment harms zooplankton and ultimately promotes algal blooms: A field mesocosm experiment

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Excess nutrient loading often promotes harmful algal blooms. Despite many past studies, few algal bloom control methods show clear, long-term improvements in water quality. For example, chemical treatments are often used to control algal blooms because they can cause large, rapid declines in algal abundance. However, these effects are often transient requiring future treatments that increase costs and risks to water quality. Moreover, empirical tests of repeated applications or combinations of different treatments are rare, even with their common use across industries, like aquaculture and drinking water. To rigorously test the effects of single or combined treatments on water quality in a eutrophic aquaculture pond, copper sulfate pentahydrate (henceforth called copper) and Phoslock were used independently (full dose) or combined (each at half dose) in a 56-day field experiment conducted in a hypereutrophic aquaculture pond. The two copper-containing treatments caused rapid declines (-83%) in algal abundance the day after treatment followed by very large (2,617%) increases after another five days. In contrast, zooplankton were reduced by 43% in the copper treatment but minimally affected (+1%) by the combined treatment the day after application. To further show that copper treatment is the mechanism responsible for the negative effects on zooplankton and phytoplankton, half of the mesocosms for all four treatments were treated with a full dose of copper on day 13 and similar effects on phytoplankton were observed as when copper was initially applied. The combined treatment was more effective at controlling phytoplankton than the copper only treatment, in part, because of lesser negative effects on zooplankton. Overall, this experiment further highlights the long-term, negative effects that chemical treatments, like copper, have on non-target taxa, such as beneficial zooplankton, which limits their potential for ecological and sustainable control of harmful algal blooms.

2.11.B.T-04 Efficacy using a copper bioavailability approach for controlling harmful algal blooms in aquaculture pond system

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Harmful algal blooms (HABs) pose significant ecological and economic risks in aquaculture ponds. Copper (Cu) has been widely used for treating HABs; however, copper toxicity depends on the Cu complexes in suspension, which are influenced by water quality. Parameters such as dissolved organic matter, pH, alkalinity, and hardness influence copper bioavailability and toxicity. Copper is often used in aquaculture systems to mitigate HABs with dosing based on alkalinity; however, there is a poor understanding of how other water quality parameters influence the efficacy of Cu treatment. The present study aimed to improve Cu treatment regimens to control HABs in aquaculture ponds. Preliminary laboratory studies were conducted to determine cyanobacterial sensitivity to Cu in aquaculture ponds. Results were used to calculate Cu doses using the Cu bioavailability approach for field trials in three different aquaculture ponds at Auburn University, based on the water chemistry of the pond water. Various water quality parameters, including Cu²⁺ concentrations (the most toxic form of Cu), chlorophyll and phycocyanin concentrations, cyanobacterial abundance, green algae abundance, and zooplankton densities were monitored at different times for 96 hr after Cu application. Results showed that cyanobacteria densities rapidly declined throughout the entire monitoring period. These trends were aligned with measured Cu²⁺ concentrations over the time of exposure. Conversely, green algal populations increased consistently, while zooplankton remained unaffected. The results suggested that the Cu treatment successfully reduced cyanobacterial densities without disrupting phytoplankton or zooplankton communities. Compared with the traditional alkalinity model, the Cu bioavailability approach uses less Cu, reducing Cu load into aquaculture systems and is more cost-effective than the alkalinity model.

2.11.B.T-05 Examining respiratory toxicity of Florida red tide in an increasingly polluted environment

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Karenia brevis create dense blooms in the waters of southwest Florida with high concentrations of neurotoxic brevetoxins. Red tide-related respiratory issues can be experienced up to 10 miles inland and increase medical costs up to \$4 million in emergency room visits. In addition to red tide events air pollution is ubiquitous, but it is unknown how exposure to multiple pollutants might affect lung toxicity. This project explores the hypothesis brevetoxins impair normal chemical sensing and metabolism in individuals exposed to multiple toxic stressors. While their mechanism of action on voltage-gated sodium channels is well-described, studies show brevetoxins have multiple intracellular targets which need greater understanding. Notably, brevetoxins can induce transcription of metabolic enzymes and BTX-6 binds to the aryl hydrocarbon receptor (AhR). These findings suggest pathway convergences between brevetoxins and other environmental toxicants that also activate the AhR. AhR plays key roles in xenobiotic metabolism, inflammation, immune/allergy response, and mediates toxicity of its classic ligands. To understand the full spectrum of red tide impacts, its molecular interactions within an increasingly polluted environment must be investigated. We focus on lung cells because inhalation is a major exposure route shared by humans and marine wildlife. Polycyclic aromatic hydrocarbons (PAHs) are air pollutants due to incineration, motor vehicle exhaust, residential heating, etc., and are well-known AhR activators. Using human AhR reporter cells, we found BTX-2 and BTX-6 alone did not activate AhR, but as expected exposure to a mixture of PAHs induced AhR activation. Surprisingly, after co-exposure of brevetoxins and PAHs no AhR activation was observed. Ongoing studies use the AhR reporter cell system to evaluate BTX-2 and BTX-6 as antagonists of the pathway. Human lung cells were exposed to brevetoxins and/or PAHs for 24 and 48 hr. AhR pathway function will be assessed by measuring transcription of key target genes by real-time quantitative polymerase chain reaction (RT-qPCR) and inflammatory cytokines by enzyme-linked immunosorbent assay (ELISA). We expect PAH and brevetoxin co-exposure will lead to reduced AhR target gene upregulation and enhance inflammatory markers. Future work will compare effects in human lung cells to sea turtle lung cells. Insights from this study will aid environmental risk assessment and support respiratory health research.

2.12.P-We Sediment Matters: Sediment Toxicity and Bioavailability Assessment, Remediation and Recovery

2.12.P-We-008 What has changed? 35 years of monitoring sediment contamination in Puget Sound

Wendy Ellyn Eash-Loucks, King County

Puget Sound is a fjord-like estuary in the northwest United States that extends approximately 230 km in a north-south direction, receives inputs from several rivers, and connects to the Pacific Ocean at the north end. Physical, biological, and chemical processes all influence sediment quality in Puget Sound. King County, located in the Main Basin of Puget Sound, has a variety of historical and current point and non-point sources characteristic of an urban environment, particularly in Elliott Bay, which is surrounded by the city of Seattle. These sources include stormwater inputs, combined sewer overflows (CSO), shipping, industry, and more. King County has monitored sediments in the Main Basin for more than three decades to better understand if chemical contamination issues exist in specific areas of interest, and if chemical concentrations are changing. During the last several decades, we routinely found chemical contamination above local sediment standards for mercury and polychlorinated biphenyls (PCBs), as well as occasional exceedances for phthalates and polycyclic aromatic hydrocarbons (PAHs). These chemicals can make their way into the food web and could eventually have negative effects on people and wildlife. This presentation will focus on the status of chemical contamination at these long-term monitoring stations as well as changes from the late 1980s to 2023. We've found that concentrations of several chemicals, including those that historically exceeded sediment standards, have decreased over time. Concentrations of some chemicals such as mercury and PCBs are still elevated in Elliott Bay. However, while problems remain in certain areas, some progress has been made over the last several decades likely due to a combination of chemical bans, cleanup efforts, and increased CSO control. While we

will continue to track long-term trends at our sampling sites, we are also modifying the program to make our data more relevant to the community we serve. Moving forward we will begin collecting data to help us better understand potential impacts of contaminants on the food web with paired benthos sampling. We will also compare these data to contaminant data from fish and crab tissue samples collected with a partner program to help us better understand potential impacts to people. In this presentation, we will share how we are modifying the program to allow us to maintain a long-term dataset, while adapting to evolving regional needs.

2.12.P-We-009 Bioaccumulation evaluation of AFFF-relevant PFAS in marine benthic invertebrates

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Per- and polyfluoroalkyl substances (PFAS) are facing growing scrutiny at Department of Defense (DoD) sites, largely due to their historical use in aqueous film-forming firefighting foams (AFFF). Due to concerns of exposure of PFAS to wildlife and human receptors, uptake of these compounds by benthic invertebrates and other low trophic level organisms is of critical importance. The limited availability of uptake data for marine aquatic life hinders the assessment of ecological risks and complicates the development of sediment quality criteria, leading to uncertainty in determining remediation and compliance requirements for AFFF-impacted sites. This study evaluated the bioaccumulation of 6 AFFF-relevant PFAS compounds (PFOA, PFDA, PFHxS, PFOS, PFDS, and 8:2 FTS) over 28 days using two standard benthic species, the clam *Macoma nasuta* and the marine polychaete *Nephtys caecoides*. Clean sediment from Carr Inlet (WA, USA) was spiked with target PFAS. Organisms were exposed to PFAS individually at mg/kg levels (1-40 mg/kg sediment dry weight) and in two mixtures at lower levels (one 10X lower and the other 100x lower in concentration than the compounds were exposed individually). Tissue (polychaete) and sediment samples were collected weekly over a 28-day period to assess bioaccumulation over time, while clam tissue was collected at 28 days. Initial sediment concentrations were within $\pm 20\%$ of nominal concentrations, and concentrations of longer chained PFAS remained within approximately 40% of initial concentrations over the course of the test. PFOA and PFHxS, more mobile species, dropped in concentration over time in the sediment, resulting in $\sim 30\%$ left at the end of exposure, likely due to partitioning to overlying water and loss during water exchanges. Concentrations in the polychaete approached steady state for four of the tested PFAS (PFOA, PFHxS, PFOS, and 8:2 FTS). There were substantial differences in PFAS bioaccumulation between the two benthic species with polychaete showing approximately an order of magnitude higher benthic sediment accumulation factors (BSAF), and higher BSAFs for shorter-chained compounds; the opposite trend was seen for clam, which showed higher BSAFs for longer-chained compounds. Sediment porewater measurements indicated that porewater-to-tissue uptake factors increased with perfluorinated chain length for both species. Uptake factors derived from this data will inform ecological risk assessment at PFAS sites. **Disclaimer/Disclosure:** This work was funded by the Strategic Environmental Research and Development Program (SERDP) ER22-3214 and the Navy Environmental Sustainability Development to Integration Program (NESDI) Project #601.

2.12.P-We-010 Field logistics for passive sampler (PFASsive™) deployment and retrieval at freshwater and marine Department of Defense sites

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Passive sampling is a widely recognized technique for understanding the availability of sediment contaminants; however, there is a limited commercial availability of passive samplers for per- and polyfluoroalkyl substances (PFAS). Demonstration of dialysis passive samplers for measuring PFAS availability is crucial for integrating passive sampling technology into PFAS risk assessments, enabling more accurate estimations of PFAS availability and risks. As part of a broader demonstration and validation study, this presentation aims to document the deployment and retrieval procedures for PFASsive™ samplers at both freshwater and marine

Department of Defense (DoD) sites. PFASsive™ samplers were deployed in sediment and surface water at 10 stations over the course of 3 to 4 days and were left to equilibrate for approximately 14 days at two different sites. Upon retrieval, PFASsive™ sampler recovery was recorded, and samplers were processed and shipped to be analyzed at a commercial laboratory. Deployment methods were adapted to site-specific conditions, including substrate type, water depth, tidal fluctuations, and wave action. For example, different deployment techniques were needed based on substrate type, and additional precautions were taken to minimize the potential for loss of samplers due to wildlife and wave action. Tidal considerations also influenced the selection of deployment locations to ensure the quality of samples at the marine site. This presentation will provide detailed methods, logistical considerations, and practical recommendations for planning and executing PFAS passive sampler field investigations, contributing to the broader application of passive sampling in PFAS monitoring and risk assessment.

2.12.P-We-011 Retrospective analysis of PFAS using sediment cores along the vulnerable coastline of Mississippi

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Per and polyfluoroalkyl substances (PFAS) are manmade toxic chemicals that have been found in water, sediment, wildlife, and humans. They are known to be extremely mobile in water and have been linked to a myriad of adverse health impacts. To date, there have been limited studies monitoring PFAS along the coastline of Mississippi. The Mississippi coastline is a model location due to its uniquely high abundance of industry located in close proximity to coastal waters. Sediment cores of 20 cm were collected at 42 sites. Each core was divided into three separate samples that represented the following time frames: present to 5-8 years, 8-20 years, and >20. The sediment extraction workflow included sonication, rotation, and centrifugation using a 0.3% methanolic ammonium hydroxide solution. A targeted method was used to monitor 31 PFAS via ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The results from these sediment samples provide a retrospective timeline of the PFAS contamination present along the Mississippi coast. The coast of Pascagoula, Mississippi has several different industrial plants on the coastline (potential PFAS sources), all near the Grand Bay National Estuarine Research Reserve. The other two main sampling areas included Bay St. Louis and Biloxi Bay. These three areas all contain different variations of potential PFAS sources, including Chemours, which is a global provider for industrial chemical products (e.g., PFAS, Bay St Louis), Kessler Airforce Base (Biloxi Bay), and a Chevron Pascagoula Refinery, a Mississippi Power Plant, a Naval Complex and Ingalls Shipbuilding (near Grand Bay). The data provided in this study compares the PFAS levels (both sum and individual concentrations) from sites near and varying distances from these sources and how those levels changed over the past 20 years.

2.12.P-We-012 Kinetic uptake factors of 15 PFAS in a marine benthic polychaete

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Per- and polyfluoroalkyl substances (PFAS) are detected in sediment globally, largely due to their historical use in aqueous film-forming firefighting foams (AFFF) and other numerous industrial and consumer uses. Due to concerns of exposure of PFAS to wildlife and human receptors, uptake of these compounds by benthic invertebrates and other low trophic level aquatic organisms is of critical importance. This study evaluated the bioaccumulation of 15 AFFF-relevant PFAS compounds (6 PFCAs, 7 PFSAAs and 2 FTSs) over 28 days using a standard benthic species, the marine polychaete *Nephtys caecoides*. Organisms were exposed in sediment to six PFAS spiked individually at mg/kg levels (1-40 mg/kg sediment dry weight (dw)) and in two multi-PFAS mixtures at concentrations lower than the individual PFAS exposures (10X lower and 100x lower); this enabled the evaluation of PFOA, PFDA, PFHxS, PFOS, PFDS, and 8:2 FTS bioaccumulation in sediment at three different concentrations. Due to trace impurities in PFAS used for spiking, bioaccumulation of an additional 9

PFAS was also possible. Tissue (polychaete) and sediment samples were collected weekly over a 28-day period to assess bioaccumulation over time. For PFOA, PFDA, PFHxS, PFOS, PFDS, and 8:2 FTS, non-linear uptake modelling demonstrated that steady state concentrations in polychaetes were reached within approximately 28 days in at least in one of the three sediments. In all cases, the sediment with the lowest concentrations exhibited the most rapid kinetics. For example, concentrations of PFHxS in polychaetes exposed to sediment containing 170, 1,900, and 18,000 ng/g, dw reached 90% of steady state within 12, 25, and 30 days, respectively. Overall, at concentrations in sediment that are approximately 200 ng/g, dw or less, it can be expected that steady state concentrations in polychaetes will be reached within approximately 1 month of exposure. Uptake factors measured at the standard 28-d bioaccumulation duration using sediments spiked at these concentrations provide accurate and conservative estimates of bioaccumulation potential in this organism. Additionally, the time to reach steady state in animals generally increased with increasing perfluorinated carbon chain length. Elimination rates ranged from 0.0002 to 0.2 day⁻¹ (median 0.035 day⁻¹) and were negatively correlated with perfluorinated carbon chain length ($r^2 = 0.23$; $P = 0.0130$).

2.12.T Sediment Matters: Sediment Toxicity and Bioavailability Assessment, Remediation and Recovery

2.12.T-01 Reeling in success: Declining pesticides in shiner surfperch in the Lauritzen Channel since the 1997 remedy

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The United Heckathorn Superfund Site in Richmond Harbor, California, includes 5 acres of uplands and 7 acres of the Lauritzen Channel. Since the 1940s, the site has been used for various industrial activities, including shipbuilding, pesticide formulation, scrap storage, and a dry bulk-cargo terminal. Listed on the National Priorities List in 1990 due to soil and sediment organochlorine pesticide contamination, the site also had elevated levels of PAHs, PCBs, and metals in the Channel. Cleanup efforts in the early 1990s included soil and sediment excavation, and by 1997, 80,000 cubic meters of contaminated sediment were dredged. Despite these efforts, EPA's post-remediation monitoring indicated that contamination levels were not maintained, leading to a 2015 draft focused feasibility study (FFS) proposing further dredging amid unresolved uncertainties. In 2023, our team designed and implemented a comprehensive multi-media dataset collection program to address key data gaps in EPA's 2015 draft FFS and support alternative (non-dredging) remedial approaches, such as the placement of an engineered blended cover with activated carbon. This investigation evaluated temporal and spatial trends in pesticides and PCBs in several fish species and other biota, with shiner surfperch being the primary focus due to their high sample numbers across four channel subareas and site fidelity. We evaluated temporal trends in fish channel-wide and across subareas using an extensive dataset ($n > 150$ fish composites created from hundreds of fish). We used boxplots, regression analyses, and focused statistical comparisons to compare the most robust historical and current shiner surfperch constituent datasets. Pesticide compositional patterns were also evaluated spatially. Shiner perch tissues showed spatially explicit decreases in pesticide (80 to 90%) and PCB (30 to 50%) concentrations over the last 15 years, with significant decreases in pesticides and PCBs in perch from the southern half of the Channel ($p < 0.05$). Channel-wide decreases in pesticide concentrations were also observed in several other fish species, ranging from 30 to 95% for smaller forage fish and 70% decreases in jacksmelt. Patterns in PCBs and pesticides varied spatially in fish suggesting different historical sources. Given the robust dataset, and when considered along with decreasing trends in sediment, these findings provide compelling evidence that natural recovery is occurring.

2.12.T-02 Rapid estuarine sediment recovery after source controls and full-scale cleanup remedies

Clay Patmont¹, Kevin Russell¹, Rob Healy² and Upal Ghosh³, (1)Anchor QEA LLC, (2)Port of Tacoma, (3)University of Maryland, Baltimore County

Recent reviews of completed sediment remediation projects reveal mixed effectiveness at reducing contaminant concentrations in surface water and biota tissue. Collecting, learning from, and incorporating new information

into practice is the best way to improve the effectiveness of remediation. Timely monitoring of source controls achieving substantial load reductions, as well as robust monitoring of completed full-scale estuarine sediment remediation projects, have revealed rapid changes in surface sediment contaminant concentrations and grain size, with natural recovery rates of 30 to 60 percent/year following completion of construction. On average, surface sediment natural recovery rates measured in estuaries are 5 to 6 times faster than projected based on measured sedimentation and surface sediment bioturbation rates. The weight of evidence reveals that natural recovery in estuarine surface sediments is accelerated by exchange across the sediment-water interface from feeding, bioirrigation, and bioresuspension activities of macrobenthos. Sediment natural recovery rates measured at full-scale sediment remediation projects across a wide range of aquatic environments in North America are highly correlated ($r^2 = 0.89$) with benthic invertebrate biomass. Control of contamination sources is the first principle of contaminated sediment management and is the logical focus of programs targeting meaningful risk reduction. This presentation will demonstrate how source controls, coupled with natural recovery processes driven by rapid sediment/water exchange processes, are the most effective means to reduce contaminant concentrations in surface sediment, surface water, and biota tissue. This presentation will also provide an overview of a collaborative source control and adaptive management program currently underway in the Pacific Northwest that can be broadly applied to reduce risks rapidly and cost-effectively.

2.12.T-03 Demonstration of a commercially available peeper passive sampler for PFAS in sediment

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PFAS are a concern at many Department of Defense (DoD) installations due to the historical use of aqueous film-forming foam (AFFF) containing PFAS. Sediment can represent a significant source of PFAS to aquatic life, aquatic dependent wildlife, and human receptors, and there is a need to understand the fate and bioavailability of PFAS in sediment for site-specific risk assessments. Passive samplers have shown to be a powerful tool for providing measurements of the freely dissolved concentration (C_{free}) that can be used to improve site-investigations; however, there are limited examples of applying commercially available passive samplers at PFAS sites. The overall objective of this project is to demonstrate and validate the use of PFASsive™, a commercially available dialysis-based passive sampler ("peeper") for measuring PFAS in sediment porewater and surface water. A comprehensive field study was conducted in nine different locations in freshwater creeks receiving PFAS from former a fire training site with historic AFFF use in May 2024. Co-located sediment, surface water, and porewater grab samples were collected at each station. In situ bioaccumulation tests were performed with a freshwater bivalve (*Corbicula fluminea*) at all stations and in situ bioaccumulation tests for a benthic organism (*Lumbriculus variegatus*) were completed at two stations. Sediment was collected from all stations to run ex situ sediment bioaccumulation assays using *L. variegatus* due to the difficulty of performing in situ sediment bioaccumulation testing at this site. This presentation will highlight benefits of passive sampling for PFAS, sample design, field work, and results of C_{free} measured in the passive samplers compared to measured PFAS in bulk media and organism tissue. In particular, PFOS was generally the most frequently detected (and highest detected PFAS) in samples. PFAS passive sampling results were well correlated with uptake. For example, passive sampling PFOS C_{free} was positively correlated (r^2 values of 0.73 to 0.88) with concentrations in clams exposed to site surface water and oligochaete worms exposed to site sediment. Overall, this data indicated that passive sampling is helpful in understanding the fate and availability of PFAS in aquatic systems. Together with data collected from a saltwater AFFF site in April 2025, the information from this project will provide a robust demonstration and validation of the use of PFAS passive samplers at AFFF sites. **Disclaimer/Disclosure:** Funding for this work provided by ESTCP (project ER23-7741) and ExxonMobil.

2.12.T-04 PCB Target tissue level development for juvenile salmonids in northwest regional sediment evaluations

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Juvenile salmonids are listed species as a part of the federal Endangered Species Act (ESA). Salmonids are exposed to PCBs in many urban estuaries (e.g., Duwamish, Portland Harbor, lower Columbia River) in the Pacific Northwest while completing their physiological transition from freshwater to marine environments. Wild out-migrating sub yearlings tend to have very low lipid values (1 to 2 percent) compared to hatchery fish or wild adults. During this sensitive stage, PCB exposure can alter physiological functions, especially thyroid hormones, which may substantially reduce the chances of successful smoltification and the ability to survive and grow in saltwater. Target Tissue Levels (TTLs) are concentrations in tissue against which sediment bioaccumulation test results are compared to evaluate the potential for adverse effects. This evaluation is included in sediment suitability determinations for in-water or other disposal options for dredging projects within critical habitat areas. The current PCB TTL of 1.4 mg/kg-lipid published in the 2018 Sediment Evaluation Framework for the Pacific Northwest was re-evaluated in this analysis. Endpoints were selected determined to be relevant to effects on salmonid fitness, and a species sensitivity distribution (SSD) was used to estimate a 5th percentile hazard concentration (HC05). Lipid normalization of tissue effects data allowed for the calculation of effects estimates (HC05) at environmentally relevant lipid concentrations, and improved comparisons across laboratory test results, fish species, and field collected tissue. The result is a revised bioaccumulation TTL for PCBs designed to be protective of salmonids during sensitive developmental stages.

2.12.T-05 How to assess and integrate low PAH bioavailability into a CERCLA marine risk assessment; Bremerton Gas Works Superfund site

Nathan Soccorsy¹, **Ariel Blanc¹**, **Masakazu Kanematsu¹**, **Jessica Goin¹** and **Kalle Kuether Godel²**, (1)*Anchor QEA LLC*, (2)*Montana-Dakota Utilities Co.*

The Bremerton Gas Works Superfund site (Site) is a former manufactured gas plant (MGP) in Puget Sound, Washington. The MGP produced a gas supply via a carbureted water-gas process using black carbon briquettes. During MGP operations, briquettes were incidentally released to sediment and are still occasionally found in the intertidal area. Sediment PAH concentrations are elevated adjacent to the former gas works, raising concern for risk to ecological receptors and human shellfish consumers. The preliminary conceptual site model (CSM) identified briquettes as the major source of PAHs in sediment. Multiple studies were conducted to confirm the CSM and demonstrate limited bioavailability and risk from the briquette-sourced PAHs. These included sediment sampling, microscopic evaluation, bioassay testing, ex situ porewater testing, bioaccumulation tests, and in situ tissue collection. Sediment PAH concentrations exceeded benthic sediment criteria by up to 16 times, but no toxicity was observed in bioassays, indicating limited bioavailability. Consistent with the lack of toxicity, sediment porewater toxic units were less than 1. The microscale texture of briquette fragments in sediment was consistent with the published characteristics of lampblack, and the site-specific Koc values were 1 to 3 orders of magnitude greater than conventional Koc values, consistent with black carbon-affected sites. Porewater and sediment PAH concentrations were positively correlated with bioaccumulation test clam tissue concentrations, suggesting a causal relationship between Site sediment and tissue PAH. However, the measured porewater and tissue concentrations were notably lower than would be expected if the Site sediment were not influenced by black carbon and related Site specific partitioning. This information, together with the lack of benthic toxicity at sediment PAH concentrations many times the benthic protection criteria, supports the CSM of limited PAH bioavailability in Site sediment due to PAH sequestration to the briquette material. Although no unacceptable risk, including wildlife consumption of prey from the Site, was identified for ecological receptors, human health cancer risks remained greater than the threshold due to relatively high area specific shellfish consumption rates. The CSM and site-specific bioaccumulation relationships will be used to inform human health-based preliminary remediation goals and alternatives development in the feasibility study.

2.12.T-06 Cleaning sediments and restoring ecosystems - Evolving towards an adaptive risk assessment and management framework

Robert K Johnston, *Applied Ecological Solutions*

Cleanup at many large contaminated sediment sites can be very costly, time consuming, and ineffective if larger scale pressures and ecological processes are not adequately addressed by the remedial design. A holistic approach is ideal - one that integrates remediation with restoration to maximize synergies, reduce costs and environmental impacts, and promote long-term sustainability. Sustainable remediation strategies should be informed not only by considerations of regulatory compliance but also by stakeholder goals, values and expectations. Traditionally, remediation efforts have focused on mass removal or reducing exposure to sediment-associated contaminants to address human health and ecological risks. However, evaluating the success of such remedies has proven challenging due to the complexity of ecosystem interactions, the lack of comprehensive monitoring data, and the need to align cleanup objectives with ecosystem recovery. Additionally, sediments are linked to processes occurring within the watershed making them susceptible to, and potential drivers of, chemical, physical, and biological stressors from larger scale social and economic pressures, climate change, and other disturbances associated with the Anthropocene. Drawing on the results of case studies, key concepts and lessons learned to better integrate sediment cleanup and ecosystem recovery goals are discussed. By integrating risk assessment and adaptive management into a unified Adaptive Risk Assessment and Management framework - incorporating the Plan-Do-Act-Check-Learn adaptive management cycle alongside an iterative risk assessment process - organizations can enhance risk management decisions, foster continuous stakeholder engagement, and ultimately improve both cleanup and restoration outcomes. This approach ensures flexibility, promotes ongoing learning, and aligns remediation strategies with evolving ecological and sustainability goals **Disclaimer/Disclosure:** This presentation reflects the personal views of the author and does not suggest or reflect the official policy, practices, programs, or doctrine of any governmental agency.

2.13.A.T Stormwater Runoff Impacts, Solutions and Innovative Research

2.13.A.T-01 A Data-driven index for evaluating BMP water quality performance

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A data-driven stormwater best management practice (BMP) performance index (PI) developed herein provides a simple, unbiased method to interpret the water quality treatment performance of a structural BMP, and provides actionable information for managers in planning, implementing and maintaining BMPs. The PI is derived from field-monitored influent-effluent pollutant event mean concentrations normalized by a user-specified water quality benchmark that may be adapted to reflect watershed-specific objectives. Benchmarking allows performance of any BMP to be investigated regardless of the treatment mechanisms, climate conditions, hydrologic performance, or site-specific pollutant concentrations. Quantitative monitoring data normalized by the benchmark are subsequently binned into performance categories of Success, Excess, Marginal, Insufficient, and Failure, which are indicators of the relative potential to achieve downstream receiving water goals. A single PI score per analyte (PI_{analyte}) is derived by compositing the categorical distribution. A simplified analytical hierarchy procedure is adapted to combine multiple PI_{analyte} scores, if/when BMP selection for future applications must address a range of pollutants, e.g., where multiple water quality objectives are present. Data from the International Stormwater BMP Database are used to demonstrate PI applications such as selecting the “right” BMP to address specific water quality concerns, comparing amongst similar types of BMP to identify beneficial design features, and the clear interpretation offered by the score providing actionable information compared to a percent-removal assessment method. An index interpretation guide provides managers, maintenance teams, and designers with a feedback mechanism to refine future project designs, operations, or maintenance needs, and informs progress towards achieving successful watershed management plans.

2.13.A.T-02 Toxicological effects of a fire retardant on early life stages of Chinook salmon

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As wildfires become more frequent and intense across the western United States, the use of long-term fire retardants, such as Phos-Chek Wildfire Home Defense, has increased substantially to protect homes and landscapes. While effective for fire prevention, widespread application raises concerns about runoff contamination, especially in urban areas where the majority of stormwater is discharged untreated into nearby rivers, lakes, and coastal ecosystems. Chinook salmon (*Oncorhynchus tshawytscha*), a threatened species in California, may be especially vulnerable during early development when exposed to such runoff. This study assessed both lethal and sublethal effects of Phos-Chek Wildfire Home Defense on Chinook salmon embryos through a 96-hr static-renewal exposure, simulating first-flush storm events following application. Test concentrations reflected potential environmental levels following post-fire runoff events. To simulate realistic conditions, both freshly prepared and UV-weathered Phos-Chek formulations were tested. Endpoints included mortality (96h-LC₅₀), hatching success, morphological abnormalities, and behavior. Preliminary results indicate concentration-dependent toxicity, with reduced hatching success and altered behavior at sublethal levels; notably, weathered formulation exhibited greater toxicity than fresh (unweathered) fire retardant. These results provide critical data for understanding the ecological risks associated with fire retardant runoff in salmon habitats. Given the pressures already facing Chinook salmon from habitat loss and climate change, fire retardants represent an additional stressor that must be accounted for in watershed and fire management strategies. This research underscores the need for environmentally informed fire mitigation practices to better safeguard sensitive aquatic species. **Disclaimer/Disclosure:** Sponsor: Delta Stewardship Council Award #DSC22082 to SB and #DSC22083 to AS.

2.13.A.T-03 Treating conventionals and contaminants of emerging concern in stormwater with a high performance bioretention soil media: A field effectiveness study

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Stormwater runoff is the largest source of contaminant loadings in the Puget Sound. Green stormwater infrastructure technologies, like bioretention, are increasingly relied upon to mitigate a wide range of stormwater pollutants - from conventional contaminants like suspended solids, nutrients, and metals, to contaminants of emerging concern (CECs) like 6PPD-quinone (6PPDQ) and PFAS. Considerable research efforts have been made to optimize bioretention soil and media mixtures to treat nutrients and prevent their export from filter media while providing effective treatment of toxic contaminants. Over the last decade in Washington State, governments, academic institutions, and environmental consultants have collaborated to develop a non-proprietary, high performance bioretention soil mix consisting of sand, coconut coir, and biochar with an additional nutrient polishing layer to meet the complex demands of bioretention media. While the mix was developed in the laboratory, it needs field validation to confirm its performance. This presentation focuses on the first known field study of a full-scale bioswale containing high performance bioretention soil mix in a residential neighborhood in Bellingham, WA. Treatment effectiveness was monitored for conventional parameters (nutrients, metals, suspended solids) and CECs (6PPDQ and 40 PFAS compounds) across 9-20 storm events (depending on the parameter). Toxicity tests were also conducted on the swale's influent and effluent over 3 events for juvenile coho salmon (*Oncorhynchus kisutch*) and *Daphnia pulex*. Results demonstrate this high performance mix's effectiveness at removing suspended sediment, nutrients, and metals. Even in this residential contributing area, 6PPDQ in the swale's influent reached concentrations above 70 ng/L, and 6PPDQ was never detected in the swale's effluent, suggesting near complete removal. Influent PFAS concentrations were very low, with 8 compounds routinely detected below 5 ng/L, but no removal of PFAS was observed. Additionally, the development of this study's experimental design revealed that monitoring treatment effectiveness for multiple CECs can present methodological challenges that warrant further study.

2.13.A.T-04 Evaluating stormwater biofilters for pollutant removal from roof runoff

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Roof runoff, a component of stormwater, is recognized as a major contributor of pollutants to surface water. This study aims to answer the question: Which type of biofilter is the most effective at reducing pollutant loads from roof runoff? To address this, biofilters were installed to treat runoff from two roof types (galvanized metal and asphalt shingle) and included: (1) zeolite media, (2) shale media, and (3) a conventional sand and compost mix (Grattix boxes). The zeolite and shale filters are proprietary products commercially available for stormwater treatment. The zeolite filter incorporates positively charged zeolite and peat, while the shale filter contains shale, biochar, and peat. Grattix boxes, developed by the Port of Vancouver, provide a low-cost alternative that uses a conventional bioretention soil mix (compost and sand) and established plants. Samples were collected from approximately 60 storm events, and effluent samples were tested for zinc, copper, nitrogen, phosphate, phosphorous, and microplastics (106–5000 μm). Metal concentrations were quantified using atomic absorption spectroscopy (AAS), and nutrient levels were tested following EPA-equivalent methods. For microplastic analysis, samples were sieved at 106 μm , chemically digested to remove organic matter, and examined under a Leica S9i stereo microscope for visual identification. Results showed that all filters increased nitrogen concentrations in the effluent compared to the influent (15–405% export). Phosphate concentrations increased in the effluent by 375–1900% and total phosphorus by 140–583%. Copper concentrations decreased only in the shale filter effluent (61.9% reduction) and increased in all others (30–1888% increase). Despite nutrient and copper export, all the biofilters reduced zinc concentrations: the galvanized metal roof Grattix showed the greatest reduction (54.4%), followed by zeolite (45.1%), asphalt shingle Grattix (33.4%), and shale (14.4%). Microplastic analysis is still ongoing; however, fibers, fragments, and beads have been detected in both roof runoff and bulk atmospheric deposition samples. Overall, while the shale filter showed lower nutrient export, the galvanized roof Grattix box achieved the highest zinc removal. These findings suggest trade-offs among filter types and highlight the importance of matching treatment media to specific pollutant concerns.

2.13.A.T-05 Removal of micro- and nano-plastics from stormwater using green infrastructure with biochar and vegetation

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Micro- and nano-plastics are contaminants of emerging concern that pose risks to the environment and human health. Green infrastructure (GI) has been increasingly implemented in cities globally to enhance urban ecosystem services, with the potential of removing microplastics from stormwater. Biochar, particularly post-pyrolysis biochar, has been suggested as an effective filter material to remove microplastics through attachment and retention. Additionally, biochar may indirectly immobilize micro- and nano-plastics through vegetation enhancement, yet plant performance may be suppressed due to plastic toxicity. However, the dynamic coupling effects of biochar and vegetation on micro- and nano-plastic removal in GI remains unclear. In this study, we investigated the effects of biochar (unprocessed vs. post-pyrolysis air oxidated (PPAO) biochars) and vegetation (presence vs. absence) on the removal of micro- and nano-plastics in a bioretention system. *Rudbeckia hirta* was planted in vegetated bioretention column to examine vegetation effects on leachate quality. Synthetic stormwater spiked with a known amount of polystyrene micro- and nano-spheres in various particle sizes was added into each bioretention column monthly to investigate the efficiency of micro- and nano-plastic removal. Results showed that biochar strongly enhanced the retention of 0.02 μm and 0.2 μm polystyrene spheres in the bioretention columns during stormwater spiking events. Moreover, the presence of vegetation reduced the leaching of micro- and nano-plastics during weekly irrigation. In addition, the amendment of biochars, particularly those with PPAO biochar, reduced the leaching of total organic carbon from the bioretention systems. However, an increase of nutrient (phosphorus, potassium, sulphur, calcium, and magnesium) concentrations in the leachate was observed for the bioretention columns amended with biochars, likely due to the high nutrient content of biochars themselves. The presence of vegetation reduced the concentrations of total organic carbon, total carbon, and inorganic carbon in the leachate relative to the columns with no vegetation. These findings advance the innovative design of green infrastructure that integrate processed biochars and

native vegetation for the removal of contaminants of emerging concern in stormwater, enhancing sustainable urban water management.

2.13.A.T-06 Fate of microplastics and tire and road wear particles in soil and sediment of two urban stormwater green infrastructure

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In this work, we present data on microplastics, including tire and road wear particles in soil and sediments of two stormwater green infrastructure, an infiltration-based system and an urban stream. Multiple studies have now confirmed the efficiency of infiltration-based systems such as bioretention cells and bioswale to trap microplastics by comparing influent and effluent concentrations. Here, we focus on concentrations in the soil media, which accumulates microplastics over time. This sampling strategy offers the advantage of characterizing the fate of microplastics within the infiltration-based green infrastructure. Concentrations in microplastics and tire and road wear particles in a bioswale media were quickly attenuated from lateral sheet flow from the road and a building wall and preceding bioswales to downgradient zones. Specifically, we demonstrated that microplastics concentrations showed decreasing trends both longitudinally along the dominant flow path and vertically with depth. In an urban stream designed to attenuate large floods, concentrations in microplastics and tire and road wear particles in stream sediments decreased from the inlet to downgradient zones under average rain conditions. However, our data showed that a large rainfall resulted in the re-suspension and deposition downstream of microplastics and tire and road wear particles. Data from these two different types of green stormwater infrastructure inform microplastic mitigation processes and management and maintenance practices.

2.13.B.T Stormwater Runoff Impacts, Solutions and Innovative Research

2.13.B.T-01 Urban bioretention systems for stormwater treatment: Targeting emerging contaminants and tire wear particles

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Urban stormwater is a major pathway for a broad range of chemical pollutants, including tire wear particles (TWP) and stormwater contaminants of emerging concern (SWCECs), such as vehicle-derived organics, pesticides, pharmaceuticals, and benzothiazoles. However, their removal or release from bioretention units during storm events is not well characterized. This study used field monitoring data of four bioretention rain gardens in San Francisco, CA, to evaluate the removal efficiencies of novel SWCECs and TWPs in bioretention systems as part of the Next Generation Urban Greening initiative—a collaboration to enhance the performance and benefits of green infrastructure. In the city's combined sewer system, green infrastructure slows runoff and acts as a first line of pollutant capture and treatment. Stormwater samples were collected at the inlet and outlet of each bioretention unit during 3 to 9 storm events over two wet seasons. Rainfall-triggered whole-water composite samples, composed of 2-6 aliquots per storm, were collected using trace-clean protocols. Concentrations of TWPs and 21 SWCECs were analyzed to assess removal efficiencies. The Norwegian Institute for Water Research quantified tire wear particles using GC/MS. The 21 SWCECs were analyzed by the University of Washington Tacoma using LC-MS/MS. Removal efficiency was calculated from direct inlet-outlet concentration comparisons. Results show wide variation in removal performance across compound classes. Vehicle-derived chemicals were detected in all inlet and outlet samples, with median removal efficiencies ranging from 50% for 1,3-dicyclohexylurea to 95% for 6PPD-quinone (6PPDQ). TWPs had a median removal efficiency of 78%. Benzothiazoles and benzotriazoles median removal ranged from 50% for benzotriazole to 88% for 2-(4-morpholinyl)benzothiazole. Pesticides exhibited the greatest median removal variability, ranging from -33% for imidacloprid (suggesting mobilization) to 92% for diuron. Median removal

efficiencies of pharmaceuticals and personal care products ranged from 43% for cotinine to 83% for caffeine; both compounds were detected in all inlet and outlet samples. These findings emphasize the variable performance of bioretention systems in removing SWCECs and TWPs. High removal of 6PPDQ suggests that current designs can be effective for some high-risk compounds, though 56% of outlet samples still had 6PPDQ concentrations that exceeded the freshwater screening value for acute exposures.

2.13.B.T-02 Meta analysis of best management practices for 6PPD-quinone stormwater mitigation

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The compound, 6PPD (N(1,3-dimethylbutyl)-N- phenyl-p-phenylenediamine), is added to tires as an antiozonant, antioxidant, and antidegradant; it is a critical component in tires that extends their lifespan and increases road safety. 6PPD transforms into 6PPD-quinone (6PPD-Q) in the presence of ozone and UV irradiation. In 2020, the structure of 6PPD-Q was first elucidated, and laboratory studies have found it to be acutely toxic to coho salmon, with variable responses found in other aquatic organisms. The discovery of this transformation product has spurred a surge of studies investigating the toxicity and environmental occurrence of 6PPD-Q. While alternative assessments are underway to find a suitable replacement for 6PPD in tires, this process will take time. In the meantime, efforts continue to focus on mitigating 6PPD-Q concentrations in stormwater runoff to reduce risks to aquatic species. Best management practices (BMPs) are designed to reduce pollutants in stormwater before they reach receiving waters and are a potentially effective mitigation strategy for managing 6PPD-Q in stormwater. Here we present a meta-analysis of BMPs that treat 6PPD-Q in stormwater. A literature review was conducted to identify peer-reviewed studies and case-study reports that contained the keywords ‘6PPD-Q’ (or analogs), best management practices’, ‘water’, ‘stormwater’, or ‘run-off’. Studies were screened for those that measured 6PPD-Q in water before and after a BMP or equivalent treatment. Eligible studies were classified into the following BMP categories: biofiltration, filtration, membrane and cartridge filters, permeable pavement, and sedimentation basin. Meta-analysis results show reduction of 6PPD-Q by biofiltration (87% reduction), permeable pavements (67% reduction), membrane and cartridge filters (38% reduction), and filtration (25% in the field and 89% in the lab). To date, data on sedimentation basins suggest low removal efficiency, and in some instances 6PPD-Q increased. Differences in removal efficacy appear between laboratory and field studies (86% and 41%, respectively), and between the source of water entering the BMP. Factors influencing variability within treatment types and the removal efficiency of co-contaminants will also be discussed. This meta-analysis provides information for stormwater managers, engineers, aquatic conservationists, and researchers to help reduce concentrations of 6PPD-Q in stormwater runoff and mitigate risk to aquatic species.

2.13.B.T-03 Evaluating 6PPD-Q in stormwater runoff in the Lake Tahoe basin

yeongkwon Son¹, Andrea Buxton², Hyebin Kim¹ and Monica Arienzo¹, (1)Desert Research Institute, (2)Tahoe Resource Conservation District

Chemicals in tire wear particles, such as 6PPD-q, are highly toxic to aquatic biota. Contaminants of Emerging Concern (CECs), including 6PPD-q, may have detrimental impacts, but there is a lack of knowledge with regard to some CECs in the Tahoe Basin. To fill the knowledge gap, this study measured 6PPD-q concentrations in stormwater runoff in the Lake Tahoe basin. Stormwater samples were collected between December 2024 and May 2025 from six sites during surface runoff events. The Speedboat (SB), Tahoe City (TC), Tahoma (TA), and the Pasadena (PO) sites discharge to Lake Tahoe. Upper Truckee (UT) and Elks Club (EC) sites are on the south shore but discharge to the Upper Truckee River before discharging to the Lake. Automated samplers were used to collect continuous flow data and individual samples across the hydrograph. All individual samples were collected within 24 hr, composited on a flow-weighted basis, and immediately transferred to amber glass bottles. Composite samples labeled FF (first flush) comprise the first 24 hr of a runoff event that lasted longer than 24 hr. Samples labeled 20 and 80 are composites from runoff events that lasted 24 hr or less where two composites were made (first 20% and last 80% of the flow volume). Once returned to the lab, the samples were extracted using HLB SPE cartridges. After spiking a labeled internal standard, the 6PPD-q concentrations were

quantified using the UPLC-MS (Waters Xevo TQ-S) method. Measured stormwater volumes ranged from 6.2 to 304.5 cubic meter. The UT site showed the highest 6PPD-q concentrations in the spring season. The UT-FF sample in January showed 83.22 ng/L while UT-20 and UT-80 samples in March showed 945.69 ng/L and 1300.98 ng/L, respectively, and 1269.99 ng/L and 1047.67 ng/L in May, respectively. Samples from other sites, SB-20 and SB-80 in December, PO-FF and EC-FF in January, and TC-FF and TA-FF in March, showed 48.83, 43.66, 12.42, 14.05, 157.86, and 303.99 ng/L, respectively. The UT site is located at the intersection of a highway and secondary road, the contributing catchment has the highest impervious coverage (72%). Lake Tahoe basin receives 90% or more stormwater runoff between October and May, thus the observed 6PPD-q loads could be extrapolated to estimate annual load from each monitored catchment. Observed data suggest that best management practices (BMPs) that treat 6PPD-q could prevent significant loads of 6PPD-q from entering the Lake via stormwater runoff.

2.13.B.T-04 Canary in the creekbed: Real-time evidence of 6PPDQ-induced mortality and developmental impairment in coho salmon

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Roadway stormwater runoff delivers episodic contaminant pulses into salmon-bearing streams, yet most toxicity studies rely on static exposures, simplified mixtures, or endpoints that fail to reflect real-world conditions. We conducted a two-phase, flow-through exposure study at Miller Creek, an urban stream in Burien and Normandy Park, WA, that receives untreated roadway runoff and has a history of coho mortality. This system provided a field-based 215 to assess acute and sublethal toxicity of stormwater in early life stage coho salmon (*O. kisutch*). Both phases used a side-by-side exposure design, with fish reared in well water (control) or unfiltered creek water (treatment). In Phase 1, three concurrent groups of juveniles were exposed to spring storms using continuous flow-through infrastructure. Stream-exposed fish exhibited characteristic symptoms of 6PPDQ exposure. Subsequent mortality in the stormwater treatment group averaged 80% and aligned with peak 6PPDQ exposure windows, confirmed by LC-MS/MS. Peak concentrations of 6PPDQ ranged from 72 to 110 ng/L in 2-3h composite samples, exceeding published LC50s for juveniles. This is the first study to demonstrate 6PPDQ-induced acute mortality under environmentally realistic conditions in a natural stream. In Phase 2, embryos were fertilized on-site and reared to fry under the same design. Stream-reared fry showed developmental impairments including delayed yolk absorption, reduced cranial growth, and a 29.9% reduction in caudal fin area, which were linked to impaired swim performance in trials. To test whether chronic exposure influenced acute sensitivity, both groups underwent a 24-hr 6PPDQ toxicity assay. Control fry showed dose-dependent mortality consistent with established LC50s. Stream-reared fry only showed mortality (40%) at the highest concentration (160 ng/L), ~400% above the reported LC50 for fry. This rightward shift in dose-response may reflect early-life selection, sublethal conditioning, or altered toxicokinetics. These findings support 6PPDQ as the primary causal toxicant driving both mortality and developmental disruption in early life stages. Real-time stormwater exposure acts not only as a chemical hazard but as a developmental force reshaping survival thresholds and performance capacity. Results highlight the need for risk frameworks that go beyond acute mortality in naïve fish to include exposure history, functional endpoints, and stage-specific vulnerabilities in wild salmonids.

2.13.B.T-06 Blood-brain and blood-gill barrier disruption in coho salmon exposed to roadway runoff and 6PPD-quinone

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The tire-derived chemical N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone)

causes acute mortality in coho salmon (*Oncorhynchus kisutch*), yet its mechanisms of toxicity remain poorly understood. We exposed juvenile coho salmon to roadway runoff or 6PPD-quinone to investigate whether disruption of the blood–brain barrier (BBB) and blood–gill barrier cause behavioral symptoms of urban runoff mortality syndrome in this sensitive species. BBB disruption was present in one-third of presymptomatic fish and in all symptomatic individuals, supporting its role in toxicity. Co-occurring brain and gill barrier disruptions at the onset of sustained surface swimming suggest a systemic vascular response rather than localized brain injury. Histological analysis of coho brains revealed red blood cell congestion within intact endothelia, consistent with circulatory collapse and plasma leakage, likely impairing oxygen delivery and disrupting neuronal signaling. Behavioral symptoms also coincided with altered expression of junctional proteins genes (ocln, cld5a, and vecad). In Chinook salmon (*Oncorhynchus tshawytscha*), exposure to a high but environmentally relevant concentration of 6PPD-quinone reduced expression of the scaffolding protein zo-1, suggesting potential sublethal effects. These findings identify BBB disruption as a key event in 6PPD-quinone toxicity and link vascular injury to behavioral symptoms in coho salmon. Ongoing work in this model will further clarify mechanisms of action and support assessments of environmental and human health risks from tire-derived chemicals.

2.13.P-Mo Stormwater Runoff Impacts, Solutions and Innovative Research

2.13.P-Mo-100 The use of unamended soils as a best management practice for mitigating 6PPDQ in stormwater: Influence of soil type and 6PPDQ persistence

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The tire-derived chemical, 6PPD-quinone (hereafter 6PPDQ), has been identified as a driver of urban runoff mortality syndrome in commercially, ecologically, and culturally important salmonid species. As such, developing effective mitigation strategies for roadway runoff has become a priority issue for stormwater managers. A review conducted by the Washington State Department of Ecology identified several best management practices (BMPs) for efficacy in removing 6PPDQ, including bioretention, street sweeping, and soil mixes. The use of unamended soils in bioretention systems represents a potential low impact BMP for 6PPDQ removal that incorporates natural on-site features, though its potential efficacy as a BMP has not been tested. As such, this study aimed to determine the effectiveness of unamended soils for 6PPDQ removal using three soils collected from the Capitol State Forest (Olympia, WA) and an amended bioretention soil mix (BSM). Five stormwater samples collected from January - March 2025 were collected from an urban area of Seattle and ran through bioretention columns containing three replicates of each soil type at rates representative of storm conditions. A suite of metals, polycyclic aromatic hydrocarbons (PAHs), and 6PPDQ were analyzed in influent and effluent and used to calculate percent removal. In addition, soil samples were analyzed for the same compounds at days 0, 7, 15, 30, 60, and 90 after cessation of stormwater application to determine potential persistence. Overall, two of the three unamended soils showed excellent removal of 6PPDQ (> 90%), with comparable performance to the commonly used BSM and effluent concentrations below state-specific aquatic life criteria. One of the collected soils, Centralia Loam, had effluent concentrations in several applications exceeding proposed WA aquatic life criteria of 0.008 µg/L. Soil types varied in 6PPDQ persistence, with the clay and loam-based soils having 6PPDQ concentrations below reporting limits 30 days after the final stormwater application, but the BSM still containing detectable 6PPDQ concentrations 60 days post-application. Overall, this study suggests that unamended soils may be effective in bioretention systems for mitigation of 6PPDQ, and that 6PPDQ persistence in soil may be variable among soil types. These findings will be used by stormwater managers to develop appropriate mitigation strategies for roadway runoff in urban areas impacted by 6PPDQ. **Disclaimer/Disclosure:** This work was funded by Washington State Department of Ecology.

2.13.P-Mo-101 Comparison of lab-scale and stormwater testing to evaluate key parameters governing environmental fate and transport of 6PPD-quinone

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6PPD-quinone, a tire-derived transformation product, is an environmentally ubiquitous contaminant with high aquatic toxicity. This study seeks to evaluate crumb rubber (a waste tire-derived product used as artificial turf infill) as a source of 6PPD-quinone and the effectiveness of existing stormwater best management practices (BMPs) in removing 6PPD-quinone. This presentation will combine lab-scale studies on (i) 6PPD-quinone leaching from crumb rubber and (ii) treatment efficacy of natural and engineered sorbents used in current best management practices (BMPs) with (iii) stormwater testing of crumb rubber filled artificial turf field runoff. Lab-scale batch leaching studies were conducted on crumb rubber to estimate leaching potential of 6PPD-quinone from artificial turf fields filled with waste tire rubber infill. Lab-scale aqueous partitioning was performed for natural soils and engineered sorbents to estimate partitioning coefficients for 6PPD-quinone. Recent work focused on sampling runoff from artificial turf fields filled with crumb rubber near salmon rearing and migratory habitat, including samples taken before and after existing stormwater management systems. The concentrations of 6PPD-quinone in stormwater will be compared to previous field impact estimates from the lab-scale leaching studies. The treatment efficacy of existing stormwater management systems will be compared to estimates from lab-scale aqueous partitioning studies, to evaluate environmental transport and treatment predictions from lab-scale results. Potential solutions for preventing or mitigating 6PPD-quinone release into habitats will be discussed.

2.13.P-Mo-102 6PPD & 6PPD-quinone: Regulatory momentum and analytical innovations

Louis Wagner, ALS Environmental Canada, Canada

6PPD-quinone (6PPD-Q)—the tire-wear transformation product now recognized as the driver of urban runoff-induced coho salmon mortality—has spurred an exceptionally rapid regulatory response in Canada and the USA. In a span of only thirteen months the USEPA released Draft Method 1634 and published acute freshwater screening values, Washington State codified a 0.012 µg/L aquatic life toxics criteria, and British Columbia issued a 0.010 µg/L water quality guideline. To support compliance and research, ALS Canada has validated an ISO 17025-accredited, isotope dilution LC-MS/MS method that mirrors Draft EPA Method 1634, achieving routine and low-level limits of reporting well below these thresholds and water quality guidelines. ALS has also extended testing to beyond the scope of Method 1634 to include accredited testing for 6PPD, the precursor to 6PPD-Q, and has developed an optimized antioxidant preservative to extend the stability of 6PPD to 14 days. This enables the quantification of both 6PPD-Q and its parent compound 6PPD at trace concentrations. An ultra-trace analysis method for 6PPD-Q to far below the current acute guidelines has also been developed, allowing for studies investigating baseline levels and quantifying chronic 6PPD-Q exposures. In addition to detailing these analytical advances, this presentation will summarize the latest regulatory updates, showcase representative field and laboratory case studies, and investigate how 6PPD-Q data—together with stabilized 6PPD measurements—can guide mitigation strategies and the potential ecological impacts of urban runoff containing these substances.

2.13.P-Mo-103 Field evaluation of rice husk biochar and pine tree woodchips for removal of tire wear particles from urban stormwater runoff in Mississippi (USA)

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Tire wear particles (TWPs), a form of microplastics (MPs) pollution, are transported into water bodies through stormwater runoff, leading to environmental pollution and impacts on associated biota. Here, we investigate the effectiveness of rice husk biochar and pine tree woodchips in reducing TWP pollution in urban stormwater runoff in Oxford, Mississippi at two sites during two separate storm events. Triplicate runoff samples were

collected upstream and downstream of the biofilters at two sites during two storm events at peak flow within minutes of the start of the storm and after 30 min. Samples were analyzed for TWPs using a combination of stereomicroscopy, micro-attenuated total reflectance Fourier transform infrared spectroscopy (μ -ATR-FTIR) and scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDX). Concentrations (TWPs/L) upstream of the biofilter were variable but highest at the start of the runoff, dropping from an average of 2811 ± 1700 to 476 ± 63 after 30 minutes at site 1 and from 2702 ± 353 to 2356 ± 884 at site 2. Biochar was more effective than woodchips ($p < 0.05$) at removing TWPs, reducing concentrations by an average of 97.6% (first use) and 85.3% (second use) compared to 66.2% and 54.2% for woodchips, respectively. Biochar was particularly effective at removing smaller TWPs ($< 100 \mu\text{m}$). Both materials became less effective with use suggesting fewer available trapping sites and the need for removal and replacement of the material with time. Overall, this study suggests that biochar and woodchips, alone or in combination, deserve further scrutiny as a potential cost-effective method to mitigate the transfer of TWPs and other contaminants to aquatic ecosystems and sensitive biota.

2.13.P-Mo-104 Toxicological assessment of road runoff-derived chemical mixtures in *D. magna* and *C. elegans*

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Road runoff has been implicated in substantial salmon die-offs observed in the Pacific Northwest. These salmon mortalities are referred to as urban runoff mortality syndrome that occurs in freshwater before the fish can spawn. One of the major toxic components of stormwater runoff are tire particles and their chemical leachates. The complex proprietary formulation of tire rubber consists of thousands of chemical additives. Of these additives, 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine), 1,3-Diphenylguanidine (DPG), dicyclohexylurea (DCU), and hexamethoxymethylmelamine (HMMM) have been prioritized for toxicity testing and analytical analysis as they are among the most predominantly detected organic compounds in stormwater runoff, tire rubber leachates, and surface water, and are potentially contributing to stormwater runoff toxicity in aquatic organisms. The main objectives of this study are to 1) test the toxicity of individual compounds and mixtures in the two model organisms; 2) test various bioretention media mixtures efficacy in the retention of several known tire additives and stormwater contaminants including zinc, 6PPD-q, DPG, DCU, and HMMM; and 3) conduct toxicity testing in relevant indicator species with unfiltered water or water filtered through bioretention media mixtures to evaluate the acute toxicity mitigation potential of the selected media. Concentration-response curves and LC50 values were established for DPG and zinc using *Daphnia magna*. *Caenorhabditis elegans* were exposed to $0.1 - 20 \mu\text{g/L}$ 6PPD-q and evaluated for behavioral and oxidative stress toxicity endpoints. DPG and zinc elicited mortality in *Daphnia* with LC50 values of $9,180 \mu\text{g/L}$ and $747 \mu\text{g/L}$, respectively. DCU, HMMM, and 6PPD-q separately did not significantly affect *Daphnia*. Equipotent mixtures with DPG and zinc revealed a slight antagonistic relationship between the compounds' toxicity. These findings demonstrate that among tire-derived contaminants, zinc and DPG are acutely toxic to *D. magna*, while other common tire additives such as DCU, HMMM, and 6PPD-q show limited acute effects. Notably, the slight antagonism observed in DPG-zinc mixtures highlights the importance of considering chemical interactions when assessing stormwater toxicity and underscores the potential value of bioretention media in mitigating these effects.

2.13.P-Mo-106 Assessing crayfish as a bioindicator for 6PPD-Q from laboratory experiments and field data collections

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Stormwater runoff is known to carry a variety of pollutants, leading to the contamination of water bodies with both organic and inorganic contaminants. (1,3-Dimethylbutyl)-N-phenyl-p phenylenediamine-quinone (6PPD-Q) is a recently defined organic pollutant that leaches from microplastic tire wear particulates and is toxic to some aquatic species, specifically Coho salmon. However, due to the transient nature of stormwater runoff events, which serve as the primary contamination pathway for 6PPD-Q, and the mobile nature of affecting

aquatic species, monitoring for this pollutant is difficult. This study aims to assess the suitability of crayfish as a bioindicator for 6PPD-Q exposure. Here, we report on findings from the first two phases of the study, a laboratory ecotoxicological experiment, and in situ sampling from three watersheds in the upper Columbia River Basin. The laboratory experimental design exposed two species of crayfish common in the Columbia River Basin (*Pacifastacus leniusculus* and *Faxonius rusticus*), to up to nine different concentrations of 6PPD-Q (0, 5, 25, 95, 250, 500, 1000, 5000, and 10000 ng/L). Replicate crayfish tissue and water samples were collected at 0, 24, 48, and 96 hr intervals, and 6PPD-Q concentrations in tissues and waters were quantified using a UPLC coupled to a triple-quadrupole mass spectrometer. Field water and tissue samples were collected from 9 sites across a spectrum of rural and urban land uses and quantified using the same methods developed for the laboratory analyses. The findings to date reveal quantifiable tissue accumulations of 6PPD-Q under high concentration laboratory exposures, but negligible accumulation under field conditions. While preliminary findings suggest that crayfish may not be ideal biomonitoring organisms for 6PPD-Q, the data do provide valuable insight into exposure durations and toxicokinetic dynamics in a common large aquatic macroinvertebrate. **Disclaimer/Disclosure:** This work is supported by EPA cooperative agreement #02J19601.

2.14.P-Mo General: Aquatic Toxicology, Ecology and Stress Response

2.14.P-Mo-062 Exposure to persistent organic pollutants (PCBs, OCs) in Greater Caribbean manatees (*Trichechus manatus manatus*) in Puerto Rico

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Greater Caribbean manatees (*Trichechus manatus manatus*) are aquatic mammals inhabiting the coastal and estuarine habitats of the Wider Caribbean Region. With fewer than 700 individuals remaining in Puerto Rico, it is critical to research the health of individual manatees to guide conservation efforts. Unlike cetaceans, sirenians possess a thinner, double layer of fat rather than a thick blubber layer. As a result, manatees are likely to face unique challenges in terms of exposure to toxic substances from human origins. This study focuses on evaluating the exposure of manatees to Persistent Organic Pollutants (POPs), particularly concerning health status. POPs, including organochlorines and polychlorinated biphenyls, are anthropogenic chemicals that persist in the environment and accumulate in marine organisms. Despite regulatory efforts, these pollutants continue to pose risks to wildlife, particularly species living in close proximity to human activities. By assessing the degree of POP exposure in manatees from Puerto Rico, we aim to correlate concentrations of these pollutants with the health of male and female manatees across different age groups (calves, juveniles, and adults). To achieve this, collected tissue and blood samples from stranded and rescued manatees (2012-2025) were analyzed for POPs using Gas Chromatography-Mass Spectrometry at the University of California, Davis. In addition, the health status of animals with significantly high concentrations of POPs was evaluated through a comprehensive metabolic panel. Preliminary results suggest that calves and juveniles are more vulnerable to higher contaminant concentrations, with notable presence of endrin aldehyde, PCB 66, and PCB 87 in samples. These findings highlight the potential for industrial activities to influence contaminant levels more significantly than agricultural practices in Puerto Rico. This study offers valuable insights into the exposure of an endangered marine species to harmful environmental pollutants and aims to enhance our understanding of the health risks posed by POPs. The results will inform conservation strategies and potentially guide future research on other environmental contaminants affecting marine ecosystems.

2.14.P-Mo-063 Influence of hypoxia on lead toxicity, calcium homeostasis and associated transcriptional responses in early life stage zebrafish (*Danio rerio*)

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Climate change is predicted to increase the occurrence of hypoxic events in freshwater habitats in the coming

decades. Co-exposure of freshwater fish to hypoxia and other environmental contaminants carried in anthropogenic runoff, such as lead (Pb), is likely to occur. Evidence suggests that hypoxia (20% air saturation) leads to hypocalcemia in early life stage (ELS) zebrafish (*Danio rerio*) likely due to a reduction in ionocytes expressing the epithelial calcium channel (ECaC). Calcium (Ca) is an essential ion involved in the development and homeostasis of the musculoskeletal and nervous systems of vertebrates. Given that Pb competes with Ca and is believed to enter fish through Ca transport pathways, such as ECaC, our goal is to determine whether Pb and hypoxia co-exposure would influence sublethal Pb toxicity as a result of Pb- or hypocalcemia-induced neurological dysfunction in ELS zebrafish. We hypothesize that reduced toxicity may occur due to diminished Pb uptake from reduced ECaC expression, whereas increased toxicity may occur due to exacerbated hypocalcemia owing to Pb acting as a Ca antagonist. To characterize the role of hypoxia in mediating Pb toxicity, we are co-exposing ELS zebrafish to Pb and 20% air saturation for 96 hr post-fertilization. Our endpoints include measuring whole-body Pb and Ca accumulation, swimming behaviors such as a startle response, and gross morphology including spinal curvature. Furthermore, we are examining the mRNA expression for target genes of interest involved in the hypoxia response, Ca homeostasis, and neurological/musculoskeletal development, including hypoxia inducible factor (HIF1 α), N-myc-downstream regulated genes (NDRG1 α), ryanodine receptor (RYR1 β) and ECaC.

2.14.P-Mo-064 Navigating challenges of low water solubility in ecotoxicity testing – A case study

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Substances with low water solubility are commonly found in various industries such as chemicals, cosmetics, and pharmaceuticals. Their low solubility presents many challenges in ecotoxicity testing, including analytical, dispersion, stability, bioavailability, and interpreting results. As part of the EU REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) regulation requirements, we conducted a series of tests including analytical method validation, water solubility, freshwater algae growth inhibition, fish early life stage, and reproduction for *daphnia magna* on a UVCB (Unknown or Variable Composition, Complex Reaction Products, or Biological Materials) substance, Di-tert-Nonyl Polysulfide. We overcame analytical challenges of measuring its low water solubility by pre-concentrating the aqueous samples with solid-phase extraction, combined with liquid chromatography–mass spectrometry analysis with post-column addition of AgBF₄ in acetonitrile. An estimated water solubility value of 63 ng/L presented significant challenges in subsequent ecotoxicity testing and solvent was used as recommended in the OECD 23 guidance document. Three different solvents (dichloromethane, dimethylformamide, dimethyl sulfoxide) were used for three trophic level species to adapt to dosing methods and issues with biofouling. Water solubility and analytical validation had to be repeated with each solvent. Despite repeated attempts, analytical confirmation of the test solution was not possible due to low water solubility. Stock solutions in solvent were measured as surrogates instead. No adverse effects were observed at the highest dose level in algae (0.78 μ g/L) and daphnia (1.0 μ g/L). The overall NOEC (No Observed Effect Concentration) for the early life stages of fish is 0.10 μ g/L, and the estimated EC10 (Effective Concentration 10%) 0.23 μ g/L based on growth (weight). We conclude that study design flexibility, including solvent choice, dosing methods, and analytical validation, is critical for the success of ecotoxicity testing with low water-soluble substances. Lessons learned from this case study can provide insights into future testing on similar substances.

2.14.P-Mo-066 Oxybenzone-induced oxidative stress and genotoxicity in *Aiptasia pallida*: A coral model for risk assessment in coastal ecosystems

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Organic ultraviolet (UV) filters such as oxybenzone (benzophenone-3, OBZ) are increasingly recognized as contaminants of concern in coastal ecosystems. Commonly found in sunscreens and personal care products, OBZ is frequently detected in nearshore waters, where it may persist and bioaccumulate. Growing evidence

suggests that even at low concentrations, OBZ can induce sub-lethal physiological and molecular changes in marine organisms, including those within coral reef communities. This study evaluates the potential of OBZ to induce oxidative stress and genotoxicity in *Aiptasia pallida*, a symbiotic cnidarian model widely used for coral toxicology. Specimens were exposed to environmentally relevant concentrations of OBZ (20, 200, 2,000, and 5,000 micrograms per liter) under static renewal conditions across three exposure durations: 5, 10, and 30 days. Biochemical responses were assessed through measurements of antioxidant enzyme activities, including catalase (CAT) and superoxide dismutase (SOD), along with DNA damage evaluated via comet assay. Preliminary results from the 5-day exposure period revealed a dose-dependent increase in CAT activity, while SOD activity was variably inhibited at higher concentrations. Notably, comet assay results showed significant DNA fragmentation in OBZ-exposed groups compared to controls, supporting the hypothesis that OBZ elicits both oxidative stress and genotoxic responses in *A. pallida*. These findings highlight the organism's sensitivity to organic UV filters and underscore its value as a sentinel species for assessing pollutant effects on reef-associated organisms. Ongoing analysis of longer exposure durations will offer further insight into the chronic impacts of OBZ and inform future ecological risk assessments.

2.14.P-Mo-067 Acute and chronic toxicity and oxidative stress by metallic nanoparticles in early life stage fathead minnows (*Pimephales promelas*)

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The objective of this research was to determine the toxicity of nZnO, nTiO₂, nFe₂O₃, nCu and nCuO to larval *Pimephales promelas* after 96-h and 28-d exposures. Endpoints included survival, growth, spinal curvature, and oxidative stress (total glutathione, GSH [reduced glutathione], GSSG/GSH ratio [GSSG = oxidized glutathione], and TBARS [Thiobarbituric Acid Reactive Substances; a measure of lipid peroxidation]). The lethal concentration 50s (LC50s) for nFe₂O₃ (28 mg/L) and nCuO, (0.66 mg/L) were greater than those for Fe⁺³ (0.039) and Cu⁺² ions (0.005), but the LC50 for nCu (0.009 mg/L) was similar to Cu⁺². There was no evidence of acute toxicity of dissolved ions in the nanoparticle suspensions, nor for nZnO or nTiO₂. In chronic exposures, both mortality and growth rate were increased at the lowest concentration of nFe₂O₃ (350 µg/L), while mortality and axial spinal curvature was the most sensitive indicator of chronic nCuO toxicity (the Lowest Observed Effect Concentration [LOEC]= 32.5 µg/L for both). The levels of TBARs and glutathione-related parameters suggested that oxidative stress increased in nFe₂O₃-exposed fish, but were decreased by nCuO exposure. Because of logistic constraints, chronic tests for nCu were not carried out. The LC10 and LC50 for nCu and LOEC concentrations of nCuO fall within the range of predicted concentrations for high-level exposure scenarios, so these effects may occur at environmentally-relevant concentrations. These nanoparticles were more toxic in fathead minnows than in other species from previous studies, and differential toxicity of pure nanoparticles vs. commercial formulations is discussed as a possible reason for difference between the present and previous works.

2.14.P-Mo-068 An overview of the process in creating site-specific water quality conditions for an industrial facility

Michael Jirsa, EA

The development of site-specific water quality criteria for individual metals through the performance of biological translators or water effect ratio studies has successfully enabled permittees to modify the National Pollutant Discharge Elimination System (NPDES) discharge permit single metal permit limits. This testing concept is based on the U.S. Environmental Protection Agency (EPA's) recognition that the metals can be substantially less toxic in an effluent/receiving water matrix than in clean laboratory water, on which the single metal permit limits are often based. Additionally, the ratio of dissolved to total fractions of the metal can greatly affect the toxicity of the specific metal. One metal in particular that has commonly been evaluated through these studies is copper. The toxicity of copper is dependent on site-specific factors such as pH, hardness, total suspended solids and dissolved organic carbon. This presentation outlines the process used to develop site-

specific copper criteria for an industrial facility that discharges non-contact blowdown cooling water. Key steps in the project included conducting a chemical translator study, performing whole effluent toxicity (WET) testing, and implementing Toxicity Identification Evaluation (TIE) procedures. Through TIE testing, EA identified multiple potential toxicants including polar organics, non-polar organics, and oxidants as contributors to observed toxicity. Further investigation, in collaboration with the client, traced the sources of these toxicants to water conditioning chemicals used for controlling corrosion, biological growth, and scaling in the cooling system. As a next step, EA is working with the facility to optimize the dosage of these treatment chemicals relative to the flow rate of the blowdown water. The ultimate goal is to bring the facility into consistent compliance with its NPDES permit and to support successful modification of the copper discharge limits.

2.14.P-Mo-069 Investigating developmental abnormalities in the imperiled gopher frog (*Rana capito*) using transcriptomics

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Amphibians face diverse threats across their complex lifecycles, which has spurred the use of a common conservation technique, ‘headstarting’, to bolster populations of imperiled amphibians. In 2021, our head-starting program for the Gopher Frog (*Rana [Lithobates] capito*) at the University of Georgia in Aiken, SC resulted in developmental abnormalities in nearly 100% of captive-reared metamorphs (severe edema, skin, jaw, skeletal, eye). Over the past decade, multiple facilities rearing Gopher Frogs from different source populations across the southeastern U.S. have observed similar co-occurring abnormalities. In 2023, we obtained experimental evidence that the native wetland grass, maidencane (*Panicum hemitomon*) that is the recommended plant for stocking headstarting mesocosms can be deleterious to the development of larval Gopher Frogs. In a monoculture-context, nearly 100% of individuals reared with maidencane developed abnormalities. Although we did not observe abnormalities when using a mixture of dominant wetland plant species that included maidencane, many wetlands in the coastal plains are dominated by maidencane to an extent that may mimic monoculture conditions, potentially causing abnormalities in wild populations and contributing to the species’ decline. To examine the physiological underpinnings of these abnormalities, we are investigating competing hypotheses regarding endocrine disruption arising from specialized metabolites originating from either (a) cyanobacteria associated with plant material or (b) the plant material itself. Given that carryover effects of atypical development on juvenile fitness are not well understood, we must clarify mechanisms and sources underlying these abnormalities to identify strategies to minimize risk of abnormality occurrence for wild individuals in maidencane-dominated wetlands. Our central objective is investigating whether abnormal larvae experienced endocrine disruption indicative of dysregulated retinoic acid signaling. To accomplish this, we are using a reference-guided RNA-seq approach with robust sequencing depth to generate liver-specific transcriptome profiles for analyzing gene expression and evaluating potential functional effects. We expect our data to not only improve basic understanding for the teratogenic potential of the *Panicum* genus to larval amphibians, but also to inform the refinement of head-starting and habitat restoration approaches supporting Gopher Frog conservation efforts.

2.14.P-Mo-070 Lethality of copper to the invasive golden mussel (*Limnoperna fortunei*)

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The freshwater golden mussel (*Limnoperna fortunei*) is an invasive aquatic bivalve, where their presence can result in costly economic and ecological damage. In October 2024, golden mussels were discovered in California’s Sacramento-San Joaquin Delta, representing the first known occurrence of golden mussel in North America. As part of a larger statewide response to contain and suppress golden mussels in currently infested waters, a chemical treatment pilot study to evaluate the efficacy of chemical treatment in open and closed water systems was implemented. As part of this study, dose-mortality relationships for golden mussel were developed utilizing two ionic copper based molluscicides (e.g., EarthTec QZ and Cutrine Plus). Laboratory based lethal

time-to-effect relationships (LT50, LT100) were established utilizing field collected adult golden mussel exposed to copper in site water. The results of this testing will be presented.

2.14.P-Mo-071 From tire to toxic? Assessing lethal and developmental effects of tire wear chemicals on sheepshead minnow and spotted seatrout

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Estuaries serve as critical habitats, providing abundant food resources, shelter, and nursery grounds for juvenile fish. Despite their ecological importance, these environments are often contaminated by urban runoff, which introduces a range of chemical pollutants. A significant source of this pollution is tire wear particles (TWPs), generated through friction between vehicle tires and road surfaces. These particles are ubiquitous in the environment and often found in aquatic systems. As tires contain numerous chemical additives, these substances are released into the environment with the dispersal of TWPs. Among these additives are 6PPD, an antioxidant and antiozonant used to extend tire lifespan, and its transformation product, 6PPD-quinone. Research has shown that the presence of such chemicals in aquatic environments can adversely affect the survival and development of various fish species. This study aims to investigate the effects of tire wear chemical exposure on the survival and development of two estuarine fish species, sheepshead minnow (*Cyprinodon variegatus*) and spotted seatrout (*Cynoscion nebulosus*). Both species are ecologically and economically significant in the South Atlantic region and rely on estuarine habitats throughout their life cycles. The study will assess toxicity thresholds for embryonic to larval sheepshead minnow and spotted seatrout, including the determination of an LC50 value, and evaluate non-lethal effects, such as impacts on growth and development. Embryos will be collected post-fertilization, exposed to varying concentrations of tire wear chemicals in a controlled laboratory setting, and monitored daily for mortality and development. It is hypothesized that chemical exposure will negatively affect survivorship and impair embryo-larval development in both species. Findings from this study will address critical knowledge gaps regarding the effects of tire wear chemicals, including 6PPD and 6PPD-quinone, on estuarine species and contribute to the growing understanding of the environmental impacts of tire wear particles. These results may inform regulatory bodies, such as the U.S. Environmental Protection Agency (USEPA), in establishing chemical safety thresholds and support fisheries management efforts which ultimately work to mitigate the impacts of pollutants.

2.14.P-Mo-072 Acute and sublethal effects of tire-derived 6PPD-quinone on salmonids and smelt species of conservation concern

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The transformation product 6PPD-quinone, derived from tire wear particles, has emerged as a high-risk contaminant in urban runoff. While its lethality to Coho Salmon is established, data remain limited for other fish species of conservation concern. This study presents a cross-species assessment of acute and sublethal effects of 6PPD-quinone on Delta Smelt (*Hypomesus transpacificus*), Longfin Smelt (*Spirinchus thaleichthys*), Chinook Salmon (*Oncorhynchus tshawytscha*), Coho Salmon (*O. kisutch*), and Steelhead (*O. mykiss*). Larval and juvenile stages were exposed to a range of concentrations in 96-hr static-renewal assays. Acute tests revealed species-specific sensitivity: Coho and Steelhead showed high sensitivity (LC₅₀ = 70 and 5,000 ng/L), while Chinook, Delta Smelt, and Longfin Smelt showed no mortality compared to controls. However, sublethal effects revealed toxicity not evident from survival alone. Behavioral assays under light-dark conditions revealed altered locomotion and spatial behavior across species compared to controls. Coho Salmon larvae and juveniles exhibited hyperactivity, freezing, and increased anxiety-like behavior at 0.1-100 ng/L. Steelhead larvae shifted from hyper- to hypoactivity between 0.5-500 ng/L, and juveniles showed concentration-related changes in activity and anxiety. In Chinook, larvae displayed increased freezing and erratic locomotion at 100-1,000 ng/L, while juveniles showed decreased velocity and altered anxiety-like behavior. Delta Smelt juveniles showed increased freezing at 1,000 ng/L, more bursting at 100,000 ng/L, and decreased thigmotaxis. Longfin Smelt

larvae and juveniles had reduced activity and anxiety changes between 100-100,000 ng/L. Physiological assays confirmed sublethal impacts. Coho and Steelhead showed altered upper incipient lethal temperature (UILT) at both life stages. Chinook juveniles had 8% lower swimming performance (Ucrit) at 100 ng/L. Delta Smelt larvae had increased UILT at 100,000 ng/L; Longfin Smelt juveniles showed marginal thermal sensitivity. These findings highlight 6PPD-quinone's capacity to impair behavior and physiology at low concentrations, even in species not acutely affected. Given their imperiled status and increasing urban runoff contamination, these results emphasize the importance of incorporating behavioral and physiological endpoints into ecological risk assessments for species of conservation concern. **Disclaimer/Disclosure:** Funding for this project was provided by California Department of Fish and Wildlife CDFW Proposition 1, Grant #Q2296002 to A.Segarra

2.14.P-Mo-073 Dynamics of trace element accumulation in North American river otters (*Lontra canadensis*) in the Indian River Lagoon inferred from stable isotope analysis

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North American river otters (*Lontra canadensis*) are semiaquatic predators occurring in both freshwater and estuarine habitats in North America, particularly in the highly urbanized Florida waterways. This species is susceptible to various anthropogenic impacts, including habitat destruction, road collisions, hunting, and chemical pollution. The dynamics of trace elements accumulation and their toxicity is not clearly understood in this species, particularly in Florida. Here, we examined the influence of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes, age, and sex on the accumulation of four trace elements—mercury (Hg), selenium (Se), lead (Pb), and cadmium (Cd)—in the muscle tissues of road-killed otters from the Indian River Lagoon (IRL) and upstream freshwater habitats. We hypothesized that age, sex, and trophic level, inferred from stable isotopes, would significantly influence trace element accumulation in river otters. Our results indicated that $\delta^{13}\text{C}$ significantly predicted Se levels but had no effect on the accumulation of other trace elements. Unexpectedly, a significant negative relationship was found between $\delta^{15}\text{N}$ and Hg, contrasting with expectations of biomagnification at higher trophic levels. This complexity suggests that multiple interacting factors may influence Hg bioaccumulation and warrant further investigation. Pb and Cd accumulation was influenced by sex, with males having significantly lower levels than females. Age only predicted Cd accumulation, with concentrations increasing with age, while Se and Hg were not affected by age or sex. Toxicity assessment using the Se: Hg molar ratios revealed that 31.3% of samples had a ratio >1 , indicating a protective effect of Se against Hg toxicity, whereas 68.7% of samples had a ratio ≤ 1 , suggesting heightened Hg toxicity risk due to insufficient Se levels to mitigate Hg effects. These findings highlight the complex interplay of ecological and biological factors influencing trace element bioaccumulation and toxicity in river otters.

2.14.P-Mo-074 Brevetoxin accumulation and depuration rates in *Mercenaria mercenaria* and the effects of microplastic co-exposures

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Harmful algal blooms (HABs) in Florida caused by the red tide dinoflagellate *Karenia brevis* pose a continuing threat to human and marine health, in addition to economic stress on local shellfish farms. Neurotoxic shellfish poisoning (NSP) caused by the consumption of brevetoxin contaminated shellfish, has led to regulations requiring shellfish farms to restrict harvesting, established by clam tissue toxin accumulation by ELISA (>1.6 ppm) and mouse bioassays (>20 MU). However, information regarding accumulation and depuration rates of brevetoxins in the commercially harvested clam species *Mercenaria mercenaria*, at differing cell densities is variable. As marine microplastic pollution escalates, the need to study microplastic exposed shellfish is paramount. These studies sought to define the accumulation and depuration rates of brevetoxins and their metabolites in *M. mercenaria*, and the effects of microplastic co-exposures. Controlled exposures to cultured *K. brevis* were conducted at low ($\sim 9.0 \times 10^4$ cells/day), medium ($\sim 2.5 \times 10^5$ cells/day), and high ($\sim 3.3 \times 10^8$

cells/day) cell concentrations and plastic microbead co-exposures at high ($\sim 3.0 \times 10^7$ cells/day) cell concentrations. We found that at low cell concentrations, toxin accumulation exceeded 1.6 ppm within 96 hr and depuration below 1.6 ppm occurred after 48 hr. At medium cell concentrations, toxin accumulation exceeded 1.6 ppm within 48 hr and below 1.6 ppm after 72 hr of depuration. At high cell concentrations, toxin accumulation exceeded 1.6 ppm within 24 hr and remained above 1.6 ppm throughout 120 hr of depuration. Microbead and *K. brevis* co-exposures altered accumulation and depuration in preliminary studies, requiring further investigation. With these findings, we aim to better understand the effects of microplastics on the accumulation and depuration of brevetoxins by shellfish in future studies, conducting more rigorous studies of microplastic and *K. brevis* co-exposures at varying densities with more field representative microplastics. This work is supported by Mote Marine Laboratory and FWC Fish and Wildlife Research Institute.

2.14.P-Mo-075 Monitoring the correlation between nutrient levels and microcystin production in an aquatic system

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Nutrients, such as nitrate and phosphorus have been identified as a cause of algal and cyanobacterial blooms in aquatic systems. The blooms can result in the degradation of drinking and recreational water due to the production of toxins as well as taste and odor. Due to human activity, the levels of nutrients in these systems are projected to continue to rise at rapid rates. Microcystis, a cyanobacterium sometimes involved in toxic blooms, produces the potent hepatotoxin microcystin. In this study, we monitored total reactive phosphorus and nitrate levels using surface and subsurface water samples collected weekly at three different locations of the east fork of the Stones River, Murfreesboro, Tennessee, USA. The samples are analyzed using HACH Nitrate TNT 835 and HACH Phosphorus: Total and Reactive TNT 843. The monitoring of nitrite and phosphorus levels in water may allow for future predictions of algal blooms. Relationships investigated were between nitrates/total reactive phosphorus and microcystin production. Data collected thus far showed no significant correlation. However, this study is ongoing and new data will be incorporated as it becomes available.

2.14.P-Mo-076 What is regulating chironomid populations? The influence of food supply and interference competition on development and mortality in *Chironomus riparius*

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Population dynamics in aquatic ecosystems are governed by complex interactions between environmental factors and density-dependent processes. While standardized toxicity tests often focus on individual-level effects, ecological risk assessment requires understanding how these translate to population-level impacts. *Chironomus riparius*, a widely used test species in ecotoxicology, exhibits strong density-dependent regulation, yet the mechanisms driving these relationships remain poorly quantified. This study investigated how food availability and larval density interact to influence *C. riparius* development, reproduction, and mortality. Laboratory experiments were conducted across multiple food levels (0.05-0.5 mg dw/larva/day) and initial densities (10-200 larvae/50 cm² beaker). Additionally, we used a recently developed DEB-IBM full life cycle model and successfully applied it under variable food conditions at the population-level under laboratory conditions. Our study showed that chironomid development and reproduction are primarily dependent on food supply, whereas larval density drives the density-dependent mortality rate. The interaction of food availability and interference competition determined the effective mortality rate over time. Cannibalism was the most likely mechanism responsible for the intraspecific mortality of the larval stages. Finally, the mechanistic model successfully predicted population trajectories under various conditions, including scenarios not used in model calibration. This work provides quantitative insight into density-dependent regulation in a key test species, demonstrating how individual-level responses to food limitation scale up to population-level effects. The validated model offers a tool for extrapolating standard toxicity test results to more environmentally relevant

conditions where density dependence may buffer or amplify chemical impacts. These findings highlight the importance of considering density-dependent processes in ecological risk assessment and provide a framework for incorporating these mechanisms into population modeling for regulatory purposes. **Disclaimer/Disclosure:** This work was supported by Syngenta Crop Protection. N. Galic and M. Vaugeois are employees of Syngenta.

2.14.P-Mo-077 Comparison of per- and polyfluoroalkyl substances (PFAS) mixture bioaccumulation in bluegill (*Lepomis macrochirus*) through water and dietary exposure

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Per- and polyfluoroalkyl substances (PFAS) are a large class of synthetic chemicals used in a wide range of industrial and commercial applications. These chemicals can enter the environment through wastewater treatment plant effluent, atmospheric deposition, landfills, biosolid application, and firefighting activities. Once in the environment, PFAS break down very slowly and can enter groundwater or bioaccumulate within the food web, leading to a variety of adverse health effects. Because of the numerous sources and applications of PFAS, these chemicals are present in the environment as complex mixtures of many different types of PFAS molecules. In aquatic ecosystems, PFAS accumulation in fishes can serve as an important route of dietary exposure to humans. Currently, knowledge of PFAS mixture exposure routes, accumulation, tissue distribution, and depuration in fishes is limited. To better understand these processes, the present study conducted a 56-day PFAS bioaccumulation and depuration experiment using young-of-the-year bluegill (*Lepomis macrochirus*). During a 28-day uptake phase, bluegill were exposed to environmentally-relevant concentrations of PFOS-only or a PFAS mixture (PFOS, PFOA, PFHxS) through water, diet, or water and diet. Following the 28-day uptake phase, fish were transferred to clean water and fed a clean diet for a 28-day depuration phase. Muscle, liver, and plasma samples were collected throughout the uptake and depuration phases to determine PFAS bioaccumulation rates, tissue distribution, and clearance rates. Mortality, hepatosomatic index, and gonadosomatic index were also recorded. Together, these data will provide important information about the uptake, tissue distribution, and depuration of PFAS mixtures in an ecologically and economically important fish species.

2.14.P-Mo-078 Physiochemical properties in six streams in the North Fork of the White River watershed

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The White River in northern Arkansas is of environmental and economic concern for the state as a site of tourism and recreation and an important source of water for agriculture. The White River has several tributaries which were on the 303(d) list for low dissolved oxygen (DO) in 2018. Phosphorus (P) levels have also been a concern as elevated P encourages the growth of toxic cyanobacteria that threaten human, livestock, and ecosystem health. This project monitored the water quality in the North Fork White River Watershed by sampling weekly at six sites for three years (2021-2024). Water samples were analyzed on site for pH, conductivity, DO, and temperature, and both filtered and unfiltered samples were transported back to the lab on ice for nutrient, total suspended solids (TSS), and turbidity analyses. Turbidity and TSS remained low throughout the 3-year sampling period. Average total P from January 2022 to October 2024 ranged from 0.199 to 0.549 mg/L, which is significantly greater than the general upper limit of 0.1 mg/L recommended by the Lehigh University Environmental Initiative. Protecting resources such as the White River is important to agriculture and recreation within the state.

2.14.P-Mo-079 Historical performance data for the USEPA oyster acute toxicity test (shell deposition) guideline

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The Eastern Oyster (*Crassostrea virginica*) is an important species from both an environmental standpoint - providing habitat and maintenance of water quality through filter feeding - as well as being a very popular seafood source for human consumption. Additionally, oysters are very sensitive biological markers for ecotoxicity testing and are a model species used for ecological risk assessment. The USEPA OCSPP 850.1025 Oyster Acute Toxicity Test (2016) is required for the registration of crop protection products in the U.S. Oysters used for these tests are sourced from marine aquaculture, resulting in a limited seasonal availability and inconsistent shell growth due to changes in environmental conditions. Consistently meeting the guideline validity criteria for shell growth (≥ 2 mm) in the control group (s) during oyster toxicity tests with the eastern oyster is difficult for contract research organizations. Test completion may be delayed a year or more if suitable test organisms are not available in a given year. We examined our lab's historical control data to determine the success rate of achieving a ≥ 2 mm shell growth per year over three decades to examine patterns and potential causes of the variability both within and between years. Potential causes include organism source, aquaculture practices, water temperature, weather, salinity, disease, water quality, and shipping. Our experience also recommends that acclimation procedures, feeding rates, and food quality all improve the chances of a successful study.

2.14.P-Mo-081 Determining trophic movement of cyanotoxins in an aquatic system

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One type of cyanotoxin found in harmful cyanobacterial blooms is a cyclic heptapeptide hepatotoxin called microcystin. It is a strong inhibitor of the serine/threonine phosphatases which control key biological pathways such as cell proliferation and cell death. This interference triggers a chain of events that leads to eventual apoptosis of animal cells. Cyanobacterial blooms that produce cyanotoxins in aquatic systems are becoming more common due to human activity, with potential movement of toxins from aquatic to terrestrial ecosystems through trophic transfer. To evaluate potential transfer of microcystin, 12-weeks of surface and subsurface water, sediment, tetragnathid spider, and aquatic emergent insect samples were taken between June 20, 2024, and December 13, 2024, across three sites near the Walter Hill Dam on the Stones River in Murfreesboro, Tennessee, USA and analyzed for microcystin concentrations by Enzyme-Linked Immunosorbent Assay (ELISA). Microcystin concentrations were found to be lowest in surface water with an average of 0.322 ng/mL (ppb). Subsurface water had slightly higher concentrations of microcystin than surface water at 0.622 ppb, and sediment samples had a much higher concentration at 18.3 ppb. Tetragnathid spiders contained an average of 407 ppb microcystin, and aquatic emergent insects contained 433 ppb microcystin. This data provides potential evidence of bioaccumulation of microcystin as well as trophic transfer from aquatic emergent insects to tetragnathid spiders, but there is no evidence of biomagnification.

2.14.P-Mo-082 Use of quantitative PCR (qPCR) on environmental DNA to determine how harmful algal bloom composition changes with seasons

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Harmful Cyanobacteria Blooms, also called Harmful Algal Blooms (HABs), are becoming an increasing concern towards water quality and produce toxins that harm people and wildlife. One of the algal toxins of concern, microcystin acts as a liver toxin. One of the primary bloom-forming genera that produce microcystins is *Microcystis*. The ability of cyanobacteria to produce the toxin depends on the presence of the microcystin synthetase gene cluster within its genome. We used quantitative Polymerase Chain Reaction (qPCR) techniques on cultured algae to amplify genes that can inform algal community structure in field samples with environmental DNA (eDNA). DNA primers that are capable of detecting genetic sequences for cyanobacteria, *Microcystis*, and microcystin synthetase E were utilized. These primer sets were then applied to field samples from the East Fork of the Stones River in Murfreesboro, TN to infer the variation in cyanobacteria communities and toxin-gene presence as seasons change. The biotechnological data was compared with microcystin

concentration, ammonia, nitrates, phosphates, pH, dissolved oxygen, conductivity, and bioaccumulation in aquatic emergent insects and spiders. The presence of target sequences for Microcystis and microcystin synthetase E were found to show a relationship with toxin concentrations. The relationship between gene presence and microcystin levels has implications towards the use of molecular tools and eDNA as monitoring methods for HABs.

2.14.P-Mo-083 Impacts of florpyrauxifen-benzyl on native Wisconsin fish

Emma Pellegrino, *University of Wisconsin, Madison*

Florpyrauxifen-benzyl (FPB) is an active ingredient in an aquatic herbicide used to control non-native aquatic plants, specifically Eurasian Watermilfoil (*Myriophyllum spicatum*). Eurasian Watermilfoil outcompetes native plant species, disrupts natural habitats, and impedes recreational activities. FPB is highly effective against Eurasian Watermilfoil at low concentrations (ppb), has a short half-life in the environment (2-6 days), and is commonly applied during the spring months to combat this species. However, there is limited literature exploring the impacts of FPB on off-target organisms with zero published papers using fish. Therefore, we investigated the impacts of FPB (0-50 ppb) on embryos of various fish species (*Sander vitreus*, *Acipenser fulvescens*, *Esox lucius*). We did not observe significant impacts on survival for any species at pH of 7 ($p > 0.05$). However, we observed a significant interaction between water pH and FPB concentrations on embryo survival of walleye ($p < 0.05$) and northern pike ($p < 0.05$) at ecological concentrations. Acidic water observed higher embryo toxicity as compared to basic water. These results highlight the need for further studies on the effects of FPB on off-target organisms, especially fish species at different developmental stages. Overall, these results can be used by management agencies to reduce the impacts of aquatic herbicides on non-target organisms when managing non-native plant species.

2.14.P-Mo-084 Application of whole effluent toxicity testing for environmental risk assessment of carbon capture treatment technologies

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As carbon capture technologies advance to support mitigation of climate change impacts, there is an increasing need to evaluate their environmental impacts – particularly the potential ecological effects of treatment technologies and carbon capture processes. Whole effluent toxicity (WET) testing, which utilizes aquatic organisms to assess the cumulative toxicity of complex mixtures, offers a robust framework for this purpose. Our approach presents the application of standard WET methodologies, including chronic assays with marine species including the Pacific topsmelt, giant kelp, and purple urchin to characterize the potential impacts from various carbon capture systems. This broad overview includes information from effluents tested, including those derived from electrodialysis and electrolysis processes, as well as other carbon capture technologies currently being piloted. Results demonstrate variable toxicity profiles linked to treatment chemistry and by-product formation, highlighting the value of WET testing for identifying environmentally compatible treatment configurations. Findings support the integration of WET testing in environmental risk assessments of carbon capture facilities, ensuring compliance with regulatory standards and promoting sustainable deployment of carbon capture technologies.

2.14.P-Mo-085 Global occurrence and aquatic hazards of sulfamethoxazole and trimethoprim in sewage influents, effluent discharges and surface waters

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Antibiotics are among the most widely used pharmaceuticals globally, and as a result are frequently detected in raw wastewater, treated effluent discharges, and receiving surface waters. Elevated levels of these compounds in wastewater and surface waters contribute to the development of antimicrobial resistance (AMR). With the United Nations projecting that AMR may cause as many deaths as cancer by 2050, understanding

environmental concentrations of antibiotics is crucial, especially for World Health Organization essential medicines such as trimethoprim and sulfamethoxazole, which are frequently used in tandem to treat a number of conditions, including bronchitis and urinary tract infections. To better understand whether the hazards posed by these compounds vary between matrices and geographic areas, we critically reviewed published global occurrence data for sulfamethoxazole and trimethoprim in wastewater influents, effluent discharges, and surface waters. We developed environmental exposure distributions (EEDs) by matrix and geographic area when sufficient data was available, and then performed probabilistic environmental hazard assessments (PEHAs) using the predicted no effect concentration for AMR (PNECamr) and adverse effects in aquatic life (PNECeco). Across all geographic areas and matrices, trimethoprim concentrations were more likely than sulfamethoxazole to select for antimicrobial resistance; for example, a predicted PNECamr exceedance of 15.4% and 2.2% was observed for trimethoprim and sulfamethoxazole, respectively, in global surface waters. Sulfamethoxazole, however, was more likely to elicit effects in non-target aquatic species, with a predicted 8.9% exceedance of the PNECeco in global surface waters. We identified the highest influent (46.1%), effluent (39.4%), and surface water (41.5%) exceedances of the sulfamethoxazole PNECeco in Africa relative to other geographic regions. Furthermore, exceedance of the sulfamethoxazole PNECamr and both trimethoprim PNECamr and PNECeco values in surface waters was highest in Africa, while minimal exceedances were observed in Europe. These findings identify that important water quality hazard differences exist among geographic areas and regions for trimethoprim and sulfamethoxazole, and underscore the need for water quality monitoring to identify hot spots and for improved wastewater treatment to mitigate the environmental and public health risks associated with antibiotic contamination.

2.14.P-Mo-087 Essential oils: A promising alternative to commercial pesticides for managing burrowing shrimp populations?

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In Washington State, Willapa Bay, along with Grays Harbor, have historically been used for on-bottom aquaculture of Oysters (*Crassostrea gigas*) and various clam species. In these areas increasingly abundant populations of the native burrowing shrimp *Upogebia pugettensis* and *Neotrypaea californiensis*, also commonly known as ghost shrimp, are causing oyster cultures to sink and suffocate. Given that oysters are an important seafood crop for the Pacific Northwest, many efforts and studies have been devoted to burrowing shrimp control. Former methods to manage shrimp populations, such as the usage of commercial carbamate and neonicotinoid pesticides have been banned from aquaculture practices to protect non-target species, environment, and human handlers. It has been proven to be a difficult task to find safer methods for ghost shrimp population control in the past. To identify novel options with fatal effects on ghost shrimp but minimal impact on non-target species, the toxicity of six essential oil-based products was tested using bioassays. Active ingredients of the products were limonene oil, clove oil, geraniol, pyrethrum or cottonseed, clove and garlic oil and shrimp were exposed to product concentrations ranging from 0.125 to 180 ul/l aqueously. The bioassays simulated three 4-hr exposures during low tides over the course of three consecutive days. Results demonstrated that all tested products had the potential to reduce juvenile burrowing shrimp populations after 72 hr, but lethal concentrations (LC50) varied greatly from $0.13 \pm 0.02 \mu\text{L/L}$ (4.5% Pyrethrum with PBO) to $39.78 \pm 25.79 \mu\text{L/L}$ (40% cottonseed oil, 20% clove oil, 10% garlic oil). Building on these findings, in further experiments potentially sensitive non-target organisms were exposed to the same chemicals. LC50s for juvenile *Metacarcinus magister* (Dungeness crab), *Americamysis bahia* (Mysida), and *Atherinops affinis* (Topsmelt silverside) were calculated for each chemical. First results reveal that the non-target species are more sensitive towards some of the applied chemicals (including essential oils) than the targeted ghost shrimp, which are resistant to treatment, making the quest towards finding a suitable population control method for burrowing / ghost shrimp that are causing issues for oyster hatcheries an ongoing one. Future research should look towards

methods that use chemistries safer for non-target species in combination with physical disruption of burrows.

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2.14.P-Mo-088 Integrating pathogen burden and contaminant bioaccumulation in juvenile salmon: A multi-stressor field study

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Out-migrating juvenile Chinook salmon (*Oncorhynchus tshawytscha*) encounter numerous stressors as they navigate from freshwater toward the ocean, including altered hydrology, variable food availability, chemical contaminants, and pathogen exposure. These stressors may act independently or interactively to impair health and survival, particularly in highly modified systems like California's Sacramento-San Joaquin Delta, a critical migration corridor for multiple Chinook runs. Fall-run Chinook Salmon, the focus of this study, is considered a species of special concern in California due to population declines. Understanding their health status can inform broader conservation efforts for Chinook Salmon and other at-risk salmonids. To investigate potential relationships between contaminant exposure and pathogen burden, Juvenile Fall-run Chinook Salmon were cage-deployed at multiple sites across the Sacramento-San Joaquin Delta in Spring, coinciding with their outmigration phase. These sentinel fish were exposed in situ for 14 days and subsequently analyzed for contaminant bioaccumulation, pathogen load, and growth. Water temperature was recorded continuously at each site to characterize local thermal regimes, which can influence both host susceptibility and pathogen dynamics. Chemical analysis focused on three classes of pesticides frequently detected in the region: legacy organochlorines (e.g., 4,4'-dichlorodiphenyltrichloroethane (DDT) and derivatives), pyrethroids (e.g., bifenthrin), and phenylpyrazoles (e.g., fipronil). Preliminary results reveal that bifenthrin, DDE, and total DDX (as a sum of DDT and degradation products) were detected across all sites, while fipronil was not. Concurrently, Pathogen burden was assessed using high-throughput Fluidigm qPCR targeting 47 salmonid pathogens from gill tissue. Early screening revealed spatial variation in pathogen prevalence and co-infection patterns.

Environmental parameters including temperature and growth will be evaluated to enhance interpretation. Finalized analyses will contribute to a more nuanced understanding of how chemical and biological stressors jointly shape juvenile salmon health in situ, supporting improved ecological risk assessments and informing evidence-based management strategies. By integrating chemical and biological stressor data, this study provides a comprehensive assessment of sublethal pressures faced by juvenile Chinook during freshwater-to-marine transition. **Disclaimer/Disclosure:** Funding for this project was provided by California Department of Fish and Wildlife and State Water Contractor.

2.14.P-Mo-089 Emerging contaminants in juvenile Chinook salmon: Patterns of exposure and implications for conservation

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Efforts to recover populations of threatened Chinook salmon in the Pacific Northwest may be hindered by exposure to contaminants in the juvenile life stage. Here we leverage a large dataset collected by the Washington Department of Fish and Wildlife's Toxic Biological Observation System to test for the accumulation of 219 contaminants of emerging concern (CECs) in juvenile Chinook salmon and identify chemicals that may affect salmon physiology, behavior, or fitness at current exposure levels. We also highlight results obtained from the region's two most urbanized watersheds, the Green/Duwamish and Puyallup/White, to demonstrate how these data can inform decision making to protect juvenile salmonids. We found that juvenile Chinook salmon sampled in the Puyallup/White watershed had the highest average concentrations of CECs, and that six chemicals appeared to be ubiquitous and were found in all five watersheds spanning a gradient of urbanization. In the subsequent detailed studies in the Green/Duwamish and Puyallup/White watersheds, we observed patterns in accumulation of per- and polyfluoroalkyl substances (PFAS) that revealed regions where certain analytes could be entering the rivers. In contrast, spatial patterns for pharmaceutical and personal care

products (PPCPs) were less clear and indicated diffuse sources throughout the migration corridor, such as stormwater or wastewater. We recommend these results be used to target areas for source tracing studies for PFAS, and that future studies test for PPCP residues in commercial fish feed. Finally, the detection of multiple emerging contaminants in almost all composite fish samples, 11 of which exceeded available biological effects thresholds, reinforces the global call for green infrastructure projects that target source control and the removal or reduction of emerging contaminants from stormwater and wastewater.

2.14.P-Mo-090 Ecotoxicity evaluation of novel water-soluble synthetic films based on PVOH and natural delivered materials

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Water-soluble synthetic polymers and films (WSSPs) are widely used in industrial products, food packaging, household items, and biomedical applications. Among them, polyvinyl alcohol (PVOH) is a typical WSSP produced by the hydrolysis of polyvinyl acetate, and it is commonly used as a base material for detergent pods in laundry cycles. Previously, we evaluated the ecotoxicity of two types of PVOH-based water-soluble films (Hi-Selon™, Mitsubishi Chemical Corporation) that include various additives. The evaluations were conducted using *Daphnia magna*, medaka fish (*Oryzias latipes*), bioluminescent bacteria (*Aliivibrio fischeri*), and the marine crustacean (*Artemia salina*). The results revealed that these films exhibited minimal ecotoxic effects in all test organisms. In the present study, we aimed to improve product performance and reduce environmental impact by developing several new composite materials that combine PVOH with natural delivered materials such as polysaccharides. These new materials possess the possibility to enhanced biodegradability and reduced environmental persistence compared to conventional fossil-delivered products. We conducted the same series of ecotoxicity tests on these newly developed materials. This presentation reports the results of those evaluations.

2.14.P-Mo-091 Analysis of PCBs, naphthalene and heavy metals in bottlenose dolphins (*Tursiops truncatus*) and their prey species in the Mississippi Sound

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Bottlenose dolphins (*Tursiops truncatus*) are native to the Mississippi Sound (MSS), a region characterized by significant human activity that can contribute to elevated levels of environmental contaminants, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals. To investigate the sources of contamination in this population, we selected five fish species that make up the bottlenose dolphin diet to compare the chemical profiles in the tissues of fish and dolphins. Tissue samples (blubber/fat, kidney, liver, and muscle) were collected from bottlenose dolphins during necropsies performed by Mississippi State University Gulf Coast Aquatic Health Lab veterinarians and staff and the Institute for Marine Mammal Studies (IMMS) staff in Gulfport, MS, and from fish species supplied by the Mississippi Department of Marine Resources (MDMR) necropsied at a separate off-site location. These samples were analyzed for 7 PCBs, naphthalene, and 9 heavy metals. We found that PCBs were readily detected in dolphin tissues; however, there was almost no detection of PCBs in fish tissues. Naphthalene was detected in all dolphin tissues and all fish tissues except fish fat, although there was no biomagnification of naphthalene from fish to dolphins. Heavy metals were detected in all dolphin tissues and almost all fish tissues, except for cadmium in fish muscle. Mercury was biomagnified to the highest degree in the kidney, followed by liver, and muscle. These results suggest that biomagnification is not a major mechanism for bioaccumulation of PCBs, naphthalene, or most heavy metals, suggesting that chemical bioaccumulation in dolphins comes from alternative sources. Regardless of the mechanisms of exposure or bioaccumulation, the presence of these contaminants might impact the overall health of this dolphin population, highlighting the importance of long-term surveillance.

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2.14.P-Mo-093 Proposal of *Ceriodaphnia dubia* and *Moina macrocopa* as new daphnid test species in OECD test guideline

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Daphnia magna has been used for decades to assess the ecological hazard/risk of chemicals, effluent, and receiving water all over the world. However, it is originally from North America, not indigenous in Japan. Considering the different diversity and geographical distribution of Cladocera species and their species sensitivity differences, there is increasing demand to use of indigenous species, as well as international standard species, in ecological hazard/risk assessment of chemicals to protect local ecosystems. According to OECD test guideline No. 211 *D. magna* reproduction test (TG 211), “other daphnids may be used provided they meet the validity criteria as appropriate”. Therefore, we propose two species as new test organisms for TG 211: *Moina macrocopa* and *Ceriodaphnia dubia*. Both species are cosmopolitan species, in particular, *M. macrocopa* is common in ponds and rice paddies in Japan. Although *C. dubia* is not indigenous in Japan, it has been widely used in effluent toxicity test in Australia, Canada, the US (also partially used in Japan) and several standard test guidelines and chronic toxicity data for numerous chemical substances are available. In contrast, a standard test guideline using *M. macrocopa* has not been established yet, although it has been used in several ecotoxicological studies and the optimal test conditions of three-brood reproduction test using *M. macrocopa* was investigated in Korea. Three-brood reproduction tests using *M. macrocopa* and *C. dubia* for 6-8 days have significant advantages over the 21 days *D. magna* reproduction test in terms of resources savings (time, cost, space, manpower). Several studies have suggested that *C. dubia* and *D. magna* have similar sensitivities in standard chronic toxicity test; however, there are limited studies comparing them with *M. macrocopa*. In this study, we conducted literature search/review of exiting toxicity data for the three species to compare the sensitivity of these species. In addition, we will present the results of the preliminary study to develop a draft test protocol of three-brood reproduction test using *M. macrocopa*.

2.14.P-Mo-094 Antibiotic resistant gene (ARG) profile through 16s-based microbiome analysis in the remote Florida Everglades

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Antibiotics have been among the most important modern advancements in public health, providing significant benefits and breakthroughs in clinical medicine. However, widespread use of antibiotics has contributed to the rise of antimicrobial resistance (AMR), which now threatens the progress made through the use of antibiotics. Aquatic ecosystems are particularly vulnerable to AMR, as they often receive high concentrations of antibiotic residue through runoff, increasing exposure and potential ecological consequences. The FL Everglades present a unique opportunity to study AMR in aquatic environments, as they contain both highly remote and highly urbanized areas. This contrast allows for the investigation of key drivers and the prevalence of antibiotic resistance genes (ARGs) across a gradient of human impact, ultimately offering insights for protecting both ecological and human health from the detrimental effects of ARGs. Water samples were taken from 12 sites in

the FL Everglades, with sites transecting the FL peninsula. Water was filtered and DNA was extracted using QIAamp DNA Mini Kit. The V3-V4 region of the 16s rRNA gene was amplified using Quick-16s Plus NGS Library Prep Kit and final library was sequenced using Illumina NextSeq 2000 215. Reads were analyzed by applying the Qiime2 workflow. Microbial community composition varied substantially across the sampling sites. Beta diversity analysis showed that sites in close proximity tended to have similar microbial profiles—for instance, sites 11 and 12, as well as sites 3 and 7—suggesting that geographic location influences species composition and may impact AMR patterns. In terms of alpha diversity, species richness decreased from site 1 to 7, then increased from site 7 to 12, except for site 9 which displayed higher richness than site 10. These trends further highlight the role of location in shaping microbial communities. Between 3 to 45% of the microbes detected at each site belonged to the Flavobacteriaceae family, with the highest abundance observed at site 7. Flavobacteriaceae are widely distributed in marine environments, and several species within this family are known pathogens in fish. Overall, the findings indicate that location plays a significant role in shaping microbial community composition, which in turn can influence patterns of antimicrobial resistance. These spatial dynamics should be taken into account, particularly when comparing urban versus remote environments.

2.15.P-Th Late-Breaking Science: Aquatic Toxicology, Ecology and Stress Response

2.15.P-Th-018 PFAS in stormwater control measures: Removal, distribution, and long-term fate

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This study evaluates the performance of various conventional stormwater control measures (SCMs) in mitigating 26 per- and polyfluoroalkyl substances (PFAS), including perfluorooctanesulfonic acid (PFOS), and perfluorooctanoic acid (PFOA). PFAS with carbon chain lengths ≥ 9 exhibited greater particulate affinity, ranging from 35% to 73% of their concentration associated with suspended solids. In contrast, only 17–35% of PFAS with $C \leq 8$, which dominated the detected compounds, were associated with particulate phases. As most SCMs studied (biofilters, media filters, hydrocyclones, and a retention pond) heavily rely on particle removal, the treatment of filtered-water PFAS ($< 0.7 \mu\text{m}$) was poor, with essentially no attenuation. Particulate phase PFAS removal varied, with some systems, such as the retention pond, effectively reducing concentrations. In contrast, others, including the biofilters and one treatment train (hydrocyclone plus cartridge filters), exhibited unexpected increases, suggesting potential remobilization of particulate-associated PFAS within the system. The retention pond SCM, influenced by evaporation, infiltration, and used for local irrigation, was monitored over a one-year period to assess PFAS persistence, precursor transformation, and the effects of water loss on contaminant concentrations. Analysis of precursor compounds in the pond, including fluorotelomer sulfonates (FTS) and perfluoroalkyl sulfonamides (FASAs), showed decreased concentrations. When coupled with increased concentrations of stable products such as perfluorohexanesulfonic acid (PFHxS), PFOS, and PFOA, these changes suggested in situ transformation due to the long retention time in the system. Additionally, the concentration of PFAS associated with the filtered water (e.g., PFBA, PFOA) increased over time due to evaporation of water during periods of minimal stormwater inputs or discharges. Perfluoroalkane sulfonic acid (PFSA) concentrations (primarily PFOS) in sediments were higher than those in the incoming particulate phase, suggesting sorption and accumulation of PFOS in sediments. The substantial filtered water fraction of PFAS and the limited removal of PFAS by any of the SCMs underscores the limitations of conventional SCMs in treating PFAS.

2.15.P-Th-019 Phytoplankton of Omi-Nla River, Agbabu, Odigbo local government area, Ondo State, Nigeria

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Omi-Nla River, Agbabu is used for artisanal fishing, ferrying and domestic activities in Odigbo Local Government Area (OLGA), Ondo State. The river receives bitumen spills, domestic and agricultural wastes,

which could impact adversely on the water quality and resident biota. In spite of anthropogenic activities, there is dearth of information on the limnology and biota of the river. Extensive bitumen spills as well as uncontrolled discharge of domestic wastes have pollution implications as they alter prevailing conditions and destroy the habitats of aquatic organisms. The aim of this research was to investigate the phytoplankton composition of Agbabu to provide information for effective management. Monthly sampling was carried out from three randomly selected sites on Omi-Nla River, Agbabu (from June, 2024 to March, 2025). Phytoplankton samples were collected with plankton net of 55µm mesh size. The net was towed horizontally along each sampling point at a distance of 20m to 30m for an approximate speed, just below the water surface to collect phytoplankton. Phytoplankton samples collected were immediately fixed and preserved in 4% formalin solution in the field according to Onyema (2007). Keys provided by Prescott (1954), Whitford and Schumacher (1973), Needham and Needham (1962), Mason (1991), Jeje and Fernando (1986; 1991), APHA/AWWA/WEF (1998) and Nwankwo (2004) were used for identification of the plankton species. The total number of organisms per milliliter for each sample was determined by simple calculation after counting the number in the 0.1mL sub-sample examined.

2.15.P-Th-020 Examining the association between copper bioavailability and copper-driven co-selection of tetracycline resistance development in *E. coli* K12 MG1655

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Heavy metals have increasingly been recognized as key contributors to the emergence and persistence of antibiotic resistance genes (ARGs) in the environment. However, the non-traditional pathway of heavy metal-driven co-selection of antibiotic resistance remains insufficiently explored, particularly with respect to the influence of metal bioavailability. In this study, *Escherichia coli* K-12 MG1655 and tetracycline (Tet) were employed as the model bacterial strain and antibiotic, respectively, to investigate the relationship between heavy metal uptake and the development of antibiotic resistance. Cultures were grown in a defined glucose minimal salts medium (GMM), where total Cu(II) and Tet concentrations were maintained at sub-lethal levels (EC₅₀ and EC₇₅, respectively) during exposure experiments. Using the free-ion activity model (FIAM), chloride concentrations in the GMM were varied from 1 to 100 mM to generate a gradient of free cupric ion (Cu²⁺) concentrations, thereby testing the hypothesis that increased Cu(II) bioavailability enhances Tet resistance. Over a 14-day exposure period, Tet-alone treatments induced tolerance and resistance in *E. coli*, but the combination of cycling Tet and Cu(II) exposures led to more rapid and pronounced resistance development. Elevated Cu²⁺ levels accelerated the onset and magnitude of Tet resistance, a trend that persisted when chloride was replaced with EDTA to modulate Cu²⁺ availability. Furthermore, comparable resistance to Tet was observed under Cu(II)-only exposure. Quantitative PCR analysis revealed that the expressions of Cu-resistance genes (*pcoA* and *cusA*) were significantly upregulated in response to Cu(II), and their expression levels were positively correlated with those of the Tet resistance gene *tetA*. Collectively, these findings highlight the pivotal role of metal bioavailability in mediating heavy metal-induced co-selection of antibiotic resistance, with important implications for environmental risk assessment and resistance management strategies.

2.15.P-Th-021 Comparisons of mercury bioaccumulation and biomagnification in boreal headwater streams before and after forest harvesting

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Forestry practices can increase the aqueous concentrations of methylmercury (MeHg) in streams, thereby potentially affecting its subsequent bioaccumulation into consumers and biomagnification through these food webs. In northern Ontario, Canada, streams were sampled for basal food sources (aquatic and terrestrial), macroinvertebrate primary and secondary consumers, and fish both pre-, during and post-harvest (temporally;

n=2) as well as across sites with different intensities of harvesting in the watershed (spatially; n=7). Samples were analyzed for [MeHg] (food sources, macroinvertebrates) or [total Hg] (fish) and for N isotopic ratios ($\delta^{15}\text{N}$) to characterize their relative trophic level. Increases in [MeHg] in water (3x) and some macroinvertebrates (2x) and [total Hg] in fish (2x) occurred after the extensive harvest (78% of watershed) at one stream but not at another stream where less harvesting and best management practices occurred. No changes in trophic magnification slopes (log Hg versus $\delta^{15}\text{N}$) were found after harvest at these sites, with slopes ranging from 0.24 to 0.36. Spatially, we found significantly higher [MeHg] in food sources and macroinvertebrates in harvested compared to non-harvested landscapes, but no difference in [THg] of fish or Hg trophic magnification slopes (ranging from 0.25-0.3 and from 0.25-0.33 in harvested and reference streams, respectively). These results suggest that harvesting can increase [Hg] in some food web components, and the magnitude of these effects appear to vary with management practices used and the % of the watershed harvested.

2.15.P-Th-022 Photochemical dominance in mercury reduction pathways in the Florida Everglades: Insights from gross and net experiments

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Mercury (Hg) reduction to volatile elemental Hg(0) removes Hg(II) from the aqueous phase and limits its methylation in aquatic systems. Although photoreduction is recognized as the dominant pathway in sunlit surface waters, microbial and dark chemical reduction have also been observed in the absence of light. The Florida Everglades, known for its high dissolved organic matter (DOM) content and elevated methylation potential, may exhibit unique controls on Hg reduction. However, the dominant reduction pathways and the fate of Hg(0) formed in this ecosystem remain poorly understood. To address this gap, incubation experiments were conducted using Everglades water collected from two sites: Pa-hay-okee Trail (PT) and Shark Valley (SHV), during both the dry and wet seasons. Samples were spiked with 40 ng/L of Hg(II) and incubated under light and dark conditions to examine gross and net Hg(II) reduction. Here, gross reduction refers to total Hg(II) reduction to Hg(0), regardless of re-oxidation, whereas net reduction reflects the overall change in Hg(II) after both reduction and re-oxidation. Net reduction results showed no measurable Hg(0) production after 12 and 24 hr of incubation, with complete recovery of the spiked Hg(II) under all experimental conditions. In contrast, gross reduction experiments revealed that photoreduction was the dominant pathway for Hg(0) production, accounting for up to 70.8% of total Hg(II) reduction during the wet season and 27% during the dry season. Although dark chemical and microbial pathways contributed less to overall reduction, their activity was notable at SHV during the wet season, likely influenced by elevated total Fe concentrations in the sediment. These findings suggest that while Hg(0) formed in Everglades water can undergo rapid reoxidation when undisturbed, the high DOM content may enhance Hg(II) reduction by facilitating electron transfer. This study further suggests that chemical species, such as Fe may play a significant role in Hg(II) reduction dynamics in Everglades waters, and this warrants further investigation.

2.15.P-Th-023 Managing harmful cyanobacteria bloom impacts on water treatment operations: Effects of prechlorination on disinfection by-product formation and role of sludge supernatant recycling

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Cyanobacteria blooms and their associated toxins are critical issues affecting water management organizations globally, and important questions remain about the best management options for water utilities faced with blooms in their supply water. In this study, the distribution and removal of cyanobacteria cells, cyanotoxins and disinfection by-products (DBPs) were analyzed across all unit operations of a water treatment plant on a lake with regular cyanobacteria blooms (Clear Lake, California, USA) using fluorometric, liquid chromatography and gas chromatography techniques, respectively. The main objectives of this study were to assess the impacts of dewatered supernatant recycling and the effectiveness of the prechlorination process. Throughout the sampling period the treated water met all relevant United States Environmental Protection Agency (US-EPA) and World Health Organization (WHO) standards for DBPs and cyanotoxins. However, substantial increases in

microcystin-LR concentration and formation of unregulated highly toxic DBPs were observed following prechlorination, and the latter were not efficiently removed in subsequent processes. The study results also showed the effectiveness of the dewatered super-natant treatment process and the management strategies applied to minimize the effects of the recycling process; however, it is advised that the regulation for supernatant recycling should be revised to include a water quality standard.

2.15.P-Th-026 Sublethal ventilatory impacts of stormwater contaminant (6PPD-Q) exposure on steelhead (*Oncorhynchus mykiss*)

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6PPD-quinone (6PPD-Q) is an ozonation product of 6PPD, an antidegradation component of tires. In 2021, 6PPD-Q was identified as the cause of urban runoff mortality syndrome in coho salmon (*Oncorhynchus kisutch*); however, the mechanism of toxicity is still unknown. Following 6PPD-Q exposure, coho and less sensitive taxa including anadromous coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) and steelhead (*Oncorhynchus mykiss*) exhibit a distinct set of abnormal behaviors including gaping at the surface, swimming in a spiraling motion, and gill splaying. In laboratory exposure studies, changes in ventilation typically precede changes in swimming behavior. Previous studies have reported increased ventilation frequency and subtle changes in aerobic metabolism. We hypothesize 6PPD-Q exposure will increase ventilation frequency at sublethal concentrations under progressive hypoxia. To investigate changes in ventilatory behavior, we designed a novel system to video record respiratory structures. Using this system, we exposed juvenile steelhead to sublethal concentrations of 6PPD-Q and captured video to determine the ventilation frequency and amplitude. Next, the fish underwent a progressive decrease in oxygen to measure the impacts of 6PPD-Q exposure on the hypoxic ventilatory response. This work was designed to improve our understanding of the sublethal impacts of stormwater and the effects of multiple stressors on Pacific salmon.

2.15.P-Th-027 Evaluating the effects of 6PPD-Q exposure on spontaneous motor behavior in steelhead (*Oncorhynchus mykiss*)

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6PPD-quinone (6PPD-Q), an oxidation product of the tire additive 6PPD, is a recently identified contaminant of concern in urban watersheds. The mechanism of toxicity has yet to be uncovered, but growing evidence suggests neurological effects may play a role. Identification of specific changes in behavior can support mechanistic hypotheses and serve as a phenotypic anchor for future molecular studies. Juvenile steelhead (*Oncorhynchus mykiss*) were exposed to sublethal concentrations of 6PPD-Q and video recorded in behavioral arenas. Computer vision video tracking software (Noldus Ethovision XT) was used to quantify a variety of behavioral endpoints, including distance traveled, time immobile, and thigmotaxis. Based on recent research by other groups and our pilot studies, we hypothesize an increase in distance traveled and a decrease in time immobile in response to 6PPD-Q exposure. These hypothesized changes in behavior may affect predator avoidance and bioenergetic balance, which can alter survival and reproduction rates at the population level. These behavioral data provide an important line of evidence in the broader investigation into sublethal impacts and potential neurological mechanisms of 6PPD-Q toxicity in Pacific salmon and trout.

2.15.P-Th-028 The living dead: Evidence for Hg bioamplification among emaciated Lake Superior Lake Charr

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Bioenergetic bottlenecks represent the condition during which organisms function in a net energy deficit. Subsequently, for pollutants such as Hg with extended half-lives in biological tissues, this energetic imbalance can result in bioamplification. The physiological condition when organisms lose body weight and chemical

portioning capacity at a faster rate than they can eliminate those chemicals. Here, we measured total Hg concentrations in emaciated Lake Charr (*Salvelinus namaycush*) collected from Lake Superior's maximum depth (400+ m) and compared against concentrations in healthy individuals. Total Hg in emaciated fish averaged 0.76 ± 0.2 $\mu\text{g/g}$ (wet wt) with all fish exceeding FDA and EPA advisory guidances. Total Hg concentrations in emaciated fish were also ~4-fold higher relative to healthy but similarly-aged charr. Interestingly, these fish were of the high lipid siscowet ecotype for which whole body lipid contents can exceed 60% (dry wt.). For emaciated fish, the consequences of such changes in proximate composition are two-fold specifically; i) the loss of lipids contributes to reduced buoyancy and their descent into the depths and obligate zombie-ism and; ii) their high Hg concentrations suggest persistence in a zombie-like state for extended periods given mercury's tendency to bioaccumulate in protein.

2.15.P-Th-029 Ecotoxicological impacts of PFAS mixtures: Mechanistic insights from *Menidia beryllina*

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Per- and polyfluoroalkyl substances (PFAS), often called “forever chemicals,” are a class of about 15,000 synthetic compounds valued for their resistance to heat, water, and oil. These same properties make them highly persistent, allowing PFAS to accumulate in organisms and biomagnify through food webs. In aquatic ecosystems, PFAS can impair growth, reproduction, and immune function, with higher-order species and ultimately humans facing disproportionate risks. Despite their widespread presence, ecotoxicological research has largely focused on two legacy compounds, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). This narrow scope limits regulatory efforts and risk assessments, leaving thousands of PFAS understudied and gaps in understanding mixture toxicity. This research aims to fill these gaps by evaluating the aquatic toxicity of PFAS mixtures through an acute ecotoxicity study on *Menidia beryllina*. Objectives include (1) determine the concentrations at which mixtures cause measurable harm to aquatic species and (2) identify toxic effects and underlying biochemical pathways. The approach integrates standard aquatic toxicity testing with molecular analyses to link organism-level responses to mechanistic pathways. We hypothesize that PFAS mixtures produce additive effects at concentrations lower than those predicted from single-compound data. Confirming this would highlight the inadequacy of relying on legacy compounds alone for ecological risk assessment and emphasize the need to consider mixture toxicity. Integrating data on PFAS mixtures with findings from single-compound toxicity studies will strengthen ecological risk assessment frameworks, improve screening thresholds, and inform more effective environmental policies. In doing so, this research will contribute to protecting ecosystems and the communities that depend on them.

2.15.P-Th-030 Effects of microfiber age on marine keystone species, *Americamysis bahia*

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Plastic pollution is a significant threat to the achievement of the United Nations' Sustainable Development Goals. In marine environments, plastics break down into tiny fragments, commonly referred to as microplastics. Microfibers, a common type of microplastic, are known to cause significant effects in marine species, including food dilution, and are an increasing concern. For keystone species such as the mysid shrimp *Americamysis bahia*, microfibers interaction could differ with differently-aged plastic fibers. Interactions could differ based on surface properties, propensity to grow biofilms, texture, and dosage. The study examines whether mysid shrimps exposed to polypropylene microfibers that had aged for three years will show different endpoints (growth and behavior) compared to those exposed to microfibers aged for five years and the controls. This study also examines the dose response of mysid interactions with different fiber concentrations (5, 10, 20 particles/mL). After running the exposure for 7 days, we will examine the differences in the growth of the mysid shrimps using ImageJ, as well as the dose-response of the mysid shrimps. The results of this study can be useful for establishing baseline information for mysid interaction with the aging of microfibers in the marine environment.

2.15.P-Th-031 Modeling population and disease dynamics of Great Lakes fish exposed to PFAS

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Per- and polyfluoroalkyl substances, otherwise known as PFAS, chemicals have been produced since the 1940s and are suspected to be harmful to humans, wildlife, and the environment. Previous research has found these compounds to have toxic effects on lipid metabolism, hepatotoxicity, immunotoxicity, development, reproduction, and growth in humans and various aquatic species. These compounds have been found in all five of the Laurentian Great lakes as well as within many of the valuable fish species that reside in the lakes. I created simulation models to explore population-levels effects on species such lake whitefish (*Coregonus clupeaformis*), lake trout (*Salvelinus namaycush*) and steelhead (*Oncorhynchus mykiss*) populations stemming from PFAS contamination effects on factors such as fish growth, fecundity, disease mortality, and egg survival. For interactions with disease, I used an infectious disease model that simulated lake whitefish exposures to viral hemorrhagic septicemia virus (VHSV), lake trout exposures to epizootic epitheliotropic disease virus (EEDV), and steelhead exposures to *Flavobacterium psychrophilum*. The population model results showed that decreases in growth in terms of weight and relative fecundity (i.e., eggs per kg) had the largest effect on population abundances across all three species. When factors were modeled in combination, all three species were predicted to experience 80% declines in abundance over a 65-year period. The results of the disease transmission model found that PFAS exposure caused the largest percentage increase in the maximum proportion of infected steelhead.

2.15.P-Th-032 Towards precision ecotoxicology: Exploring cross-species behavioral sensitivity to selective serotonin reuptake inhibitors (SSRIs)

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Selective serotonin reuptake inhibitors (SSRIs) are a class of antidepressants widely used to treat depression and various other mental health disorders. Due to widespread usage, they are commonly found in freshwater and coastal ecosystems. Growing evidence indicates that these pharmaceuticals can influence the behavior of aquatic organisms at environmentally relevant levels. However, the range of taxa potentially susceptible to SSRI exposure remains largely limited. Thus, our study examined potential cross-species sensitivity of 36 known human SSRI targets, including molecular initiation events plausibly linked to behavioral perturbations. We used Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) analyses to identify susceptible species, and then developed aquatic species sensitivity distributions (SSDs) based on evolutionary conservation of targets across species. Potentially affected fraction (PAF) of aquatic species based on level 1 (full sequence) SeqAPASS analyses ranged from 2.3% for sigma-1 receptor (SIGMAR1) to 92.35% for muscarinic acetylcholine receptor m5 (CHRM5). Level 2 (functional domain) analyses for eight molecular targets showed minimal change in PAF for serotonin transporter (SERT) from 91.16% to 93.72%, a marked decrease for dopamine transporter (DAT) from 56.75% to 24.5%, and a substantial increase for norepinephrine transporter (NET) from 54.36% to 96.47%. Ongoing level 3 analyses is further evaluating critical amino acids for each target with susceptibility focused on aquatic life. Our study identifies that coupling comparative bioinformatics and probabilistic hazard assessment approaches provide a unique opportunity to improve the precision of environmental assessments to identify and prioritize compounds and species for advanced behavioral ecotoxicology research.

2.15.P-Th-033 Global occurrence and hazards of retinoic acids in aquatic matrices

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Retinoic acid (RA), a major derivative of vitamin A, is essential for normal physiological functions such as development, growth, and reproduction. However, due to widespread presence of RAs in aquatic ecosystems, there is a growing concern of potential ecological and health risks. RAs enter water bodies through both natural

and anthropogenic sources, including direct excretion from humans and animals, and discharge from pharmaceutical and cosmetic industries. Elevated RA levels have been detected in eutrophic environmental waters, and experimental studies indicate that RAs, particularly all-trans retinoic acid (ATRA), elicit teratogenic effects in aquatic vertebrates. However, there is limited global understanding of the environmental distribution and potential water quality hazards of RAs to aquatic life. To address this knowledge gap, a critical literature review was conducted to understand the global occurrence of retinoids in aquatic systems. Environmental exposure distributions (EED) were developed for nine retinoid compounds, including ATRA, detected in surface water and wastewater matrices. We observed 62.4% of reported RA concentrations from Asia and 37.6% from Europe, highlighting the scarcity of environmental occurrence data for RAs from other regions of the world. We identified 95th percentile concentrations of all retinoic compounds to be higher in wastewater (56.5 ng/L) than in surface water (11.3 ng/L), suggesting sewage treatment plant discharges may be a major source of retinoic compounds in aquatic ecosystems. A Probabilistic Environmental Hazard Assessment (PEHA) was then conducted using therapeutic hazard values (THVs) of ATRA to evaluate the potential ecological hazard. PEHA analysis found that ATRA concentrations exceeded the THV in 99.9% of both sewage and surface matrices, suggesting that ATRA could be present in the environment at levels potentially hazardous to aquatic ecosystems. Our findings warrant further investigations on global monitoring of retinoic compounds. Given their potential for bioactivity at low concentrations, further investigations are needed to understand the global occurrences and water quality risk to public health and the environment.

2.15.P-Th-034 Polyunsaturated aldehyde production and presence in the Salish Sea

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Polyunsaturated aldehydes (PUAs) are a class of secondary metabolites produced primarily by diatoms, unicellular algae that play a central role in aquatic food webs. These bioactive compounds, including 2,4-heptadienal, 2,4-octadienal, and 2,4-decadienal, have been shown to decrease the reproductive potential in some predators, including lowering copepod egg production and viability and causing embryo deformities. While much of this work has focused on planktonic interactions, there is increasing recognition that PUAs may also influence near-shore ecosystems where benthic diatoms dominate primary production. In coastal habitats, forage fish such as Pacific herring and surf smelt, along with numerous benthic invertebrates, deposit their eggs directly onto submerged vegetation, mudflats, and intertidal substrates. This potentially brings developing embryos into direct contact with benthic diatoms, creating the possibility of PUA-mediated impacts on early life stages. Given the ecological importance of forage fish as a critical link between planktonic production and higher trophic levels, understanding PUA exposure in near-shore habitats is essential for assessing ecosystem health and resilience. In this study, we report PUA production by eight species of benthic diatoms commonly found in near-shore environments of the Salish Sea. In addition, we present preliminary field measurements of PUA concentrations in surface waters from selected sites in the Salish Sea, providing an indication of environmental exposure levels in this region.

2.15.P-Th-035 Using NAMs to evaluate water quality improvements from street sweeping

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Bioassays present the advantage of integrating the effects of all regulated and unregulated pollutants on organisms. Although toxicity evaluation is a component of stormwater discharge permits in California, bioassays are not consistently used to assess the effectiveness of existing or new best management practices (BMP). Instead, the assessment of stormwater BMPs typically focuses on their capability to remove a limited suite of pollutants including metals and nutrients. The present study utilized new approach methodologies (NAMs) to rapidly assess the effectiveness of street sweeping to improve water quality. To do so, we used a

custom-built rainfall generator to simulate three ~15-min synthetic rainfall events on unswept and swept sections of a parking lot, generating three separate event pairs. The synthetic rainfall events were preceded by a two-week period of dry weather. Whole-of-event composite runoff samples were collected for toxicity testing using several in vitro bioassays that screen for chemicals capable of causing oxidative stress (antioxidant response element (ARE)), tissue damage and carcinogenic effects (aryl hydrocarbon receptor (AhR) activation bioassay), and neurotoxicity (neurite outgrowth inhibition). Cell bioscreening results showed ~25% reduction in AhR activity, 40% reduction in ARE response, 46% reduction in neurotoxicity after street sweeping for two out of the three street sweeping events. Event 2 results, however, showed little to no reduction in bioassay responses. The findings were corroborated by the chemistry data. Positive correlations were observed between AhR bioassay responses and the occurrence of polycyclic aromatic hydrocarbons (PAHs) and ARE bioassay responses and dissolved metal concentrations.

2.15.P-Th-036 Metabolomic and transcriptomic changes in larval zebrafish after developmental exposure to Aroclor 1254

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Polychlorinated biphenyls (PCBs) are long-lived synthetic compounds that were widely used until 1979. The neurotoxicity of Aroclor 1254, a commercial mixture of PCBs, has been well documented within mammalian species, however, the underlying mechanisms of toxicity have not been fully characterized, particularly in aquatic organisms. Previous research has shown that developmental exposure to Aroclor 1254 induced dose dependent tremors in the eyes of larval zebrafish (*Danio rerio*), indicating novel neurotoxic effects. Here, we used targeted metabolomics and transcriptomic profiling to investigate the neurotoxicological effects of Aroclor 1254 exposure at nominal concentrations of 500 µg/L to embryonic zebrafish for 96 h. There were significant differences between controls and Aroclor 1254 treated zebrafish larvae in proline, glutarate, asparagine, glycerol 3-phosphate, sucrose, threonine, and fructose 6-phosphate levels. Metabolites involved in neurotransmitter signaling pathways, such as glycine, tyrosine, and glutamine, had significantly different levels in Aroclor 1254 treated larvae, with top enriched pathways involved in lipid metabolism and xenobiotic response. Bioinformatic analyses utilizing transcriptomic data found that top enriched biological processes were involved in visual function indicating a disruption of these pathways. These underlying pathways were supported by eye tremor phenotypes that were present in Aroclor 1254 exposed larvae. These results suggest that changes in the levels of metabolites involved in neurotransmitter function and dysregulations in genes within visual function pathways are likely responsible for the underlying eye tremor behavior and may serve as novel biomarkers in early life stage fish exposed to PCBs.

2.15.P-Th-037 CEC toxicity benchmark derivation for freshwater and marine fish

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Contaminants of Emerging Concern (CECs) in the Washington Salish Sea were recently prioritized for further consideration using a risk-based approach that relied on several lines of evidence. One line of evidence was a benchmark, called Screening Values (SVs), that synthesized available toxicity data for freshwater fish. A limited number of SVs were available in the original work; to improve our prioritization of the CECs in the Salish Sea, we derived SVs for 42 chemicals previously identified as potential risks to marine wildlife. Additionally, we expanded the SV database by developing saltwater SVs for 19 of the CECs. Using the new CECs, we updated the previous prioritization and changed the categorization of six CECs. We also considered how to best adjust freshwater toxicity information to marine systems by comparing the fresh and saltwater SVs. This comparison showed that the commonly applied safety factor of 10 to freshwater toxicity information may

be overprotective for many CECs. Our results support that more lines of evidence along with specific consideration of marine toxicity will increase our understanding of hazards posed by CECs.

2.15.P-Th-038 Characterizing the effects of acute exposure to 6PPD-quinone on Walleye (*Sander vitreus*) and Bluegill (*Lepomis macrochirus*)

Ahmed Dualeh, Bryna Garey and Dalma Martinovic-Weigelt, University of St. Thomas

6PPD-quinone (6PPDQ), a transformation product of the tire rubber antioxidant 6PPD, is acutely toxic to certain salmonid species, most notably coho salmon, in which it causes mortality at environmentally relevant concentrations. Conversely, 6PPDQ is not very toxic to white sturgeon, fathead minnow and medaka. To better understand the taxonomic variability in sensitivity, we tested the effects of 6PPDQ on two ecologically and economically important species: walleye (*Sander vitreus*, family Percidae) and bluegill (*Lepomis macrochirus*, family Centrarchidae). We used bluegill fibroblast cell line (BF-2) to characterize the effects of 6PPDQ on mitochondrial functioning, one of the proposed mechanisms of toxicity of 6PPDQ. Basal respiration, spare capacity, and ATP-dependent, maximum, and proton leak-dependent respiration were measured using a Mitochondrial stress test and Seahorse XFe extracellular flux analyzer. Mitochondrial stress test responses of BF-2 cells, exposed to known mammalian mitochondrial modulators (i.e., oligomycin, FCCP, rotenone/antimycin-A), were congruent with those reported for other fish and mammalian cell models. Oligomycin showed a reduction in OCR - consistent with its role as an ATP synthase inhibitor. FCCP increased the mitochondrial respiration rate, which is consistent with its uncoupling action – a dissociation between mitochondrial membrane potential generation and its use for mitochondria-dependent ATP synthesis. Administration of rotenone (Complex I inhibitor) and antimycin-A (Complex III inhibitor), as hypothesized, resulted in full shutdown of the mitochondrial respiration. Our pilot data indicates that acute exposure to 6PPDQ (60, 120, 240 ng/L) did not significantly affect mitochondrial respiration in BF-2 cell line – ANOVA, $F(3,36) = 0.28$, $p > 0.05$. This differs from the effects observed in salmonid cell lines where exposure to 6PPDQ leads to increased oxygen consumption - consistent with the hypothesized uncoupling activity of 6PPDQ. In vivo acute toxicity test (following OECD Test Guideline 203) was used to determine the concentration of 6PPDQ that causes lethality in 50% of the test population. Juvenile walleyes (90 days post hatch) were exposed to 6PPDQ (nominal concentrations - 52, 103, 207, 455, 1000 ng/L) for 96 hr. There was no mortality in any of the controls or the treatments after 24 and 48 hr indicating that walleye is less sensitive to 6PPDQ. Bluegill in vivo experiments are underway.

2.15.P-Th-039 Characterizing the effects of quinone outside inhibitor fungicides and their metabolites in human and rainbow trout cell lines

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Quinone outside inhibitors (QoIs) are widely used fungicides, representing 15% of the fungicide market share. Due to their efficacy, QoIs are used extensively throughout the USA, particularly in the agricultural Midwest. QoI occurrence in water, combined with findings that some cause mortality and developmental malformations in fish, has heightened concerns over ecological impacts. Reactions with chemicals used in water and wastewater treatment (i.e., disinfection with free chlorine) often produce new chemical species, or QoI transformation products. Because transformation products can retain structural similarity to their parent QoI compounds, we hypothesized that these QoI transformation products would retain biological activity. High-resolution, animal-free, and highly controllable testing approaches are needed to rapidly explore mechanisms of cellular toxicity of numerous QoIs and their metabolites. We used the rainbow trout cell line RTgill-W1 [.] to characterize mitochondrial function of three QoIs (kresoxim-methyl, azoxystrobin, pyraclostrobin) and five of their chlorinated transformation products. Basal respiration, spare capacity, and ATP-dependent, maximum, and proton leak-dependent respiration were measured using a Seahorse XFe Extracellular Flux Analyzer. Exposure to QoIs and their chlorinated products reduced oxygen consumption and respiratory function parameters in a

dose-dependent manner; pyraclostrobin and its chlorinated transformation products were the most potent QoIs tested. The use of fish-based cell lines as a platform for mitochondrial toxicity testing can aid in the assessment of risks that fungicides pose to fish health, as well as in the development of safer fungicide alternatives. The bioactivity of transformation products was also tested using an in vitro multiplex reporter system that evaluates modulation of the activity of 46 transcription factors in the human hepatoma cell line (HepG2); analyses are underway.

3.01.P-Tu DDT+: New Perspectives on a Historic Contaminant

3.01.P-Tu-073 Biological and ecological determinants of contaminant loading in recreationally important fishes in the Southern California bight

Toni Sleugh, University of California, San Diego

Decades of ocean dumping by Montrose Chemical Corporation, the world's largest DDT manufacturer, and other municipal and industrial sources have made Southern California one of the most highly polluted regions in the world, especially with respect to DDT+. Current monitoring efforts rely on composite sampling, and consumption advisories are informed only by species and location, ignoring other critical biological and environmental factors that determine exposure risk. This project uses Bayesian modeling techniques to identify the key drivers of contaminant burden in five recreationally important fish species (Kelp Bass, Pacific Mackerel, Pacific Bonito, Vermilion Rockfish, and Pacific Sanddab). Working with recreational anglers and researchers across Southern California, we collected comprehensive data from each fish, including DDT+ and PCB concentrations in both muscle and liver tissue, bulk and compound-specific stable isotopes, otolith age estimates, exact catch location, and total length. DDTs and PCBs were detected among almost every fish in our sample, but with incredibly high variability that was not explained by just species and location. Preliminary modeling results suggest that fish size, sediment contamination, and benthic resource utilization are the primary determinants of contaminant burden, along with species. This research is a first step in improving contaminant risk assessments by considering the holistic biology and ecology of the target species. Although we do not provide specific consumption recommendations, our findings will provide public health agencies and recreational anglers alike with the tools needed to better understand patterns in contaminant burden and navigate safe seafood consumption in one of America's most polluted marine ecosystems.

3.01.P-Tu-074 Connections between watershed applications, sediments and fish to understand legacy DDT pollution in remote Canadian lakes

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Global use of synthetic chemicals, such as pesticides, has increased markedly since at least 1970. Do insecticides applied in the 1950s-60s influence the structure and function of contemporary lake ecosystems? Forestry stakeholders in New Brunswick, Canada, ran arguably one of the world's largest insecticide spray programs beginning in 1952 to control spruce budworm populations. We studied remote lakes and their sediments using dated cores (n=12) and a "top-middle-bottom" (n=31) paleolimnological approach to investigate legacy DDTs and their impacts on key trophic levels. Fish, macroinvertebrates, and zooplankton were used to assess contaminant levels and reconstruct aquatic food webs. Total DDTs ($\Sigma\text{DDT} + \Sigma\text{DDE} + \Sigma\text{DDD}$) were higher in Brook Trout from lakes where applications greater than ~0.5 kg/ha occurred. About six decades after the last application, Total DDTs in fish muscle tissue now exceed wildlife guidelines by up to 20 times. Legacy DDTs were widely distributed in lake sediments and modern biota across presumably hundreds (~20%) of lakes in New Brunswick. Total DDTs in the lake surface sediments from the profundal zone were correlated with the cumulative amounts applied to each watershed. The metabolite DDE, a product of aerobic degradation, dominated and exceeded probable effect levels in 90 of 92 sediment samples. A significant association between Total DDTs in lake surface sediments and Brook Trout suggests that contemporary sediments behave as a source, not sink, of DDTs to the modern food web. Further analyses will focus on benthic

invertebrate and zooplankton responses to legacy DDTs across decades and will also include the consideration of co-occurring drivers of ecosystem trajectories, such as land-use change from forestry activities and climatic trends. Ultimately, our goal is to link processes at the watershed, lake, and food web scales to understand cumulative effects of legacy DDTs used during the mid-20th century.

3.01.P-Tu-075 Geographic variation and molecular mechanisms of marine pollutant contamination in yellowfin tuna

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Marine environments serve as global reservoirs for persistent organic pollutants (POPs), creating significant exposure risks for humans through seafood consumption. This synthesis presents findings from five interconnected studies conducted over nearly a decade (2016-2025), investigating marine pollutant contamination in yellowfin tuna (*Thunnus albacares*) and its implications for human health. These comprehensive investigations examined both the geographic patterns of contamination and the underlying molecular mechanisms that govern pollutant bioaccumulation in this commercially important species. Our multi-year research program demonstrated that geographic origin serves as a primary determinant of contamination levels, overshadowing traditional predictors such as fish size or lipid content. Both heavy metal and persistent organic pollutant concentrations exhibited substantial spatial variation, with contamination hotspots identified at specific geographic locations. This geographic clustering of highly contaminated fish challenges current seafood safety assessments that typically focus on species-level recommendations without considering capture location. Parallel mechanistic investigations revealed the critical role of P-glycoprotein (P-gp), an evolutionarily conserved cellular defense transporter, in pollutant bioaccumulation. Through a series of studies, we identified specific congeners of organochlorine pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers that inhibit both mammalian and tuna P-gp. Advanced structural analysis using cryo-electron microscopy provided unprecedented molecular-level insights, revealing how environmental pollutants like DDT bind to tuna P-gp in conformations that prevent normal transport cycling, thereby facilitating bioaccumulation. This decade-long research synthesis demonstrates that transporter inhibition represents a fundamental mechanism enabling pollutant bioaccumulation in marine food webs, with direct implications for improving human health risk assessment and seafood safety protocols.

3.01.P-Tu-076 Targeted and nontargeted approaches to uncover complex halogenated pollutants in the historically endangered California Brown Pelican

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In the late 1960s, California Brown Pelicans (BRPE) experienced reproductive failure and population decline induced by the pesticide dichlorodiphenyltrichloroethane (DDT). DDT was banned in the United States in 1972, whereafter brown pelican populations began to recover. Since then, nontargeted analytical methods have identified over 45 DDT-related compounds (DDT+) in regional upper trophic level marine species, presumably originating from the persistent and chemically-complex DDT manufacturing waste deposited in the southern California Bight (SCB). In addition to DDT+, the SCB has been contaminated with legacy pollutants as well as other typically unmonitored halogenated organic compounds (HOCs). Despite these findings, contaminant monitoring for brown pelicans has been sparse, with the most recent contaminant data from 2005. Further, there is limited data on DDT+ and other unmonitored HOCs in the pelican population, including known toxic contaminants like tris(4-chlorophenyl)methane (TCPM). This study used nontargeted analysis to identify 183 HOCs in seven BRPE liver samples (131 ± 48 , mean $\pm$ SD). Seventeen DDT+ compounds were identified, including four TCPM-related compounds, and 17 polychlorinated terphenyls (PCTs), all of which were not

assessed in prior pelican research. Quantitative targeted analysis was conducted for three of the most abundant classes: DDT+, polychlorinated biphenyls (PCB), and polybrominated diphenyl ethers (PBDE). Among these three classes, DDT+ was the dominant chemical class ($\sum 13\text{DDT+}$, 0.56 - 197 $\mu\text{g/g lw}$), followed by ($\sum 14\text{PCB}$, 0.24 - 96 $\mu\text{g/g lw}$), and ($\sum 6\text{PBDE}$, 0.01 - 5.61 $\mu\text{g/g lw}$). $\sum 13\text{DDT+}$ primarily consisted of p,p'-DDE (77% $\pm$ 15%), 4,4',4''-TCPM (12% $\pm$ 10%), p,p'-DDMU (6% $\pm$ 3%), and 4,4',4''-TCPMOH (5% $\pm$ 6%), of which only p,p'-DDE is traditionally monitored. These results demonstrate the continuous exposure of known historical HOCs and a chemically diverse set of under-monitored HOCs in BRPEs at similar levels, suggesting pervasive contamination of a unique profile of HOCs throughout the SCB food web.

3.01.P-Tu-077 Identifying trends of DDT+, PCB, and PBDE concentrations in the endangered California Condor from 2009–2024

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The critically endangered California condor (*Gymnogyps californianus*) is a managed population that has increased in size through intensive recovery efforts. Currently, there are approximately 560 condors in the population, with ~100 inhabiting the coastal regions of California. The coastal population consumes dead-stranded marine mammals, which is linked to reduced hatching success and increased dichlorodiphenyltrichloroethane (DDT) concentrations compared to condors inhabiting inland regions. Further, new research shows that DDT contamination is more complex than previously thought, with > 45 DDT-related compounds detected in marine mammals from southern California. This expanded suite of DDT chemicals is collectively known as DDT+, which includes the typically monitored DDX compounds (p,p'- and o,p'-DDT, DDE, DDD), as well as typically unmonitored compounds such as tris(4-chlorophenylmethane) (TCPM). This study aims to quantify a subset of DDT+ compounds (n = 13), as well as 14 polychlorinated biphenyls (PCBs), and 6 polybrominated diphenyl ethers (PBDEs), in condor whole blood from 2009 to 2024 using targeted chemical analysis by gas chromatography-mass spectrometry (GC-MS). Across all samples, we detected 6 of the 13 DDT+ compounds (p,p'-DDE, -DDD, -DDMU; o,p'-DDT; and 4,4',4''-TCPM), as well as 13 of the 14 PCBs and all 6 PBDEs. We found average $\sum\text{DDX}$ concentrations of 156 ± 214 ng/g wet weight (ww) and $\sum\text{DDT+}$ concentrations of 160 ± 222 ng/g ww (which includes $\sum\text{DDX}$) across all timepoints, showing that the typically nonmonitored DDT+ compounds contribute little to overall DDT load. The compound p,p'-DDE contributed $97 \pm 3\%$ of $\sum\text{DDT+}$ load, indicating that it is the primary driver of DDT contamination. p,p'-DDMU was the second highest contributor ($1.6 \pm 1\%$), followed by o,p'-DDT ($0.6 \pm 3\%$), p,p'-DDD ($0.5 \pm 0.6\%$), and 4,4',4''-TCPM ($0.4 \pm 0.8\%$). $\sum\text{PCB}$ and $\sum\text{PBDE}$ concentrations were generally lower than $\sum\text{DDT+}$, with average concentrations of 48 ± 85 ng/g ww and 47 ± 84 ng/g ww, respectively. Further analysis will be performed to test whether a condor's coastal behavior summarized over different time frames (e.g., 1 year, 5 years, lifetime) predicts hatch probability and DDT+ concentrations, and we will investigate for a direct predictive link between DDT+ concentrations and hatch probability.

3.01.P-Tu-078 Wide area survey and assessment of dichlorodiphenyltrichloroethane (DDT) dumpsite with autonomous vehicles

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The increasing demand for accurate assessment of underwater large-scale dumpsites—ranging from legacy munitions disposal areas to industrial waste deposits—requires advanced and comprehensive survey methods. These sites often span vast areas, making it difficult to assess their full extent, composition, and associated environmental risks using traditional survey methods. This study explores a known dumpsite of dichlorodiphenyltrichloroethane (DDT) off the coast of Southern California in the San Pedro Basin, CA, with an autonomous underwater vehicle (AUVs) equipped with synthetic aperture sonar (SAS), electronic still camera (ESC), and laser line scan (LLS) to provide a wide-area environmental risk assessment. Surveys covered >300 km² of the known dumpsites, revealing spatial patterns of objects and seafloor variations that

could be related to bioturbation, a potential vector for DDT from the sediment to the marine food web. A remotely operated vehicle (ROV) conducted surveys simultaneously over several areas of interest to provide high-resolution imagery. Results from this work highlight the significance of high-resolution wide-area surveys that help inform assessments of environmental risks.

3.01.P-Tu-080 Toxicity and benthic fauna associated with sediment DDX concentrations and waste barrels in the Santa Monica and San Pedro deep sea basins

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Industrial dumping of organic pollutants such as DDTs in the deep ocean within the San Pedro Basin (SPB) and Santa Monica Basin (SMB) off the coast of southern California was a recurrent practice in the past decades, leaving a legacy of chemical pollution whose impacts on the benthic ecosystem is still being studied. The overarching goal of this ongoing research is to understand the effect of DDX (DDT and metabolites DDE, DDD and DDMU) on the structure and function of the benthic biota and the roles of fauna in contaminant redistribution and trophic transfer. This includes (1) characterization of the benthic faunal communities at designated dumpsites located in the SPB and SMB in relation to the distribution of DDX contaminants in sediments; (2) linking macrofaunal characteristics to fine-scale sediment properties, contaminant distributions and life habits, and (3) an evaluation of DDX concentrations in tissues of benthic biota at SMB Dumpsite 1, SPB Dumpsite 2, and in the surrounding areas. This presentation will focus on the third objective in addition to an assessment of toxicity using embryos of the purple sea urchin at select sites. Intact sediments were collected using a coring device and invertebrates were collected by suction from barrel surfaces with the ROV SuBastian. Samples were collected in Aug. 2021 in SPB and in Dec. 2023 and May 2024 in both the SMB and SPB. Toxicity was documented using purple sea urchin embryos exposure to both whole sediment and sediment elutriates suggesting the potential for ongoing direct effects near the waste barrels. Concentrations of DDX in tissues from a wide range of benthic species ranged from non-detect to 310 ng/g ww with a wide range of variability noted. Interestingly concentrations of DDX in the nemertean *Cerebratulus ruber* (310 ng/g ww DDX) were elevated outside Dumpsite 1 in SMB compared to inside (6.3 ng/g ww). The biota-sediment accumulation factor (BSAF, lipid-TOC normalized) for DDX was highest for the serpulid polychaete *Protis pacifica* (15.4) followed by *C. ruber* (11.1) and the gastropod *Astyris permodesta* (4.2), although there was high variability within species and among sites. These collective findings raise concerns about the potential for direct toxicological effects as well as the transfer of DDX to higher trophic levels related to legacy hazardous waste disposal activities in the deep sea, both within and outside designated dumpsite location more than 50 years after dumping ceased.

3.01.T DDT+: New Perspectives on a Historic Contaminant

3.01.T-01 Updating research concerning continuing ecological effects of legacy deepwater-disposed DDT wastes into the Southern California bight

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Records show that the nation's largest DDT manufacturer, Montrose Chemical, manufactured approximately 800,000 tons of the broad-spectrum insecticide DDT at its plant located near Los Angeles, California between 1947 to 1982. Two distinct manufacturing by-product waste streams were produced, including the alkaline ("caustic") and the acid waste streams from the process. Disposal of the caustic waste stream is well documented; toxic wastes were discharged in high volume through a County wastewater treatment plant to offshore ocean outfalls near the Palos Verdes (PV) Shelf until the early 1970s. A multi-agency Natural Resources Damage Assessment and USEPA Superfund listing in the 1990s led to a subsequent "DDT trial" and monetary settlement (\$140M) from Montrose Chemical in 2000, focused on restoration and limited cleanup of the PV Shelf and Santa Monica Bay. Significantly, no regulatory action has ever been taken to address the

deepwater ocean dumping of the DDT acid waste stream, believed to occur into the early 1960s. Historical review has shown that ocean dumping of the acid wastes occurred at two loosely designated deepwater dumpsites located near Catalina Island, at depths of approximately 800m to 1,000m. Various estimates have reported a total mass of up to 1,535 tons of ocean-dumped DDT acid waste, containing up to approximately 0.5 to 2.0% of technical grade DDT, which is roughly equivalent to the estimated volume of caustic DDT waste released to the PV Shelf. In addition, measured total DDT concentrations in sediments from both the PV Shelf and the deeper basins are also approximately equivalent (frequently more than 100 µg/g-1 for both locations). Moreover, DDT-contaminated fish tissue at both locations (greater than 1,000 µg/g-1 wet wt), further substantiates the ecological importance of this independent DDT waste stream. We will summarize the past practice of ocean dumping of DDT wastes, along with an overview of data summarizing what is known about ongoing toxicity and ecological effects of ocean-dumped DDT wastes. Several investigations have emphasized DDT effects from the lowest trophic levels of the marine food web to fish, as well as upper trophic level predators and scavengers, attempting to link together effects of ocean-dumped DDT wastes to this marine ecosystem. Our DDT session will also summarize what is known on potential health effects of DDT in humans potentially occurring from exposure to DDT in southern California.

3.01.T-02 Seven decades of DDT contamination in mesopelagic fishes of the Southern California bight

Lillian McGill, Brice Semmens, Lihini Aluwihare, Raymmah Garcia and Tran Nguyen, Scripps Institution of Oceanography

In southern California, extensive dumping of DDT via sewage outfalls and offshore barging occurred for most of the last century, yet the magnitude and distribution of these contaminants remains poorly characterized for organisms not directly utilized by humans. Mesopelagic fishes, which undergo diel vertical migrations, provide an essential link between surface and deep ocean environments, making them ideal indicators for understanding the extent of pollution across ocean compartments. Using archived specimens from the California Cooperative Oceanic Fisheries Investigations (CalCOFI) program, we present the longest time series of contaminant concentrations in mesopelagic fish to date, analyzing DDT and its metabolites (DDX) in two species of myctophid from the Southern California Bight from 1948-2023. Our dataset reveals that DDX rapidly dispersed within the region, with total DDX concentrations peaking in the mid-1960s, followed by a steady decline through the present day. We also found that DDT concentrations peaked first, followed by its degradation products DDE and DDD. Furthermore, despite more than half a century since the cessation of industrial dumping in the region, DDX concentrations in mesopelagic fish remained detectable throughout our time series, highlighting the persistent nature of hydrophobic contaminants in marine food webs and demonstrating that industrial pollution can rapidly penetrate open ocean ecosystems and persist for decades.

3.01.T-03 Modern inventory of dichloro-diphenyl-trichloroethane (DDT) in Southern California bight sediments reflects historical waste disposal practices

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Sediments in the deep San Pedro Basin, California, were polluted during the 20th century by disposal of a variety of wastes, resulting in modern adverse impacts to local marine ecosystems. Such waste disposal efforts reportedly released millions of gallons of concentrated sulfuric acid wastes from the manufacture of a widely used pesticide – dichloro-diphenyl-trichloroethane (DDT) – into the surface ocean across San Pedro Basin between 1947 and 1961. Because the sulfuric acid wastes contained trace amounts of hydrophobic DDT product, a substantial mass of DDT was thought to have been deposited in the deep basin sediments. Our earlier work suggested the deposition of DDT and its primary degradation products, dichloro-diphenyl-dichloroethane (DDD) and dichloro-diphenyl-dichloroethylene (DDE), were also widespread in these sediments across a simple transect. In this study, we ask how expansive DDX (collectively DDT, DDD, and DDE) contamination is across San Pedro Basin and how much DDX still resides in these sediments today. We hypothesize that elevated DDX concentrations are deposited throughout, and extend beyond, San Pedro Basin sediments, contributing to a large mass of resident DDX pollution along the Southern California Bight (SCB). We present

sediment DDX concentrations at 126 sites across the SCB, including 54 stations within the ~800 km² footprint of San Pedro Basin. Sediment was sampled at fine (1-2 cm) depth resolution, then 2,4'- and 4,4'- isomers of DDT, DDD, and DDE were extracted and quantified by gas chromatography. Concentration profiles were mapped bight-wide at estimated age of deposition intervals using natural neighbor spatial interpolation methods. Integrated contaminant burden masses were then mapped within San Pedro Basin using the same spatial interpolation methods. Bight-wide concentration maps showed DDX was indeed expansive within San Pedro Basin and extends west into Santa Monica Basin and the shallower Catalina Rise. We report that conservatively 29.8 metric tons of DDX still resides within San Pedro Basin sediments today. Both the spatial extent of contamination and burden estimates are critical for understanding current and future bioavailability potential and thus ecological risk in Southern California related to pesticide DDT contamination.

3.01.T-04 DDT+ in waters off Southern California: Concentrations, spatial and temporal distributions, sediment-water fluxes and ramifications

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The coastal marine ecosystem offshore southern California is heavily contaminated with DDT, its degradates, and related contaminants (DDT+). This contamination has led to the Palos Verdes Shelf (PVS) being designated a Superfund site by the USEPA, as well as more contamination recently discovered further offshore from previous open-ocean dumping. Understanding the spatial and temporal extent of DDT+ contamination is essential in guiding cleanup efforts at PVS, as well as characterizing the extent of impacts at the dump sites. To this end, passive water samplers were deployed at the PVS and further offshore to determine depth profiles of DDT+ concentrations, as well as magnitudes of fluxes from contaminated sediment to overlying waters. Total DDT concentrations at PVS in 2022 ranged from 98-7300 pg/L, primarily from p,p'-DDE and p,p'-DDMU. Levels were greatest 4 km northwest of wastewater treatment plant outfalls, following the primary current direction in the Southern California Bight in this area. Concentrations, spatial distributions, and fluxes were consistent at PVS with observations made up to a decade previous. These observations are consistent with USEPA Monitored Natural Attenuation evaluations at PVS, suggesting slow declines in concentrations that remain high enough to be of concern in the water column, sediments, and biota. At dump site #2 (900 m depth), concentration gradients indicate transport of DDT compounds from sediment to the water column, consistent with observations at the much shallower PVS (tens of m in depth). These measurements help to evaluate what hazard assessment is appropriate to protect ecosystem and human health, and to evaluate suitability of treatment and remediation options.

3.01.T-05 A "deeper dive" into DDT+: Comparative responses to DDT+ chemical species in vitro and in zebrafish

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DDT is a legacy pesticide with many existing environmental and biomonitoring programs, the recent research conducted in Southern California has demonstrated the persistence of many lesser-studied DDT metabolites and related products (DDT+) in marine environmental and coastal biota. For many of these DDT+ compounds, almost no toxicity information was yet available or was highly limited. During this presentation, we will build upon the existing knowledge of DDT+ biomonitoring by presenting novel toxicological data for these understudied compounds. We will present in vitro research comparing cytotoxicity and estrogen receptor activity of DDT+ compounds, followed by aquatic toxicology research in zebrafish investigating impacts in developmental success, transcriptomics, and behavior. We will present data that suggest that the DDT byproducts tris(4-chlorophenyl)-methane and -methanol may be of particular interest for ecotoxicological and

human health research due to conserved toxicity with DDT and similar bioaccumulation patterns. Likewise, estrogen receptor agonism is moderately conserved across humans and California condors, and there are clear differences in estrogen receptor activity between DDT+ species. Transcriptomics for exposed zebrafish embryos reveal distinct clusters of DDT+ compounds based on molecular response, and p,p'-DDT, -DDE, and -DDD have unique responses compared to the other DDT+ compounds assessed. Overall, we categorize these contaminants into groups of higher or lower priority for future investigation, mitigation efforts, and conservation. **Disclaimer/Disclosure:** This research was supported by the California Sea Grant Deep Ocean DDT+ Research Program (2023-2025). The authors and presenter have no other conflicts of interest to disclose.

3.01.T-06 Cancer in California sea lions: A naturally occurring model for persistent organochlorine-associated carcinogenesis

Alissa Deming, Pacific Marine Mammal Center

Due to the historic deep ocean DDT disposal that occurred off the Southern California coast from the 1940's to the 1980's, marine mammals in the region have high burdens of DDT identified in their blubber. Sea lions in the region are amongst the highest recorded levels of persistent organic pollutants in any marine mammal species. As a result, wild California sea lions along the west coast of the United States have one of the highest prevalence of cancer amongst all wildlife species. The unusually high prevalence of cancer, specifically urogenital carcinoma, in this species has been strongly linked to a combination of high organochlorine burdens (DDT and PCBs) and infection with an oncogenic herpesvirus. Multivariate regression models controlling for age, sex, and stranding location show that animals in the top quartile of DDT and PCB exposure have higher odds of developing urogenital carcinoma. In addition, California sea lions in managed care that have familial lineage to previously wild sea lions from the California coast, have been documented to succumb to mammary carcinoma, potentially because of the transgenerational impacts of DDT exposure as has been described in humans. Studying cancer in these sea lions provides a unique naturally occurring model for investigating the mechanisms organochlorine exposure contributes to the development of cancer. Stranding network veterinarians and collaborative researchers collect and analyze samples from these animals to provide a deeper look into the pathophysiologic mechanisms. Continued disease surveillance and contaminant monitoring in marine mammals are critical to understanding long-term environmental health risks, informing policy, and guiding restoration efforts in contaminated marine habitats.

3.01.T-07 Defining biomagnified DDT+ chemical profiles and investigating the health impacts on sentinel wildlife species and humans

Nathan Dodder¹, Margaret Stack¹, Kari Sant² and Eunha Hoh¹, (1)San Diego State University, (2)Michigan State University

During its production prior to 1972, dichlorodiphenyltrichloroethane (DDT) waste was discharged via a marine outfall to the Palos Verdes Shelf (PVS) near Los Angeles, CA. Accordingly, the historical narrative and environmental monitoring focused on the PVS as the primary source of DDT to the local environment. Although offshore dumping of industrial waste (by ship) was legal at the time, this alternate disposal route was largely dismissed until sedimentary DDT waste was (re)discovered further offshore in the San Pedro Basin in 2019. At approximately the same time, nontargeted analysis (NTA) based on comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry of regional marine mammal blubber identified not only the typically monitored DDT compounds (DDX) but also a suite of 45 DDT related compounds (DDT+). It was hypothesized the DDT+ profile reflects the persistent and transformed waste contaminants and is unique to the region. Regional biological impacts in cetaceans, California sea lions, and California condors were found to be associated with present-day (ca. 2022) DDT levels. At this point, there was evidence of relatively high levels DDX in the southern California ecosystem, and a unique and chemically complex mixture of additional related compounds and structurally unidentified halogenated organic contaminants. There was evidence of biological impacts, but also a lack of contaminant monitoring data in historically endangered or impacted species. Therefore, to systematically assess southern California wildlife and human exposure to DDT+

and address data gaps, the objective of our study was to measure current levels, temporal trends, the geographic distribution, and the association of DDT+ with biological outcomes. The aims were to measure DDT+ in the following regional species. 1) The common sportfish species chub mackerel, sanddab, and CA halibut. 2) The piscivorous brown pelican, common murre, and Brandt's cormorant, each of which dive to different depths. Additional aims were to assess the following. 3) The temporal trend in the short-beaked common dolphin, a marine mammal sentinel species. 4) The geographical distribution in California sea lion pups from different Channel Island colonies. 5) The association of urogenital carcinoma prevalence and DDT+ body burden in California sea lions. 6) The association of reproduction success with DDT+ exposure in the California condor.

3.02.P-Tu Ecotoxicology and Risk Assessment in Marine Coastal Ecosystems

3.02.P-Tu-007 Reviewing laboratory culturing for *Laminarian* kelp and their use for ecotoxicology

Marianne Elizabeth Glascott, *Life Sciences, University of Sussex, United Kingdom*

This review synthesizes current laboratory cultivation methods for three kelp species—*Laminaria digitata*, *Laminaria hyperborea*, and *Saccharina latissima*—relevant to ecotoxicology research. Growing concern over kelp loss in many regions, driven by climate change, pollution, and other anthropogenic stressors, has prompted the development of active restoration strategies. These, in turn, necessitate reliable cultivation protocols for both conservation and commercial applications. Ecotoxicological testing using kelp addresses significant gaps in environmental risk assessments—particularly in evaluating how pollutants may contribute to kelp declines—while also offering an alternative to vertebrate testing in toxicological studies. However, the complexities of kelp life cycles and the historical challenges of maintaining viable laboratory cultures have limited the widespread use of kelp in ecotoxicology. Through an integrative review combining published literature and semi-structured practitioner interviews, this article consolidates successful cultivation techniques across academic, commercial, and restoration sectors, detailing how to establish and maintain kelp cultures as spores, gametophytes, juvenile sporophytes, and mature sporophytes. Findings highlight common cultivation parameters, including optimal temperature ranges (5–15 °C), recommended nutrient enrichments (e.g., Provasoli Enriched Seawater), and careful light intensity and wavelength management to control life-stage transitions. Strategies to address recurring laboratory challenges—such as diatom and bacterial contamination, genetic drift, and extended culture longevity—are also identified. Finally, the review critically examines current efforts to standardize kelp ecotoxicology protocols and underscores the need for universally accepted methods to enhance comparability across studies and integration into regulatory frameworks. By drawing on robust cultivation practices and advocating for standardized test methods, this synthesis supports the broader adoption of kelp-based ecotoxicological assays, offering the potential to improve our understanding of pollution impacts on kelp forests and advancing both conservation and sustainable management of coastal marine ecosystems.

3.02.P-Tu-008 Evaluating the potential effects of artificial light in sea turtles

Beatrice Hernout, *Laura Lilly, Kirt Rusenko, Stephane Zsoldos, Boris Spokoyny, Erwin Lau and Charlie Menzie*, *Exponent*

Artificial lighting can have negative impacts on sea turtle nesting as it can drown out the natural cues from celestial light reflected on the ocean. Hatchlings may become disoriented (or "misoriented") by land-based lights and move toward them instead of toward the ocean, leading to hatchling death from dehydration, predation, or road kill. Under the Endangered Species Act, it is unlawful to "take" a protected species, which includes actions that "harass, harm, or kill". Artificial light, when it alters migration, nesting, or foraging behavior, constitute such harm. Shorter wavelength (blue-violet) light appears to cause the most hatchling disorientation in most species, including green turtles, while loggerhead turtles are also averse to yellow wavelengths. Red-amber wavelengths do not have the same impact and are thus considered more "turtle-friendly". Hatchlings appear to seek the brightest light within a hypothetical cone with a horizontal angle of 180° and a vertical angle of between 10° below and 30° above the horizon. While there is substantial evidence of widespread commitment across the US to address light pollution represented by the implementation of light ordinances, further research

is needed to understand the potential light thresholds affecting sea turtles to better alienate the risks of hatchling disorientation. In this case study located in Florida, we will present an evaluation of potential effects of artificial lights on sea turtle by assessing previous and current hatchlings success in relation to measure of ambient lighting, evaluate the importance of site-specific cues such as dunes, vegetation, and distance, and simulating ambient light vision through the eyes of a turtle. The overall approach is an attempt to draft an environmental risk assessment framework adapted to sea turtles and light pollution. **Disclaimer/Disclosure:** Exponent, inc

3.02.T Ecotoxicology and Risk Assessment in Marine Coastal Ecosystems

3.02.T-01 Integrated monitoring and toxicological assessment of PFAS in aquatic ecosystems: Human exposure risks in UK and Spanish populations and insights from cellular bioenergetic profiling

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Per- and polyfluoroalkyl substances (PFAS) are persistent, bioaccumulative and potentially toxic contaminants increasingly detected in coastal and marine ecosystems. This study combines extensive monitoring of PFAS in aquatic environments and biota with mechanistic toxicity assessments to better understand environmental exposure pathways and human health risks. Comprehensive field campaigns were conducted between 2020 and 2024 across the United Kingdom (UK) and in 2024 in Spain, analyzing PFAS concentrations in over 10,000 freshwater, groundwater and saline water samples, and 288 edible fish specimens from 16 species. Water samples were sourced from the UK Environment Agency's Monitoring Programme, while fish were selected to reflect local dietary habits. PFOS and PFOA emerged as dominant compounds, with regional variation: Spanish fish showed higher levels of long-chain and emerging PFAS (e.g., PFNA, 6:2 FTS), whereas UK fish had elevated PFOS. Average dietary intake in Spain exceeded EFSA's threshold by over fivefold, highlighting the urgency of mitigation in high-consumption regions. In the UK, freshwater hotspots for PFAS such as Moreton-in-Marsh underscored the need for targeted remediation. To complement field data, in vitro toxicological profiling was conducted using zebrafish embryo fibroblast (ZF4) cells exposed to 19 PFAS, assessed via Seahorse XF extracellular flux analysis and CCK-8 viability assays as a potential new approach methodology (NAM). Mid- and long-chain PFAS - particularly PFOA, PFDA, and Nafion BP1 - caused significant mitochondrial dysfunction and glycolytic shifts at concentrations as low as 250 - 600 μ M. Effects were dose-dependent and followed known chain-length toxicity trends, confirming mechanistic links between PFAS structure and bioenergetic disruption. Work to integrate the findings into an Adverse Outcome Pathway for PFAS-induced mitochondrial disruption is underway. By integrating environmental monitoring with NAMs, this research provides a multidimensional evaluation of PFAS risks. These findings inform ecotoxicological models and regulatory strategies, while supporting the shift toward non-animal, mechanistic testing frameworks for complex contaminants. **Disclaimer/Disclosure:** This work was supported by the Horizon 2020 SCENARIOS project (Grant No. 101037509), the Spanish Ministry of Science and Innovation (IMAGE Project PID2020-116789RB-C41), the Generalitat de Catalunya (ON-HEALTH 2021 SGR 01150), the UK Environment Agency and DEFRA.

3.02.T-02 Motility matters: A novel zoospore-motility assay reveals copper-speciation-dependent toxicity in *Laminaria* spp.

Marianne Elizabeth Glascott, Life Sciences, University of Sussex, United Kingdom

Kelp forests rely on the successful dispersal and settlement of motile zoospores to maintain population resilience, yet rising coastal pollution alongside an unexplained decline in some kelp populations highlights a need to investigate the ecotoxicological impact of widespread pollutants such as copper on habitat-forming kelp. Here, we introduce the first quantitative motility assay for kelp zoospores—a high-throughput, image-

analysis approach that captures sub-lethal shifts in swimming behavior. Using this novel endpoint, we establish baseline motility for three *Laminaria* species across seasons and sites, then quantify their responses to copper delivered in three environmentally relevant forms: dissolved copper sulfate (CuSO_4), copper oxide (CuO), and elemental copper powder (Cu^0). Exposure concentrations mirror those measured in the eastern English Channel, ensuring ecological relevance. Motility proved highly sensitive and strongly dependent on copper speciation. CuSO_4 produced a biphasic (hormetic) response, with motility ratios rising at moderate concentrations before collapsing at higher doses. In contrast, particulate CuO and Cu^0 caused rapid, monotonic motility loss even at the lowest concentrations tested, suggesting more immediate toxicity, possibly via nano-specific surface interactions or micro-scale copper release at the spore interface. By demonstrating that (i) motility is an early-warning, sub-lethal endpoint and (ii) particulate copper poses a greater instantaneous hazard than dissolved copper, our study provides a new tool for ecotoxicological risk assessment. Incorporating both chemical form and seasonally driven biological behavior into management frameworks is essential if we are to safeguard kelp forests under intensifying coastal-development pressures.

3.02.T-03 Evaluating the effects of anticoagulant-containing bait pellets to early life stage Pacific Salmon **Melissa Driessnack**, Lillian Pavord and Jenifer McIntyre, Washington State University

Invasive species have cascading negative effects on community structure and are detrimental to many ecosystems. Rat species have been especially problematic on island ecosystems. The USDA engages in whole-island eradication of invasive rats using aerially dropped cereal bait pellets containing anticoagulant rodenticide chemicals to support the conservation efforts of native species. 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. Currently, the USDA and Alaska Fish and Wildlife are developing eradication plans to target invasive Norway rats (*Rattus norvegicus*) on the Aleutian Island, Great Sitkin Island. This island includes spawning and rearing habitat for Pacific salmon. As part of this planning, surveys are being conducted on targeted islands to evaluate the potential for non-target organism mortalities, which have identified Pacific salmon, including coho (*Oncorhynchus tshawytscha*) and pink (*O. gorbuscha*), as potentially impacted species due to aerially broadcast pellets unintentionally entering waterways. The timing for these eradication efforts is important as they often occur in late summer to early fall and overlap with the spawning and developmental timeline for early life-stage salmon. There is a large information gap for the aquatic toxicology of brodifacoum and diphacinone. To address this gap, we exposed pink and coho salmon embryos to bait pellets during early development and monitored survival and developmental impairments until swim up. Despite the very low water solubility of brodifacoum and diphacinone, embryos and alevin accumulated both chemicals. Brodifacoum reduced survival and increased developmental defects (e.g., cranial hemorrhaging); these effects were not observed from exposure to diphacinone. These data will help inform whether extra precautions are necessary during eradication efforts to reduce AR pellets from entering the aquatic environment to protect Pacific salmonids.

3.02.T-04 A tale of fish and wildfires at the Californian coast: Impacts of virgin and weathered fire retardant on early life stages of a model salmonid species (*O. mykiss*)

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As across much of the Western U.S., the San Francisco Bay-Delta (SFBD) has experienced considerably worsening wildfires. 907,550 acres were burned in 2020 alone, partly in the direct vicinity of this unique aquatic ecosystem. The region serves as a key migratory route for sensitive salmonid species. While wildfires directly threaten the area, wildfire suppression techniques, such as the application of ammonium phosphate-based fire retardants, may pose an additional ecological risk to it. Fire retardant chemicals can enter waterways during first-flush events, sometimes after having been weathered through exposure to ultraviolet light (UV), potentially altering their toxicity. Acute toxicity of virgin fire retardant has previously been evidenced in Rainbow Trout

(*Oncorhynchus mykiss*), a commonly used model species for evaluating impacts on endangered salmonids, at 60 days post-hatch. But little is known about lethal and sublethal effects on earlier life-stages and the effect of UV-weathering on fire retardant toxicity. Here we address these gaps by establishing the acute toxicity (LC50) along with behavioral and developmental impacts for *O. mykiss* embryos, larvae, and alevins exposed to two ammonium phosphate-based wildfire retardants (Phos-Chek LC95W-HD, Phos-Chek LCE20W). Preliminary results reveal a particularly high sensitivity of embryos and larvae compared to alevins and suggest an increased toxicity of weathered fire retardant. These findings demonstrate that early life stages of salmonids are particularly vulnerable and some fire retardants, especially when altered through UV-weathering, may pose an additional risk to populations already in decline due to cumulative anthropogenic stressors. Therefore, this study can help inform future fire management strategies near SFBF waterways to minimize their ecological impacts.

3.02.T-05 Acute lethal and sublethal toxicity of anticoagulant rodenticides on Pacific Salmon

Lillian Pavord, Melissa Driessnack and Jenifer McIntyre, Washington State University

Island ecosystems are vulnerable to the predatory pressure of invasive rodents such as the Norway rat (*Rattus norvegicus*). Eradication efforts typically include aerial applications of food pellets containing anticoagulant rodenticides. Two of the most commonly used anticoagulant rodenticides are brodifacoum and diphacinone. While aerial applications aim for precision, pellets inevitably enter aquatic environments. Trace amounts of these rodenticides have been detected in several fish species after pellet applications on rat infested islands. Upcoming eradication efforts on the Aleutian Islands of Alaska require assessment of risks to Pacific salmon. However, both acute and sublethal toxicity data for aquatic species are limited, and are absent for Pacific salmon. To address this gap, we used coho salmon (*Oncorhynchus kisutch*) to generate dose-response curves via intraperitoneal (IP) injections in corn oil, estimating 96-hr LD50 values of 80.1 µg/g (95% CI: 65.8–94.4 µg/g) for brodifacoum and 54.0 µg/g (95% CI: 52.4–55.7 µg/g) for diphacinone. Acetone was initially used as a carrier with corn oil. However, we found that the presence of acetone in the injection solution significantly reduced the toxicity of diphacinone but not brodifacoum. Tissue analysis supported a difference in the metabolism of diphacinone when acetone was used. exposed to a sublethal dose of diphacinone, concentrations in liver, muscle, and plasma significantly decreased over time, unlike in tissues from fish dosed with brodifacoum, which did not show a significant decrease. Clotting time was measured over time after administering a single sublethal dose. When exposed to 53.9 µg/g (LD13) of brodifacoum, coho had significantly impaired blood clotting at 72- and 96-hr post-injection but returned to baseline by 120 hr. We did not observe significantly altered clotting times within 144 hr after dosing coho with 45.5 µg/g (LD4) of diphacinone. This suggests that diphacinone may affect coho through a different mode of action. These data will help inform policies regarding the use of these chemicals for rat eradications on the Aleutian Islands as well as increase our understanding of anticoagulant rodenticides toxicokinetics and toxicodynamics in aquatic species.

3.03.P-Mo Herplandia!

3.03.P-Mo-020 Per- and polyfluoroalkyl substances (PFAS) in wild amphibians: A call for more research

Jeremiah Harvey Tennant¹, Sujan Fernando², Eric Levanduski¹, Sydney Burg³ and Roxanne Razavi¹,

(1)SUNY ESF, (2)Clarkson University, (3)University of Georgia

Amphibians serve a central role in food webs and provide a link between aquatic and terrestrial habitats. Because of their dependence on water, amphibians are susceptible to legacy and emerging contaminants. We investigated mercury (Hg) and per- and polyfluoroalkyl substances (PFAS) concentrations in wild populations of two amphibian species—the green frog (*Lithobates clamitans*) and the American toad (*Anaxyrus americanus*)—which exhibit different life histories. We evaluated if proximity to PFAS point source (State Superfund site) impacted PFAS exposures in amphibians and assessed length, mass, sex, diet, and trophic position as predictors of contaminant concentrations. We found higher Hg concentrations in the more aquatic species (i.e., green frog); but higher PFAS concentrations (summed PFAS, PFDA, PFUnA, PFDoA, PFTrDA, and PFTeDA) in the more terrestrial species (i.e., American toad). Proximity to the PFAS State Superfund site

did not strongly affect PFAS exposures in amphibians. Of the biological predictors tested, diet best explained PFAS concentrations, but other factors (e.g., physiological differences) are likely contributing to the difference in PFAS bioaccumulation between aquatic and terrestrial species. Only three reports on PFAS concentrations in wild amphibian populations exist, all from contaminated sites in China, reporting much higher concentrations than found here. Additionally, laboratory studies have only investigated the effects of exposure to a few PFAS compounds, of which PFDA, PDUa, PFDoA, PFTrDA, and PFTeDA detected here are not represented in the literature. To truly understand the effects that detectable PFAS impose on wild amphibians, research is needed on the effects of more PFAS compounds, and the elimination of PFAS via their skin to different media.

3.03.P-Mo-021 Mercury concentrations in the spotted turtle (*Clemmys guttata*): Assessing the impacts of multiple stressors in coastal and inland populations

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The spotted turtle (*Clemmys guttata*) is currently under review for federal listing, occurs on 40 U.S. Department of Defense (DoD) installations and potentially occurs on another 48. Spotted turtles are exposed to a variety of stressors, including anthropogenic contaminants, varying hydroperiods (e.g., wetland loss), roads, saltwater intrusion from major storm events, and poaching. Through recent funding from the DoD, our project aims to promote the resilience of spotted turtle populations on military installations. Mercury (Hg) is a heavy metal that bioaccumulates in wildlife and may elicit a variety of impacts across taxa, including reptiles. Spotted turtles possess many traits that may make them susceptible to the bioaccumulation of Hg and other legacy contaminants. These turtles are long-lived, have relatively small home ranges, and are important predators of aquatic invertebrates within their respective wetlands. In 2025, we initiated the collection of spotted turtles at six installations across South Carolina, Virginia, and Maryland. For this presentation, we will compare Hg concentrations collected from spotted turtles among all locations. Our objectives are to (1) quantify total mercury (THg) in turtle blood, (2) quantify THg in turtle nails, and (3) compare turtle THg concentrations among life stages and sampling locations. We will also present predictive relationships between the two tissue types. Our dataset presents a unique opportunity to provide critical Hg bioaccumulation data for a reptile species currently under review for federal listing. In addition to providing baseline contaminant data for future monitoring efforts and risk assessments, our work will quantify stressors at each site and identify which stressors the turtles are most sensitive to, helping prioritize management actions and best management practices for the species. **Disclaimer/Disclosure:** This work is supported by grant through the Department of Defense's Strategic Environmental Research and Development Program (#RC24-4117 [TDT]).

3.03.P-Mo-022 Bioaccumulation and biological effects of per- and polyfluoroalkyl substances (PFAS) in eastern mud turtles (*Kinosternon subrubrum*) and spotted turtles (*Clemmys guttata*)

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PFAS are a large group of approximately 15,000 synthetic compounds that are persistent, bioaccumulative and globally distributed across environmental matrices—including surface water, groundwater, and sediment—as well as in biological samples such as wildlife tissues, human blood, breast milk, and urine, with documented adverse health effects in humans and wildlife. Reptiles remain critically understudied in ecotoxicology despite serving as excellent bioindicators and sentinel species, with no established adverse outcome pathways or biomarkers for PFAS exposure and effects. This study quantifies PFAS bioaccumulation and biological effects in Eastern Mud Turtle (*Kinosternon subrubrum*), a common southeastern species serving as a potential important sentinel for aquatic ecosystem contamination. We also evaluate the potential of *K. subrubrum* as a surrogate for the spotted turtle (*Clemmys guttata*) -- an enigmatic turtle species currently under review for

federal listing. If validated as a surrogate species, *K. subrubrum* could enable assessment of potential risks of PFAS exposure to *C. guttata* without invasive or destructive sampling. PFAS bioaccumulation will be quantified in water, sediment, and turtle plasma. Point-of-care analysis will measure 12 physiological analytes in plasma across liver, kidney, immune, bone, and metabolic systems in turtles exposed to varying contaminant concentrations to establish exposure-response relationships and identify sensitive plasma analytes. Blood smears will evaluate red and white blood cell counts and differentials for immunological exposure-response relationships. PFAS analysis will employ solid phase extraction with linear and branched standards across all matrices. We hypothesize that long-chain PFAS will preferentially bioaccumulate in plasma and sediment, increasing with turtle age and higher in males than females. Kidney, liver, and immune analytes are expected to be most sensitive indicators of PFAS exposure, with sublethal effects correlating with plasma biomarker levels and environmental PFAS concentrations. This study provides insights into chemical exposures and plasma analyte and hematological responses in the two turtle species. If validated as a surrogate species, *K. subrubrum* could inform PFAS risk assessment for species of greatest concern *C. guttata* by contributing to the development of adverse outcome pathways and ecological risk assessment frameworks for evaluating cumulative stressor impacts.

3.03.P-Mo-023 Freshwater turtle species vary in their mercury exposure from golf courses in urbanized landscapes

Jeremiah Harvey Tennant¹, Bradley J Cosentino², Kristin Winchell³ and Roxanne Razavi¹, (1)SUNY ESF, (2)Hobart and William Smith Colleges, (3)New York University

Turtles are long-lived, high trophic predators that serve as biomonitors for anthropogenic pollutants such as mercury (Hg). Previous research assessed the urbanized landscape as habitat for turtles and found that golf courses offer suitable habitat that can be improved with management. Here, we assess the risk posed by Hg pollution to the urban turtle habitat using a causal modelling framework. We investigated the direct and indirect effects of species, biological traits, waterbody characteristics, and landscape composition on Hg bioaccumulation in two common and widespread turtle species in New York State—the common snapping turtle (*Chelydra serpentina*) and the Eastern painted turtle (*Chrysemys picta*) from 70 waterbodies. Habitat characteristics, including waterbody type, conductivity, and land cover composition, affected Hg exposure in turtles. In snapping turtles, Hg concentrations were significantly lower in urban ponds than wildlife management areas or golf courses. In painted turtles, Hg concentrations were significantly lower in urban ponds than wildlife management areas, but not golf courses. We found significant effects of conductivity and proportion of wetland on Hg concentrations in snapping turtles, but we did not find any significant causal predictors of Hg concentrations in painted turtles. This suggests that the habitat variables we investigated have a greater influence on Hg bioaccumulation in snapping turtles than in painted turtles. Species differences in Hg bioaccumulation were pronounced, but sex did not affect Hg concentrations. This research provides insights into how habitat management of golf courses may affect turtle species differently in the urban landscape.

3.03.P-Mo-024 The effects of per- and polyfluoroalkyl substances (PFAS) on amphibian disease dynamics

Olivia Biasetti and Jason T. Hoverman, Purdue University

Emerging infectious diseases and environmental contaminants are increasingly recognized as co-occurring stressors that influence host-pathogen interactions. Per- and polyfluoroalkyl substances (PFAS) are widespread, persistent pollutants that negatively impact both human and wildlife health. Previous studies in amphibian models show that chronic PFAS exposure can impair immune function and delay development. However, most toxicological research focuses on constant, single chemical exposure scenarios, which do not reflect the variable and intermittent nature of real-world contamination. To address this gap, we investigated how the timing and duration of exposure to an environmentally relevant PFAS mixture influences fitness metrics and susceptibility to ranavirus infection in amphibian larvae. We evaluated this across three species, bullfrogs (*Lithobates catesbeianus*), wood frogs (*L. sylvaticus*), and crawfish frogs (*L. areolatus*), using 30- and 42-day laboratory experiments. A 4×2 full factorial design (PFAS x ranavirus) was implemented, with eight treatment groups and

a total of 720 tadpoles. PFAS treatments included early (days 0-20 or 0-28), late (days 20-30 or 28-42), and continuous (days 0-30 or 0-42) exposure; ranavirus was introduced on day 20 or 28. We hypothesized that continuous PFAS exposure would lead to higher ranavirus prevalence and load compared to early or late exposure and that both continuous and early exposures would reduce fitness metrics, such as scaled mass index (SMI), snout-vent length (SVL), and mass. Preliminary results from bullfrogs show no significant differences in SVL or mass among treatment groups, but SMI was significantly lower in all PFAS and ranavirus treatments relative to controls. Quantitative PCR for viral prevalence and load using liver and kidney tissues is ongoing for all species. This study illustrates the importance of incorporating ecologically realistic contaminant scenarios into disease ecology research, highlighting how these contaminants may exacerbate disease risk and alter host-pathogen dynamics in amphibians.

3.03.T Herplandia!

3.03.T-01 Monitoring accumulation and potential effects of per- and polyfluoroalkyl substances (PFAS) in diamondback terrapins (*Malaclemys terrapin*) along the Atlantic coast

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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 and potential effects 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*) are useful for monitoring various pollutants in estuarine habitats. Terrapins exhibit many characteristics that make them ideal for monitoring PFAS contamination, 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. To better understand bioaccumulation of PFAS and potential physiological effects, we collected blood samples from terrapins throughout waters along the Atlantic coast in New York, New Jersey, Delaware, Maryland, and North Carolina. Plasma was analyzed for PFAS compounds using HRMS-LC and multiple indicators of immune and thyroid system status. The results indicate variations in PFAS bioaccumulation and profiles across the sampling locations. These novel ecotoxicological and health data provide a foundation for understanding PFAS exposure, as well as identifying factors that may dictate exposure risk and will assist state natural resource and wildlife agencies with management of terrapin populations.

3.03.T-02 Alligator-ingested microplastic: A contaminant of concern in a top predator

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Plastic pollution is a global concern and poses risks to water resources and wildlife. Despite national and international pressures to curb plastic and microplastic pollution, data regarding contamination and toxic effects on wildlife are lacking. Therefore, research on this emerging contaminant is critical for addressing data gaps to quantify and understand potential impacts on wildlife. American alligators (*Alligator mississippiensis*) are ubiquitous in wetlands throughout the southeastern United States. They are long-lived top predators with high site fidelity and broad diets. Throughout their range, management programs use alligators as bio-monitors for

aquatic contaminants, including metals and persistent organic pollutants. We hypothesize that alligators can be used as sentinel species for bioavailable microplastics in aquatic systems. We aim to explore microplastic ingestion in alligators and the utility of alligators as bio-monitors for microplastics in aquatic systems by leveraging ongoing state- and university-led research and the legal harvest of alligators across the region to collect alligator samples range-wide (nine states). We provide an overview of novel methodology for analyzing plastics from alligator diet samples using a two-step chemical digestion, density separation, and laser direct infrared analyzer. We present information on alligator diet and ingested microplastics from a subset of 30 alligators from sites throughout South Carolina, and our preliminary results comparing regional alligator-ingested microplastics from samples collected across nine states. We found variation in diet and recovered microplastics among inland freshwater habitats and coastal wetlands. Additionally, our initial analyses of ingested microplastics show measurable differences among alligators. Our results infer that diet, as well as location in relation to roadways and urbanization, may impact the prevalence and characteristics of ingested microplastics in alligators.

3.03.T-03 Refining ranges of amphibians and reptiles to support endangered species risk assessment

John Marton, Corteva Agriscience

Amphibians and reptiles have the highest proportion of threatened species across all vertebrate taxa, with 85 species currently listed as threatened or endangered (i.e., listed) in the United States. Registration and re-registration of plant protection products in the United States requires assessing potential risk to listed species. A critical component of this process involves determining the spatial overlap between the species' range and/or critical habitat and the appropriate use data layers (UDLs), which represents the spatial footprint of a specific crop or crop group. The overlap assessment is used within the weight-of-evidence to inform the predicted determinations of jeopardy or adverse modification, potentially necessitating general label mitigations or the implementation of pesticide use limitation areas (PULAs) with species specific mitigations to reduce potential exposure to listed species. Even if the initial risk assessment predicts no direct toxicological risk to a certain taxon, there may be potential indirect effects if a species' food source or habitat is impacted (i.e., insecticides potentially impacting the diet of insectivorous amphibians). Outdated or overly conservative species range maps can lead to overestimation of overlap, thereby leading to unnecessary mitigations that can place undue burden on growers with little to no benefit to the species. This presentation will explore the refinement of species' range maps for a select number of amphibians and reptiles, and highlight how simple geospatial refinements can be used to enhance the precision of range maps for use in the endangered species risk assessment process without compromising species protection and allowing growers greater access to crop protection tools.

3.03.T-05 Where the amphibians are: Differences in community composition among wetland reserve easements of the Mississippi Alluvial Plain

Taylor Thomisee, Helena Grace Wheeler, Stephen Mullin and Jennifer Bouldin, Arkansas State University

The Mississippi Alluvial Plain (MAP) within northeastern Arkansas has experienced fragmentation and degradation of historic wetland habitats on account of industrial-scale agriculture and other types of alteration. Amphibians reside in wetlands because of the high availability of food sources and appropriate habitat for breeding. The federal Agricultural Conservation Easement Program-Wetland Reserve Easement (ACEP-WRE) supports land-use conversion from agricultural fields to wetland habitat for preservation of native species and improvement of landscape functionality. The efficacy of conservation efforts can improve with a more complete understanding of how amphibian species utilize restored wetlands when compared to both natural or heavily impacted lands. We employed a variety of survey techniques uniformly across 40 wetlands that are categorized into three types: (1) Reference, sites over 25 years of age within wildlife refuges that present ideal habitat conditions for amphibians; (2) Control, agricultural ditches that sustain a population of at least one amphibian species; and (3) Restored, parcels under 20 years of age enrolled in the WRE program. Each of the Restored parcels was selected to represent a range of characteristics such as age, wetland size, and variability in other habitat parameters (e.g., water turbidity and conductivity, canopy closure, surrounding habitat matrix). We

quantified the richness and evenness of the amphibian community at each site, and assessed the relative abundances of different life-history stages of the species present. Differences in community composition were found to be driven partially by site age relative to site size, particularly involving Restored parcels of larger size. Predicted abundance of amphibians was more reliable at sites with lower overall conductivity, with interactive effects driving abundance patterns for select amphibian species. We discuss our results in the context of understanding the relationships between habitat characteristics and amphibian diversity, with a primary focus on modeling ecosystem characteristics and amphibian occupancy in these re-established wetland parcels.

3.04.P-Mo Wildlife Toxicology Across the Full Annual Cycle

3.04.P-Mo-019 Ecological disturbance in the anthropocene: Legacy effects of unplugged oil wells on deer mouse physiology and vegetative disturbance

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Persistent organic pollutants have been studied intently for the last thirty years, and many are known to be mutagenic, with recent studies focusing on the more carcinogenic compounds. Polycyclic aromatic hydrocarbons (PAHs) are commonly studied environmental organic contaminants. There are 16 PAHs listed by the United States Environment Protection Agency (USEPA) as priority contaminants of concern. Hazardous PAHs are naturally occurring components of crude oil and can remain in the environment long after crude oil spills have occurred. While catastrophic, large, marine oil spills often receive considerable media attention, smaller-scale inland spills occur much more frequently, resulting in legacy PAH contamination. Oklahoma has over 15,965 documented orphaned, unplugged oil wells (UOWs). Oklahoma Energy Resources Board (OERB) is working diligently to plug orphaned wells however, lasting effects on surrounding ecosystems are not well understood. Using two study locations in Cushing, Oklahoma, multiple ecosystem parameters will be observed. To assess site-specific disturbance, we will conduct vegetative surveys to generate Floristic Quality Assessments (FQAs). Soil sample contaminant concentrations will be measured. Resident deer mice metabolic rates and hematological indices will be collected. In accordance with substantial data showing PAH exposure causes mammalian hematological damage, altered immune function, and metabolic rate shifts, we hypothesize legacy PAH contamination will lead to physiological differences in free-living *Peromyscus* individuals collected most proximal to UOWs. We will measure white blood cell differentials, packed cell volume, hemoglobin concentration, and organismal resting metabolic rate. Collectively, this data will allow us to evaluate legacy effects of unplugged oil wells on multiple ecosystem components.

3.04.T Wildlife Toxicology Across the Full Annual Cycle

3.04.T-01 PFAS dynamics in American Black Bear (*Ursus americanus*) physiological phases (pre, during and post hibernation) and temporal trends from 1989 to 2016

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Perfluoroalkyl substances (PFAS) have been detected in wildlife globally, yet limited research exists on their dynamics in terrestrial vertebrate hibernators, such as the American Black Bear (*Ursus americanus*). Our objectives were twofold: first, to determine the possible fluctuations in PFAS among the same bear (n = 38) experiencing different physiological phases, including hibernation, and second, to document the temporal trends in PFAS levels across multiple bears (n = 97) over a period of three decades. We hypothesized that depleted body reserves and slow metabolic state during hibernation could influence PFAS concentrations in this species, and that bear plasma would reflect temporal PFAS industrial use. To test these hypotheses, we analyzed plasma samples (n = 169) repeatedly collected across the different hibernation stages, from early Fall to the subsequent Spring in Virginia, USA. We measured a myriad of PFAS (n = 21), including long-chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSA), which exhibited different patterns across the physiological phases. During hibernation, bears displayed increasing concentrations of PFCAs such as

perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA), while perfluorooctanoic acid (PFOA) and perfluorotetradecanoic acid (PFTeDA) showed a significant decreasing trend. Interestingly, other PFCAs such as (C11-C13) remained consistent throughout. In contrast, among the PFSA, both linear and branched isomers of perfluorooctane sulfonate (PFOS) showed a significant increase during hibernation, followed by a marked decrease post-hibernation arousal. Our results indicate that the metabolic activity during hibernation, associated with fasting conditions, is sufficiently involved in biotransformation and increases the overall PFAS burden in *U. americanus*. Preliminary analysis of temporal trends showed that Σ PFAS concentrations increased from 1993 to 2004, followed by a declining trend from 2006 to 2015. Notably, PFSA were consistently present at higher levels than PFCAs during the period of rising Σ PFAS concentrations in these bears.

3.04.T-02 Life-stage specific toxicity of lambda-cyhalothrin to the painted lady butterfly (*Vanessa cardui*) via contact and dietary exposure

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Pollinators in agricultural landscapes may be exposed to insecticides via direct overhead spray, off-field drift, or contaminated plant material. For new test species, it is important to understand how sensitivity to insecticides may vary across developmental stages and routes of exposure, as these factors can influence toxicity outcomes. This consideration is particularly relevant for non-*Apis* pollinators such as Lepidoptera, which remain underrepresented in ecotoxicological research. The painted lady butterfly (*Vanessa cardui*) is a widespread species for which laboratory rearing methods are well-established, making it an ideal model for evaluating life stage-specific insecticide toxicity. We assessed the effects of lambda-cyhalothrin, a commonly used pyrethroid, on *V. cardui* across five developmental stages—three selected larval instars, pupae, and adults—using separate contact and dietary exposure assays. This approach captures differences in sensitivity that could be missed if only a single developmental stage or exposure route were evaluated. To determine whether formulation influences toxicity, we conducted parallel experiments using both the formulated product (Warrior II) and the technical grade active ingredient dissolved in solvent. We compared LD₅₀ values and dose-response curves across developmental stages, exposure routes, and product types. To place *V. cardui* sensitivity in context, we compared toxicity estimates to published data for honey bees (*Apis mellifera*), a common model species in pollinator risk assessments. The identification of vulnerable life stages, critical routes of exposure, and formulation-dependent effects could inform more nuanced ecological risk assessments and guide the development of targeted strategies to mitigate pesticide impacts on non-target Lepidopteran pollinators.

3.04.T-03 In-situ ecotoxicology – Peru: biodiversity and mercury 6-year study

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The Madre de Dios region of the Peruvian Amazon is heavily gold mined outside of protected conservation concessions and national parks. Mercury is used to bind the fine gold dust and separate it as an amalgam. The mercury is then burned off, resulting in mercury contamination of land, water, and air, leading to impacted human health. Yet there is very little known about the impact on biodiversity and wildlife health. A 2022 study in the region demonstrated intact forests next to artisanal and small-scale gold mining (ASGM) sequester mercury at levels comparable to industrial cities. At the Estacion Biologica Rio Los Amigos in Madre de Dios, a Peruvian amazon (2018-2024) mercury monitoring program is in its seventh year, collecting across 250+ neotropics species (primates, bats, birds, small and medium mammals) and has tested >3000 hair and feather

samples. In this “protected” conservation concession, almost all samples have a detectable mercury burden. Maximum burdens (with average) in ppm ranged from 1.7-87.8 (0.2-1.22). In humans, the World Health Organization has set a 2-ppm threshold in hair to prevent reproductive and neurological toxicosis, though we do not know the thresholds in these neotropics species. Case studies will be briefly described for keystone species, such as non-human primates (often microchipped and tracked annually for behavior and breeding endpoints), ocelots, and bats (also both chipped for annual tracking). Together, this data highlights the exposure risk wildlife have to heavy metals globally, and different strategies to monitor these exposures.

3.04.T-06 Ecological and behavioral complexity in birds: Implications for chemical exposure

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Birds are morphologically and phylogenetically distinct from other classes of vertebrates. However, current risk assessment protocols often evaluate environmental chemical exposure in a relatively simplistic manner, without adequately considering avian life stage, ecological traits, and other species-specific physiological and behavioral characteristics. While such protocols may provide a basic screening-level assessment, they fail to account for the complex interplay of biological and ecological factors that influence actual exposure in birds. As a result, exposure estimates rarely reflect real-world field conditions. To address this challenge, a subgroup of the technical committee on Next Generation Ecological Risk Assessment of the Health and Environmental Sciences Institute worked to establish the state-of-the-science on how avian life-history traits influence chemical exposure. Here, we propose a fresh re-examination of avian chemical exposure by exploring how field exposure is shaped by the diverse life-history, behavioral, and ecological traits of birds. A literature review revealed that characterizing avian exposure to environmental chemicals is particularly challenging given their species diversity, ecological and physiological plasticity, complex life histories, niche partitioning and feeding ecology, as well as physiological and behavioral variations. The scientific need and value to integrate species-specific biological, behavioral, and ecological traits and behaviors (e.g., habitat preferences, migratory and dispersal capacity, foraging guild, seasonal changes in food choices) that can modulate chemical exposure and biological responses will be discussed and demonstrated with species-based field exposure studies as well as ornithological and ecological works. Case studies on selected metallic and organic contaminants will be used to illustrate the importance of life-history and ecological traits for realistic avian exposure assessment. We will conclude with implications on next-generation exposure assessment practice, as well as various theoretical and experimental research needs for advancing our understanding and capacity to assess contaminants exposure and their biological fate realistically in the aves.

3.05.P-Mo General: Wildlife Toxicology, Ecology and Stress Response

3.05.P-Mo-012 Environmental quality through science

Tony D LeBlanc, *The Conference Exchange*

SETAC is dedicated to advancing environmental science and science-informed decision-making through collaboration, communication, education and leadership. We fulfill that purpose through events, publications, awards and education programs.

3.05.P-Mo-013 Analysis of 40 per- and polyfluoroalkyl substances in sand, soil, sediment, biosolid and fish samples by USEPA method 1633

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A LC-MS/MS workflow was developed based on USEPA method 1633 and applied to analysis of PFAS in solid, biosolid and fish samples. Low level of PFAS were detected from sand and sediment samples, while relatively higher amount of PFAS were determined from biosolid samples and a garden soil purchased. To

realize high-throughput PFAS analysis from complex sample matrices, it is desirable to have a robust LC-MS/MS system that can provide consistent results without instrument downtime for maintenance. In this study, the robustness of a QSight 500 LC-MS/MS system was evaluated by continuously analyzing fish samples for over 750 injections. The results showed that spiked PFAS internal standard signal intensity remained stable during analysis, demonstrating good robustness of the system. Methods The mixed PFAS standards (EPA-1633STK) and isotope labelled standards including extracted internal standard (EIS) mix and non-extracted internal standard (NIS) mix were purchased from Wellington Laboratories. EPA-1633 proficiency testing samples for soil and fish were obtained from Absolute Standards Inc. Beach sand and sediment samples were collected from a local lake beach area. A black garden soil and fish sample were bought from a local store. Biosolids were obtained from Environment and Climate Change Canada. Samples were prepared following EPA method 1633 procedures with an automated SPE system. Using an automated solid phase extraction (SPE) with dual-layer cartridges can reduce human operational errors and improve lab work efficiency. QSight LC-MS/MS system provided great sensitivity for all PFAS studied with LOQs below the requirement limits. In addition, QSight LC-MS/MS system showed exceptional robustness for PFAS analysis from complex sample matrices without the need for instrument maintenance, evidenced by the PFAS signal stability after continuously analyzing PFAS samples for a whole year, which is important for high-throughput PFAS analysis from complex sample matrices. In this study, the robustness of a QSight 500 LC-MS/MS system was further evaluated by continuously analyzing fish samples for over 750 injections at the end. The results showed that spiked PFAS internal standard signal intensity remained stable during analysis, demonstrating good robustness of the system.

3.05.P-Mo-014 Assessment of risks to wildlife from lead and arsenic at former orchard lands at the Hanford Site, Washington

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Historical use of lead arsenate as a pesticide in orchards at the Hanford Site has resulted in legacy lead and arsenic contamination in surface soils within 100-OL-1 OU. Risks to 8 birds and mammals in 133 DUs in the 100-OL-1 OU from lead and arsenic were evaluated. To the extent possible, chemical-specific parameters were developed based on lead and arsenic associated with lead arsenate. Site-specific bioaccumulation models for lead and arsenic were developed for plants and terrestrial arthropods; literature-derived models for earthworms and small mammals were developed from lead arsenate treated soils. Soil bioavailability of lead and arsenic was evaluated using literature-derived in vivo and in vitro measures of lead arsenate bioavailability. Tissue-based small mammal exposure to lead was estimated using literature-derived data from orchards treated with lead arsenate. Dietary effects data consisted of ED10s, ED20s, and ED50s for survival, growth, and reproductive derived from published literature. Tissue-based levels for lead in mammals were extracted from published literature. A data review to quantify the relative contribution of lead and arsenic to lead arsenate toxicity was performed. Species sensitivity distributions developed from acute toxicity data show: lead salts < lead arsenate < arsenate salts. Combined exposure to lead and arsenic presents minimal risk to localized populations of the 8 birds and mammals in the 100-OL-1 OU. No risks are estimated for six receptors. Risks from lead or lead and arsenic in combination are estimated for 2 receptors and only in a single DU. Risks associated with kidney lead concentrations in small mammals are estimated only within a single DU and only to a limited degree. Probabilistic analyses performed for the five most highly exposed receptors and the DU with the highest concentrations indicated that deterministic exposure estimates may overestimate lead risks and underestimate arsenic risks when estimated exposure for each COPEC approaches the lowest ED20 threshold. Because risk conclusions based on deterministic and probabilistic HIs were in agreement for all five species evaluated, calculation of HIs that integrate the combined exposures of lead and arsenic appears to mitigate the directional uncertainties associated with the evaluation of each COPEC independently. Risk conclusions based on the HI may therefore be more robust and less uncertain than those based only on individual COPECs.

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3.05.P-Mo-015 Refining risks to wildlife at a former mining site to support habitat enhancement and ecology-focused remedial design

John Gondek¹, John Lucero¹, Katherine Thomas² and Matthew LeFauve², (1)Jacobs, (2)U.S. Environmental Protection Agency

Historical mining and milling operations at the Bautsch-Gray Superfund Site in Galena, Illinois have resulted in elevated metal concentrations in soils throughout the site. Concentrations of metals in soils exceed ecological benchmarks within a large unvegetated area of mine tailings, as well as throughout mature secondary growth woodlands surrounding the tailings. Remedial actions to be considered at the site must therefore weigh balancing reducing ecological exposures to metals and preserving the existing high-quality habitat. Previous bench scale studies noted limited leachability of metals in the tailings and low potential to cause adverse effects to plants and invertebrates. However, risks to wildlife could not be ruled out based on desktop bioaccumulation modeling. A study was undertaken to better understand site-specific bioavailability of metals in prey and forage consumed by wildlife through the collection of plant tissues and earthworm bioaccumulation tests. This study was also coupled with a detailed natural resources inventory, documenting habitat structure, terrestrial plant and invertebrate communities, wildlife observations and potential nesting areas, and other indicators of habitat quality. Cavity-nesting bird colonies are found within the tailings pile and the plant communities at the site do not appear to be adversely affected by elevated metals in the soil. Similarly, the bioaccumulation studies indicate uptake of metals into plant and invertebrates is far lower than what would be predicted by literature-based bioaccumulation models. The findings from this study were used to support a weight-of-evidence approach for risk-based decision making and refining ecological risk-based remediation goals. Ultimately, the revised remediation goals indicate a much smaller portion of the site would exceed wildlife refined risk-based thresholds, reducing the need to disturb existing habitats. Furthermore, by coupling the bioaccumulation study with detailed habitat surveys, decisions on ecological risk can be evaluated on a very granular scale, allowing the existing habitat quality to inform the remedial design. The habitat surveys are also being used to inform where habitat could be further enhanced, including creating new grassland habitat to support sensitive invertebrate species and wildlife. This work highlights how site-specific bioavailability and understanding of the greater ecological community can help refine mine reclamation activities.

3.05.P-Mo-016 Legacy organochlorines, polycyclic aromatic hydrocarbons and polyfluoroalkyl substances in pre-fledgling double-crested cormorants of the Great Lakes, including in contaminated areas of concern

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Colonial waterbirds have been monitored as sentinel species for assessing effects of organic pollutants in the Great Lakes for six decades. These studies elucidate issues managed by environmental agencies, including considerations about legacy contaminants and chemicals of emerging concern at specially designated Areas of Concern (AOCs), in contaminated areas not designated as AOCs, and in lake-wide regions. The objective of this study was to assess exposure to organic pollutants in pre-fledgling double-crested cormorants (*Nannopterum auritum*) throughout the Great Lakes, including comparisons in/near AOCs to nonAOC locations. Blood plasma samples (pooled by site and year) were analyzed for contaminants from 355 cormorant chicks at 16 colonies across the Great Lakes in 2016 and 15 colonies in 2017. Polychlorinated biphenyls (PCBs), organochlorine pesticides, and polycyclic aromatic hydrocarbons (PAHs) were measured by gas chromatography-mass spectrometry. Polyfluoroalkyl substances (PFAS) were measured by ultra-performance liquid chromatography-tandem mass spectrometry. Mean concentrations of sumPCBs, PCB 126, sum perfluorosulfonic acids (PFSAs), and perfluorooctanesulfonic acid (PFOS) were approximately 3X higher for the AOC group compared to the nonAOCs. Mean concentrations of p,p'-dichlorodiphenyldichloroethylene

(DDE) and sumPFAS were approximately 1.6X higher in the AOC group. Of the sampled AOC colonies, highest PCBs were found in western Lake Erie and Hamilton Harbor, Lake Ontario. PCBs at nonAOC colonies in eastern Lakes Erie and Ontario were lower than the AOCs for their respective lake but higher than most other nonAOC colonies on other lakes. The mean concentration of perfluoro(4-ethylcyclohexane)sulfonic acid (PFEtCHxS) was 5X higher at AOCs compared to nonAOCs, driven by high values at both Lake Michigan AOCs (Green Bay and Indiana Harbor) and the Lake Ontario AOC (Hamilton Harbor). PFEtCHxS was highest in Lake Michigan overall. Mean sumPAHs did not differ significantly between AOCs and nonAOCs, but elevated concentrations were found at some sites in both AOC groups and across all lakes except Superior. The mean concentration of sum perfluorocarboxylic acids (PFCAs) was approximately 2X higher at nonAOCs compared to AOCs, with highest concentrations in eastern Lake Superior (Whitefish Bay and upper St. Marys River). Overall, this study showed many elevated contaminants at AOC colonies, with some contaminants higher in nonAOC locations.

3.05.P-Mo-017 Multi-decadal assessment of per- and polyfluoroalkyl substances (PFAS) in North American alligators from the Kennedy Space Center to Florida Lakes

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Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent contaminants with known bioaccumulative and expanding list of adverse toxicological associations. The American alligator has been shown to be a useful environmental sentinel, since they are long-lived apex predators that have high site fidelity, with a history of being utilized as models to study the impact of endocrine disruptors. In this study, we revisited alligators in the Merritt Island National Wildlife Refuge, surrounding the Kennedy Space Center, this time investigating the “individual” change in PFAS in >30 re-captured alligators with over a 5-year gap between capture dates. Next, we explored “population” changes by comparing PFAS levels in alligators from 5 different Florida lakes with a >20-year gap between sampling events (the initial sampling events occurred in the mid-to-late 1990s). These longitudinal assessments will provide a unique perspective as to whether specific PFAS shifts in production and consumerism, which have been shown in human populations, are also reflected in wildlife populations. Lastly, this study explored the PFAS profiles between plasma, serum, and whole blood from matching individuals, highlighting the importance of proper blood preparation protocols.

3.05.P-Mo-018 Mercury exposure in central California peregrine falcon population

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As apex predators, Peregrine Falcons (*Falco peregrinus*) are especially vulnerable to contaminants like mercury (Hg). Legacy contamination from historic gold mining and industrial activities in the San Francisco Bay watershed puts Central coast peregrine falcons at increased risk of mercury accumulation. From 2019 to 2024, we collected primary feather clippings from 52 nestlings, at 24 different nest sites in our study area. Samples were weighed and total Hg measured by Direct Mercury Analyzer (DMA-80). We determined mean nestling feather total Hg concentration was 3.34 ± 2.50 µg/g (range= 0.62 µg/g - 11.3 µg/g). We found that nestling feather total Hg concentrations within brood were measurably different from one another, which has implications for future study design when determining effects of nest site location on Hg exposure. Nestling feather total Hg also varied between nest sites and across years, which we hypothesize are due to parent replacements, prey availability, and temporal fluctuations in Hg bioavailability. Given Hg exposure can cause immune system dysfunction in birds, we recommend further investigation into potential links between Hg exposure and susceptibility to emerging diseases, like highly pathogenic Avian Influenza (H5N1) which has been impacting avian populations in California since 2022.

3.05.V General: Wildlife Toxicology, Ecology and Stress Response

3.05.V-01 Forecasting *Karenia brevis* harmful algal blooms (HABs) and associated economic impacts in Florida coastal waters using predictive modeling

Kelly Liu and Amanda He, Valley Christian High School

Harmful algal blooms (HABs) caused by *Karenia brevis* (“red tides”) threaten Florida's coastal ecosystems through brevetoxin release, with climate change and nutrient runoff intensifying bloom severity. Brevetoxins cause mortality in marine life and respiratory illness in humans, disrupting fisheries and local economies. Currently, there is a gap in multi-year predictive models used to forecast HAB trends. Thus, we developed a 5-year forecasting framework combining: (1) a Long Short Term Memory (LSTM) neural network for *K. brevis* cell count prediction and (2) a Seasonal AutoRegressive Integrated Moving Average with exogenous regressors (SARIMAX) model for economic impact assessment. The LSTM forecasted red tide intensity through 2030 using historical red tide cell count, time, and location data from the Florida Fish and Wildlife Conservation Commission (FWC), combined with nutrient, salinity, and precipitation records from the National Oceanic and Atmospheric Administration (NOAA) and the National Water Quality Monitoring Council (NWQMC). We input statewide fisheries economic data (FWC) into a SARIMAX predictive model to project fish stock losses and revenue impacts through 2030. All data fed into the models dated from 2010-2024. LSTM networks achieved cross-validated $R^2 = 0.813$ in predicting *K. brevis* cell counts. Projections show 4.5% annual increases in red tide intensity, measured by *K. brevis* cell concentrations, resulting in 71.3 million pounds of lost fish stock and an estimated \$282.8 million loss in revenue by 2030. These long-term models advance HAB forecasting by providing 5-year trend predictions that enable more effective targeted mitigation and early-intervention strategies in coastal, at-risk communities. Additionally, these models are scalable to coastal systems globally

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

3.06.P-Th-040 Early indicators of environmental stress: Preliminary findings on freshwater disease in bottlenose dolphins in Galveston Bay, Texas

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Bottlenose dolphins (*Tursiops truncatus*) are widely recognized as sentinels for coastal and estuarine ecosystems due to their sensitivity to environmental stressors and close proximity to human activity. One emerging health concern in these populations is freshwater disease (FWD), a condition characterized by skin lesions and physiological stress resulting from prolonged exposure to low-salinity environments. As flooding and extreme weather events increase in frequency and intensity due to shifting environmental conditions, estuarine ecosystems like Galveston Bay, Texas, are experiencing more frequent and severe freshwater influxes. These changes make such environments critical for investigating the potential impacts of low-salinity on dolphin health. This study presents preliminary findings from a long-term assessment of a dataset (2014–2019) collected by the Galveston Bay Dolphin Research Program (GDRP), which conducts photo-identification (photo-ID) and environmental monitoring surveys of bottlenose dolphins in the region. During the study period, 135 individual dolphins were identified and observed year-round, each with a minimum of 15 confirmed sightings. Of these, 126 individuals exhibited visual signs consistent with FWD, with lesions observed during between seven and 40 sighting occasions per individual. Images of these 126 dolphins were analyzed using a newly developed visual assessment protocol, which applies a standardized criteria to evaluate the progression and severity of FWD in bottlenose dolphins. Across these dolphins, a total of 2,034 confirmed lesion sightings were documented, each representing a distinct 'case' of FWD. We analyzed lesion characteristics and their

progression in relation to low salinity exposure, considering variables such as event timing, geographic location, and salinity extremes. These findings will offer valuable insights into the health impacts of shifting environmental stressors on estuarine dolphin populations.

4.01.A.T Advancing Science Through Current Approaches in Environmental Monitoring

4.01.A.T-01 Development of pesticide response spectrum models for Chinook Salmon

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Our goal was to develop response spectrum models for selected pesticides that associate measured internal pesticide residues with adverse biological effects in migrating juvenile Chinook salmon. The internal pesticide concentration in the fish was chosen as the dose metric because it is more accurate than aqueous pesticide concentrations which don't account for pesticide bioavailability or routes of uptake. Previous studies conducted by our research team in waterways in the Delta have found that two current-use insecticides, bifenthrin and fipronil, and the ubiquitous degradation product 4,4'-dichlorodiphenyldichloroethylene (DDE) represented the most frequently detected pesticides in sediment, invertebrates, and outmigrating Chinook salmon. These compounds, due to their prevalence and toxicological significance, are the focus of this study. Mixture studies have shown that bifenthrin and fipronil act additively while DDE residues in the fish contribute to the noted effects. Bifenthrin imparts the highest risk of acute and sublethal toxicity in juvenile Chinook salmon among the pesticides tested, and residues in field-collected hatchery origin fish reach levels found to cause sublethal effects on routine swimming behavior and shoaling. These data suggest that pesticide residues are high enough in juvenile Chinook salmon to impact their predation risk and likelihood of migratory success. The newly developed model offers the advantage of enabling direct comparisons of pesticide bioaccumulation data for juvenile Chinook salmon obtained from routine biomonitoring activities to residue-effects models. This information will guide future management decisions regarding the effects of pesticides on sensitive salmonid species.

4.01.A.T-02 EDT screening: Leveraging effect-based spectral libraries to accelerate semi-quantitative non-target analysis

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Sediments contain complex chemical mixtures. While effect-directed analysis (EDA) combined with non-target screening (NTS) is promising, its large-scale application has been limited by time-consuming workflows. Here, we developed an event-driven taxonomy (EDT)-Screening strategy to effectively identify and semi-quantify non-target bioactive contaminants in sediment, taking aryl hydrocarbon receptor (AhR) activity as an example. To accelerate EDA and NTS workflows, this strategy integrated fractionation, bioassay, identification, and quantification into a single step by embedding two novel effect-based spectral libraries into LC-HRMS screening templates. The event driver (ED) library was assembled from data-mined AhR-active compounds, and event driver ion (EDION) library contained effect-related fragment ions predicted by deep learning. Compared to conventional databases (e.g., ChemSpider), AhR-ED library improved identification accuracy with more complete AhR-agonist list and fewer false positives, while AhR-EDION library uncovered additional AhR agonists, particularly industrial intermediates and transformation products often missed due to limited prior knowledge. With the multimodal learning-based semi-quantitative module, the EDT-Screening strategy increased the explained bioactivity contribution from 7.1% to 82%, significantly expanding the detections of "unknown unknowns". Our findings show that effect-based HRMS libraries provided a rapid solution for identifying and prioritizing bioactive contaminants in complex chemical mixtures, advancing EDA-NTS workflows for environmental risk assessment.

4.01.A.T-03 Nontarget prioritization using cell bioassays: A spatial study of stream water and sediment across California

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With the increasing diversity of anthropogenic contaminants entering ecosystems, monitoring programs are looking to integrate techniques that identify unmonitored chemical drivers of toxicity. One such program, California State Water Resources Control Board's Constituents of Emerging Concern Program (CEC Program), focuses on proactively protecting against contaminants through optimizations in monitoring and management. In collaboration with the Stream Pollution Trends Monitoring Program (SPoT) – which performs sediment monitoring to inform impaired waterbody listings and regional monitoring efforts – the CEC Program is testing the application of nontarget analysis (NTA) to expand beyond an established list of analytes. NTA is an a priori method capable of detecting and identifying contaminants beyond the traditional targeted capacity; however, elucidating toxicity drivers is made challenging by the +1,000 features generated from NTA per sample. With complete elucidation not yet feasible due to the need for manual validation, methods are needed to prioritize features of concern. Here, we prioritize features of concern using the response of five in vitro chemically activated luciferase gene expression cell bioassays (CB-CALUX) (aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptor alpha (PPAR α), estrogen receptor alpha (ER α), glucocorticoid receptor (GR), and anti-androgen receptor (AR)), to direct identification efforts towards compounds correlated with toxicity. To test this approach, in 2023, 18 water and sediment samples were collected for NTA & CB-CALUX response. Nontarget features were detected using gas chromatography with high-resolution mass spectrometry (GC-HRMS) and prioritized by correlating peak height and bioassay response using Spearman's Rank Correlation. Initial results show AhR response is significantly correlated ($p < 0.05$, all variables) with urban land use and ER α activation in both water and sediment samples. Seven standard-matched PAHs, including chrysene and phenanthrene, are significantly correlated ($p < 0.01$) with AhR response in sediments. Ongoing work focuses on confirming additional highly correlated features, with a specific focus on those predicted to be assay activators based on USEPA's ToxCast software or PubChem's bioassay results. These results show how prioritizing nontarget features using CB-CALUX response endpoints can enhance the detection of toxic emerging contaminants, improving monitoring.

4.01.A.T-04 Temporal variability of airborne endocrine disruptors in Montreal, (QC) Canada: Evidence from 2021-2025 campaigns

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Atmospheric pollution is a critical public health concern with well-documented associations with various human diseases, including cancer. Our research, along with that of others, has established that outdoor air contains a complex mixture of anthropogenic chemicals, including volatile and semi-volatile industrial compounds, pesticides, and combustion by-products. Many of these are recognized or suspected endocrine-disrupting chemicals (EDCs), capable of interfering with key hormonal pathways regulating metabolism, reproduction, and development, primarily through EATS-mediated mechanisms (Estrogen, Androgen, Thyroid, Steroidogenesis). Building on this evidence, we have assessed the endocrine-disrupting potential of these airborne mixtures using well-established human-relevant cell-based assays. Forty passive air samplers (PAS) were deployed across the Island of Montreal for three summer months in 2021 and 2024 and three winters months in 2025. The collected samples were chemically extracted to (1) characterize the presence and spatial distribution of organic contaminants and (2) assess their endocrine-disrupting activities. A non-targeted chemical analysis of the 2021 extracts identified thousands of molecules, including nitrophenols, pesticides/repellents, plasticizers, other industrial chemicals, and natural products. This database serves as a

reference for comparing 2024 and 2025 samples, allowing assessment of temporal variability. In parallel, we employed seven cell-based bioassays to evaluate the extracts' potential to induce (anti)estrogenic and (anti)androgenic activities, disrupt thyroid function by targeting thyroperoxidase (TPO) and the sodium/iodide symporter (NIS), and alter steroidogenesis. Our findings reveal persistent and variable sites of estrogenic activity across sampling years. We also detected anti-estrogenic and anti-androgenic effects but no androgenic activity. Notably, some sites showed disruptions in thyroid hormone signaling. Together, these findings provide the first integrated assessment combining non-targeted chemical profiling with endocrine activity characterization of urban outdoor air extracts over two sampling periods. Our approach enhances understanding of the presence, distribution, and bioactivity of airborne endocrine contaminants and their potential health implications.

4.01.A.T-05 Spatio-temporal estimation and health risk assessment of polycyclic aromatic hydrocarbons and metals using machine learning in an industrial city

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Monitoring hazardous air pollutants (HAPs), such as polycyclic aromatic hydrocarbons (PAHs) and metals, is critical for evaluating long-term exposure and health risks. The spatial and temporal coverage of HAP monitoring networks is limited, whereas criteria air pollutants (CAPs) are continuously monitored at air quality monitoring stations (AQMSs). This study develops a practical method to estimate concentrations of PAHs and metals using CAP and meteorological data. A random forest model was trained on a decade of data from 2013 to 2022 in Pohang, Korea, a city that hosts one of the world's largest steel-making complexes. The model was used to predict concentrations of 16 PAHs and 8 metals at each AQMS. Model performance, evaluated using the correlation coefficient (R), root mean square error (RMSE), and normalized mean bias error (NMBE), demonstrated good agreement for PAHs (R = 0.73), As (R = 0.91), and Fe (R = 0.77). Although the peak concentrations were slightly underestimated, the predicted concentrations were elevated near industrial areas compared to residential areas, consistent with previous studies. Estimated PAH levels were higher in winter, consistent with seasonal patterns in the monitoring data. On the other hand, metal concentrations showed little seasonal variation in both estimated and monitoring data, reflecting persistent industrial emissions throughout the year. These results indicate that the model reliably captured both temporal and spatial patterns for different pollutant groups. Principal component analysis and diagnostic ratios suggested that vehicle and industrial combustion were the dominant sources. Cancer risk maps identified high-risk areas for both PAHs and metals near the steel industrial complex and major roadways. While cancer risks from PAHs were below the USEPA threshold of 1×10^{-6} , risks from metals exceeded this level at several industrial sites due to high concentrations of As and Cr. Population-weighted risk analysis revealed that older adults living in the old city center and near industrial areas faced elevated cancer risks. This study provides a practical machine learning-based approach to estimate concentration distributions and assess associated health risks in areas with limited HAP monitoring data. This approach can also be applied to other areas with limited monitoring coverage.

4.01.B.T Advancing Science Through Current Approaches in Environmental Monitoring

4.01.B.T-01 Advancing our understanding of environmentally driven selenium speciation and bioaccumulation through a coal mine monitoring program

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A monitoring program for a metallurgical coal mine in western Canada is investigating elevated concentrations of reduced and organic selenium species observed from the outflow of mine sedimentation ponds. These selenium species are highly bioavailable and can impact growth and reproduction in various organisms, including fish. Existing literature documents biological drivers as one mechanism that generates reduced and

organic selenium species but there are limited examples of organoselenium behavior in surface water or of risk management strategies to mitigate generation of these species and their subsequent discharge into the receiving environment. The purpose of this monitoring program is to identify the spatial and temporal scale of selenium speciation, and its execution has created data-rich opportunities to investigate mechanisms driving the generation of reduced and organic selenium species with the goal of informing adaptive management. The study design is updated every three years, allowing the principal investigators to refocus study questions based on the learnings from previous cycles. This monitoring program has thus far gathered four years of data investigating mechanisms of selenium speciation and bioaccumulation across an active mine, tracking seasonal patterns, and most recently, using piezometer-type standpipes to monitor in-pond compartmentalization of speciation behavior. It incorporates non-standard monitoring approaches that document annual variability while serving adaptive management through science.

4.01.B.T-02 Nitrogen source fingerprinting in the Hillsborough River using organic chemical tracers and multivariate analyses

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Excess nitrogen is a major contributor to water quality degradation in Florida's estuarine ecosystems, promoting algal blooms, seagrass loss, and ecological imbalance. While traditional monitoring identifies elevated nutrient concentrations, it often lacks source specificity. This study aimed to enhance nitrogen source apportionment in the Hillsborough River using nutrients, chemical tracers, isotopic, and multivariate analysis. Between May 2023 and January 2025, we conducted 21 sampling events across 23 sites, resulting in water samples ($n = 460$). Nutrients and a suite of organic chemical tracers were measured to identify inputs from wastewater (legacy and new), stormwater runoff, agriculture, and industrial activities. Total nitrogen (TN) concentrations at specific sites frequently exceeded the numeric nutrient criterion (1.01 mg/L) for the Hillsborough Bay, with dry season TN ranging from 0.26–1.49 mg/L and wet season from 0.21–1.69 mg/L. Other nitrogen species, including nitrate, nitrite, and ammonium, also exhibited seasonal variation and spatially elevated concentrations at several sites. Targeted chemical tracers included, but were not limited to, 6PPD-Q, DEET, and Prometon (stormwater); sucralose and carbamazepine (legacy wastewater); caffeine (new wastewater); atrazine and desisopropyl-atrazine (agriculture); and TCP and Irgarol (industrial/boating). Tracer concentrations ranged widely, with sucralose peaking at 22,670 ng/L in the wet season, suggesting strong influence from treated or reclaimed wastewater. Spearman rank correlations revealed significant associations between tracers and nitrogen species, such as 6PPD-Q with NH_3 ($r = 0.70$), caffeine with NH_3 ($r = 0.68$), and TCP with NH_3 ($r = 0.75$). Principal Component Analysis (PCA) further resolved source contributions in the wet season, where “new” and “legacy” wastewater tracers separated, and distinct site groupings emerged to aid hotspot identification. This work demonstrates how integrating targeted chemical tracers, isotopic analysis, and multivariate analysis can advance environmental monitoring efforts. The approach provides finer resolution of nitrogen sources across time and space, supporting more targeted nutrient reduction strategies and restoration planning in the Tampa Bay watershed.

4.01.B.T-03 Understanding pesticide dynamics in Alberta's irrigation water using complimentary sampling techniques

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Alberta's irrigation networks provide critical water for both crop and livestock production. For over 15 years the Irrigation District Water Quality (IDWQ) program has monitored irrigation water to examine long-term patterns of pesticide occurrence. Our lab has recently revamped the pesticide analysis methods and are currently

researching complimentary sampling approaches to strengthen the IDWQ program. In monthly grab samples at all 72 IDWQ sites from June to September, the herbicides atrazine and pyrooxasulfone, fungicides azoxystrobin, fluxapyroxad, and tebuconazole, and the insecticide chlorantraniliprole (of 91 current-use targets) were measured with >70% detection frequency in both 2023 and 2024. Overall detections were greater in 2023, with 47 pesticides having detection frequencies >10% compared to 32 in 2024. Average and median concentrations of all detected pesticides was 9.1 and 1.3 ng/L in 2023 (n=5504) and 25 and 0.28 ng/L in 2024 (n=4820), respectively. At six IDWQ sites, the diffusive gradients in thin films (DGT) passive samplers were co-deployed continuously for three week periods between May and October. Of the total pesticide detections made by grabs and passives, 36 were common between the two techniques. Compound detections unique to only one sampling technique had detection frequencies <10%. By pairing these two complimentary sampling tools, the known relationship between derived grab and passive sampling concentrations can be used to predict when a monthly grab sample may be capturing peak inputs, baseline levels, or missing an input event entirely. We observed systematically higher grab sample concentrations (4.8 ng/L average of all detections) compared to passives (0.91 ng/L) in June and to a lesser extent July (1.4 vs 0.66 ng/L) when pesticide use and precipitation events tend to be greatest. Over the growing season this concentration difference between the two techniques narrowed (Aug: 0.75 vs 0.61 ng/L; Sept: 0.19 vs 0.23 ng/L), coinciding with fewer expected inputs. Parsing individual compound data revealed when a grab sample had likely captured a peak input event (grab >> DGT concentration), baseline levels (grab $\approx$ DGT concentration), or missed an input event (grab << DGT concentration). This work provides a framework using monthly grab sampling and continuous passive sampling in a complimentary manner to greatly enhance our understanding of pesticide dynamics and ultimately inform more accurate risk assessment.

4.01.B.T-04 One extraction, dual instrumental analysis of pesticide suites in water and passive samplers: Multi-laboratory method validation and SOP development

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A new method enables the detection and quantification of over 100 pesticide analytes at trace levels in ambient water and passive sampler matrices (e.g., Chemcatcher disk). This multi-residue, multi-class screening method employs a single extraction process compatible with both GC/MS and LC/MS/MS, enabling broad pesticide analyte coverage, including organophosphates, pyrethroids, and neonicotinoids, with Log KOW values ranging from below 2 to 7. Building on existing USGS methods, we validated the approach in-house, conducted a multi-laboratory evaluation, and established EPA-style Standard Operating Procedures (SOPs). Multi-laboratory validation across three laboratories yielded Data Quality Objectives (DQOs), including recoveries (50–150% for laboratory control samples and matrix spike samples) and precision (Relative Standard Deviation of $\pm 30\%$ for aqueous matrices and $\pm 50\%$ for Chemcatcher disks). While some analytes performed poorly, as expected for a screening method not optimized for specific analyte sets, the SOPs provide clear DQO criteria, enabling laboratories to define in-house lists of suitable and unsuitable analytes based on performance data. This performance-based method can be adapted for other SPE and Chemcatcher stationary phases and modified instrumental analysis conditions, provided that DQOs are met. The method supports both instantaneous (aqueous phase) and time-weighted-average (Chemcatcher disk with calibration) pesticide concentration measurements, making it a valuable tool for environmental monitoring programs. As one of the first EPA-style methods applicable to passive sampler sequestration media, this approach addresses a critical need for innovative analytical techniques. Currently being incorporated into training programs for California's Environmental Laboratory Accreditation Program (ELAP) assessors, this method is set to support compliance monitoring and pesticide surveillance efforts across statewide environmental laboratories.

4.01.B.T-05 Low-cost technology alternative to monitor hydrophobic organic contaminant level in stormwater

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Hydrophobic organic contaminants (HOC) in stormwater runoffs can be a major source of total loading to waterbodies. Quantifying storm-driven inputs is critical for understanding HOC mass balance of impaired waterbodies and identification of ongoing sources from land. Extensive monitoring is however limited by the current tools available like automated samplers and their cost to monitor storm events. Here we propose a dual approach of passive sampling and sediment traps as cost-effective way to monitor HOC in the water column and suspended sediment during storm events. The approach was tested at the Lower Beaverdam Creek (LBC), MD, which was identified as a major source of ongoing PCB inputs to the Anacostia River. A novel short-term (48h) passive sampling tool was used, and several variations of passive sediment traps were tested and results used to optimize the design. The samplers were deployed downstream of a recycling facility that was identified as a PCB land source. Preliminary results showed that PCB level measured in water column during storm events reached levels at least 10 times higher than recommended levels by US EPA for a cancer risk of 10 in a million, and were similar to that measured with passive sampler deployed for 3 months. The absence of dilution effect suggests increased mobilization of PCB sources during the storm event. The similarity of PCB homolog profile between short-term and long-term, suggests that the contamination observed during the storm event originates from the same PCB source. PCB concentrations measured in the suspended sediments collected by the sediment traps were close to previously measured values using ISCO sampling and support the use of our optimized sediment traps as a low-cost alternative sampling technology

4.01.P-Tu Advancing Science Through Current Approaches in Environmental Monitoring

4.01.P-Tu-022 Investigating the elemental composition of magnetic nanoparticles in wildland-urban interface fire ashes

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This study investigates the magnetic separation and elemental composition of magnetic nanoparticles (MNPs) in eleven wildland-urban interface (WUI) fire ash samples, including one vegetation ash, six structural ashes, and four vehicle ashes, along with three fire-impacted soil samples collected after the 2020 North Complex (NC) Fire and the Sonoma-Lake-Napa Unit (LNU) Lightning Complex Fire in California. The efficiency of MNP separation was confirmed using time-domain nuclear magnetic resonance (TD-NMR) spectroscopy, which showed a significant decrease in relaxation rates (0.29 to 2.02 s^{-1}) due to the removal of magnetic particles. Single-particle inductively coupled plasma time-of-flight mass spectrometry (SP-ICP-TOF-MS) was employed to determine the particle number concentrations, size distributions, and elemental compositions of the MNPs. The predominant nanomaterials (NMs) identified in the magnetically separated fractions included Fe, Ti, Cr, Pb, Mn, and Zn. Iron-bearing NMs accounted for 3.2% to 83.5% of the MNPs, primarily in soils and vegetation ashes, while Ti-bearing NMs accounted for 3.3% to 66.1%, mainly in structural and vehicle ashes. Most NMs were detected as multi-metal (mm)NMs, suggesting their existence as heteroaggregates and/or due to sorption of metals onto the surfaces of nanomaterials (NMs) during combustion. Elemental ratios (e.g., Al/Fe, Ti/Fe, Cr/Fe, Zn/Fe) in the magnetically separated MNPs in structural and vehicle ashes were higher than those in soil and vegetation ashes, indicating enrichment of metals in magnetically separated NMs from vehicle and structural ash compared to vegetation ash. Overall, this study demonstrates that MNPs generated from WUI fire ash are associated with potentially toxic elements (e.g., Cr and Zn), highlighting their potential environmental and human health risks. These findings advance understanding of the nature and properties of fire-generated MNPs and emphasize the need for further research on their environmental fate, transport, and interactions with biological systems during and after WUI fires.

4.01.P-Tu-023 Atmospheric concentrations of per- and polyfluoroalkyl substances in Michigan's ambient air using passive sampling

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As a part of Michigan's efforts to identify and address per- and polyfluoroalkyl substances (PFAS) contamination statewide, more knowledge of atmospheric PFAS concentrations is needed to better understand their atmospheric transport and impact on other environmental media. This research aimed to measure atmospheric concentrations of PFAS in Michigan's ambient air using low-cost and easy-to-use passive samplers, identify relationships with environmental factors, and increase understanding of their gas-particle partitioning. Passive samplers, consisting of polyurethane foam (PUF) discs and sorbent-filled polyethylene tubes (radiello-XAD samplers), were deployed for a month at 27 sites across Michigan and analyzed for both ionic and volatile, neutral PFAS. While previous studies have at times reported inaccurate concentrations of short chain perfluorocarboxylic acids (PFCAs) in air and biological samples, mainly due to matrix interferences, this study achieved accurate quantification through the use of multiple analytical methods and high-resolution mass spectrometry (HRMS) data. PFCAs, specifically perfluoropropanoic acid (PFPrA) (d.f. 60 %), perfluorobutanoic acid (PFBA) (d.f. 96 %), and perfluorooctane sulfonic acid (PFOS) (d.f. 63 %) were the most frequently detected compounds, with the Σ 3PFAS ranging from non-detect to 15.5 pg/m³ for the PUFs and from non-detect to 248 pg/m³ in the radiello-XAD samplers. 6:2 fluorotelomer alcohol was also frequently detected (d.f. 45 %) and had a positive correlation with both population density ($r=.52$, $p<.05$) and industrial sites' density ($r=.48$, $p<.05$). Detection of specific compounds on each type of sampler provided insight into the preferential transport pathway of atmospheric PFAS. For example, PFOS was observed mostly in the gas-phase, predominantly captured by the radiello-XAD samplers, while PFBA was mostly in the particle-phase, predominantly captured by the PUFs. This study highlights the importance of developing detection tools for measuring atmospheric PFAS across a vast geographic area to identify contributing factors to ambient concentrations.

4.01.P-Tu-024 Advancing the state of algal bloom science via western science and indigenous knowledge: A Moose Lake case study

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Since 2016, the Moose (Gardiner) Lake water quality monitoring program has supported community-driven environmental stewardship through braiding western science and Indigenous knowledge. Initiated in response to rising community concerns about algal blooms, the program has evolved into not only assessing long-term water quality trends and identifying drivers of bloom events but also advancing the state of science regarding nutrient loading and algal bloom assessment. The monitoring program, which initially focused solely on water quality, has evolved to include real-time measurements of thermal stratification (via data sondes), algal taxonomy, cyanotoxins and satellite-based remote sensing. This information coupled with indigenous knowledge of both the area and the historical changes in that area have led to a more robust understanding of the Moose Lake watershed. Notably, phosphorus, chlorophyll a, cyanobacteria, and microcystin levels peaked during the 2021–2022 heat dome (but have since declined). These reported findings were confirmed by the community and remote sensing in the area. As such, this monitoring program has not only advanced the state of science regarding algal blooms but also provides tangible findings into the growing effects on climate change on the lake. By aligning community priorities with scientific tools, and training and employing community members in water monitoring, the program exemplifies how integrated knowledge systems can respond to changing environmental conditions and help protect culturally and ecologically significant waters.

4.01.P-Tu-025 Vegetative sampling of airborne toxics: Green analytical protocols to measure PAHs and nitro-PAHs

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Polycyclic aromatic hydrocarbons (PAHs) are a large group of compounds distinguished by their many chemical structures, multiple sources, ubiquitousness, toxicity, difficulty and expense in monitoring. PAHs can undergo atmospheric reactions and form more toxic compounds, including nitro polycyclic aromatic hydrocarbons (NPAHs). These lipophilic and semi-volatile organic compounds (SVOCs) are adsorbed into the

waxes and oils of pine needles, which can serve as passive samplers, potentially providing a cost-effective and flexible monitoring approach that shows spatial distribution and temporal trends. Such sampling also allows long-term measurements and applications in remote locations where the deployment of conventional samplers is challenging. This study aims to detect and quantify a wide range of PAHs and 8 NPAHs in pine needles from *Pinus strobus*, a native species to eastern North America, using a "green" analytical approach that reduces the quantity of solvents and disposable materials. Sample preparation included freeze drying and sonication; cleanup included adsorbents to remove waxes and pigments; and analysis used gas chromatography/mass spectrometry (QqQ) with 16 deuterated internal standards for PAHs and 2 deuterated internal standards for NPAHs. Method optimization to address separation, recovery, and detection limits included tests of various extraction solvents, times, different adsorbents for cleanup (e.g., Florisil, alumina), and other parameters. During method development, several compounds tended to volatilize (e.g., naphthalene); others (e.g., benzo[e]pyrene, benzo[a]pyrene, and some dibenzopyrenes) were degraded or retained with Florisil cartridges. Ultimately, we were able to quantify 27 PAHs using alumina cartridges with solvent use reduced several folds from previously published methods. The method was applied to late winter samples collected at 27 sites in rural, urban, and industrial sites in two consecutive years. Elevated concentrations were noted at locations where high levels of other persistent organic pollutants had been measured, showing the influence of both local and regional sources. The use of natural passive samplers and the minimum use of disposable material and solvents make this approach an excellent tool for green analytical chemistry to measure PAHs and other air contaminants. **Disclaimer/Disclosure:** NIH grant number T32 ES007062

4.01.P-Tu-026 Integrating CO₂ Dynamics and monitoring tools to understand carbonate mineral behavior in mine-influenced waters

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Carbonate mineral dissolution and precipitation in mine-influenced surface waters are fundamentally governed by the geochemical behavior of carbon dioxide (CO₂), which regulates pH and carbonate equilibria through acid-base reactions. These processes are critical in shaping the formation or inhibition of calcite (CaCO₃), a common mineral that can accumulate in aquatic systems and mining infrastructure. The presence and transformation of CO₂—whether from atmospheric exchange, microbial respiration, or geochemical reactions—directly influence the saturation state of calcium carbonate, thereby controlling calcite dissolution or precipitation. Calcite precipitation can significantly impact streambed integrity, aquatic habitat quality, and operational systems through scaling. While chemical or operational strategies are often employed to manage these impacts, optimizing such interventions within routine environmental monitoring frameworks remains challenging due to the complexity and variability of site-specific geochemical conditions. To address this, a conceptual model was developed to better understand the role of CO₂ in carbonate system behavior, supported by a monitoring approach that integrates standardized industrial corrosion coupons and saturation indices. These industrial coupons, designed with consistent geometry and surface area, provide a direct, empirical measure of calcite accumulation under ambient field conditions, offering insight into real-time mineral deposition. In parallel, saturation indices, such as the Langelier Saturation Index (LSI) and Calcium Carbonate Precipitation Potential (CCPP), are calculated from routine water chemistry data to assess the potential for calcite precipitation or dissolution. These indices, influenced by pH, temperature, calcium concentration, alkalinity, and total dissolved solids, help predict whether conditions favor scaling or dissolution. Together, these tools offer a complementary framework for interpreting carbonate system dynamics and evaluating the effectiveness of mitigation strategies. This integrated approach enhances the resolution and interpretability of calcite-related processes within existing monitoring programs, supports adaptive management of carbonate control measures, and informs broader environmental risk mitigation strategies. The conceptual model will be presented to illustrate the practical application of this integrated approach within a mine-influenced watershed.

4.01.P-Tu-027 Mapping temporal trends of pesticide use and identifying potential non-occupation population exposure using a geospatial approach

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Abstract Background: Estimating pesticide exposure in environmental settings is complex due to limited data, evolving agricultural practices, and increasing use driven by weed resistance and genetically engineered crops. One of the most widely used herbicides, 2,4-dichlorophenoxy acetic acid (2,4-D), is a concern for populations near agricultural fields due to its rapidly increasing use. **Objectives:** We used a geospatial approach to show spatio-temporal trends of pesticide use and identify at-risk populations for non-occupational pesticide exposure. **Methods:** 2,4-D application on soybeans in Illinois during the years 2017, 2020 and 2023 were used. We calculated the rate of change on the county level using reported 2,4-D use and soybean crop area. Then we created a crop area, pesticide density buffer model using 1 km buffer zones correlated with 1000m x 1000m gridded census data to identify where populations were at risk of non-occupational exposure in Champaign County. **Results:** Between 2017 and 2023, there was a median increase of 341% in 2,4-D application on soybeans in each county in Illinois. We found that 98.9-99.7% of the population of Champaign County lived within 1 km of at least .04 km² (10 acres) of soybean crops from 2017 to 2023. Using 4.4 kg of 2,4-D as a threshold within the 1km buffer zone, 24.5% of the population was potentially exposed in 2017, this increased to 44.5% of the population in 2023. In 2017, the area where the most 2,4-D (30 kg) was applied to soybean crops represented 0.01% (14 people) of the population potentially exposed. Using 30 kg as a reference point, in 2023, 20.2% (~47,000 people) of the population were at potential risk of this level of exposure. **Conclusions:** Previous research has shown increasing 2,4-D agricultural use has been associated with increased 2,4-D physiological levels. We mapped at-risk populations for 2,4-D exposure due to 2,4-D application on soybeans that has increased from 2017 and 2023 using GIS. This is a cost-effective method that identifies areas to focus further studies.

4.01.P-Tu-028 Contaminants of emerging concern in San Francisco Bay: A strategy for future investigations

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Since 2001, the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) has monitored contaminants of emerging concern (CECs), unregulated or underregulated contaminants that are not commonly monitored in environmental media, and which have the potential to enter the environment and cause adverse ecological or human health impacts. The recently revised RMP CEC Strategy consists of four major elements. First, for those CECs known to occur in the Bay, the RMP establishes priorities using a tiered risk-based framework. This framework guides future monitoring studies for these contaminants. Consideration of broader chemical or functional classes is an integral approach responsive to both manufacturing substitutions, market shifts, and data limitations. Second, the RMP focuses on monitoring and modeling in contaminant pathways (i.e., municipal wastewater and urban stormwater runoff), in an effort to track Bay contaminants back to their sources and better inform management. This element recognizes the unique characteristics of pathways, including higher contaminant concentrations that can enable more reliable and timely observations of temporal trends and the appearance of new contaminants, and the ability to tie contaminants more directly to potential sources. Third, the RMP uses novel approaches to identify additional CECs, including nontarget analysis and new approach methodologies (NAMs) for hazard assessment. Fourth, RMP staff review the scientific literature and engage with scientists around the world as a means of identifying new contaminants for which no Bay occurrence data yet exist. Studies of these newly-identified CECs may be needed to evaluate the risks posed to the Bay. The RMP's multi-faceted approach to addressing the challenge of CECs is designed to be flexible and adaptive to new data from both the RMP and other sources. Based on the Strategy, a multi-year plan indicating monitoring and science priorities is updated annually, in service of the overarching goal to develop cost-effective CECs science in support of management efforts to minimize impacts to the Bay.

4.01.P-Tu-029 Application of passive sampling for time-integrated measurement of per- and polyfluoroalkyl substances in aquatic and sediment porewater systems using the Passivex™ sampler
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Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants frequently detected in groundwater, surface water, stormwater, and sediment porewater. Traditional discrete sampling methods may underrepresent temporal variability and require high sample volumes and handling efforts. Passive sampling offers a time-integrated, low-maintenance alternative. This work evaluates the performance and utility of the Passivex™ passive sampler, a small integrative device containing modified organosilica sorbent enclosed in open mesh, for quantifying PFAS in diverse aqueous environments. The sampler, calibrated under controlled laboratory conditions for groundwater and surface water application, has detection limits of 1–2 ng/L, with relative standard deviations between 15–30% across replicate samplers. Validation studies demonstrated linear uptake of target compounds over typical field deployment durations (3–45 days), with field results showing strong correlation with conventional grab samples analyzed via modified United States Environmental Protection Agency Methods 537.1 and 1633. Uptake of PFAS onto the sampler was found to be dependent on flow rate and temperature, but is largely unaffected by variations in ionic strength, pH, and dissolved organic carbon concentrations. Current research extends this application to sediment porewater monitoring under the Environmental Security Technology Certification Program. Laboratory static tank and flowing packed-column experiments are being conducted to characterize PFAS uptake in sediment matrices with differing grain size distributions, organic carbon content, and salinity. A custom stainless-steel deployment stake was developed to enable vertical profiling of sediment porewater to 50 centimeters, permitting spatially resolved sampling and replicate comparison. Field demonstration will compare sampler-derived concentrations to concentrations in co-located porewater and sediment samples and assess precision, agreement and bias. This presentation will include evaluation of passive sampling results for PFAS in both water columns and sediment matrices. By providing time-integrative data, facilitating flux estimation, and reducing logistical burdens, the method supports more representative monitoring of PFAS transport and bioavailability in complex hydrological settings. Findings will inform field deployment guidance and best practices for environmental investigation and remedial performance monitoring

4.01.P-Tu-030 Trace-element particle characterization in moss samples used as biomonitors of air emissions using single particle inductively coupled plasma time of flight mass spectrometry (spICP-TOF-MS)

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Epiphytic mosses are often used as biomonitors of air pollutant deposition in terrestrial environments, including for trace elements. While deposition of particulates on mosses has long thought to be of importance, particularly for trace-elements, few studies have characterized the size distribution and composition of particulates from moss samples. We characterized trace element containing particulates in mosses exposed to emissions from a waste incinerator and a landfill receiving combustion waste from the incinerator in the Willamette Valley, Oregon, USA using novel methods. Thirty representative samples from each source were selected based on their distance from the incinerator and landfill and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) for 23 trace elements. We then extracted particulates and fractionated them using glass fiber filters by size into <10 µm, <2.5 µm, and <1 µm fractions. The < 1 µm fractions were further characterized for particle number and particle composition using single-particle time-of-flight ICP-MS (sp-ICP-TOF-MS).

Approximately 16,000 particle types were identified; 19 particle types which were counted at least 50 times in at least one sample were retained for further analysis. All 19 types contained Pb alone or in combination with other elements, except for particles containing only Ag. Particle counts were significantly higher for most types near the incinerator, except particles containing Co, Mn, and Pb, which had higher particle counts near the

landfill. While particle mass distributions were similar between the incinerator and landfill sites, suggesting that particles from the landfill site originated from incinerator waste disposed there, the mass percentage of elements differed for some of the particle types (Co/Mn/Pb, and Mn/ Pb particles, which had higher percentages of Pb near the incinerator relative to the landfill sites. Samples from sites that were more distant from these sources (>4.37 km) generally had <1% of the maximum particle count for most particle types, and <8% for Ag particles. This is the first study to provide detailed characterization of the composition and particle size distribution of metals in moss biomonitoring samples and the first application of sp-ICP-TOF-MS to these samples. These tools can add another dimension to moss biomonitoring data, contributing to a better understanding of pollutant sources, deposition mechanisms, and implications for human health. **Disclaimer/Disclosure:** This work was supported by the United States Forest Service (USFS) under joint venture agreement 22-JV-11261979-053. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and should not be construed to represent any official USFS or U.S. Government determination or policy.

4.01.P-Tu-031 Sampling of various air matrices from per- and poly-fluorinated alkyl substance (PFAS) destruction systems

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Evaluating the effectiveness of remediation of per- and polyfluoroalkyl substances (PFAS) containing matrices relies, in part, on the evaluation of any gas, vapor, or aerosol phase emissions from the remediation process to complete the fluorine mass balance. Herein we describe the air sampling techniques and analyses used to quantify air-borne PFAS and PFAS degradants from bench, pilot, and production scale remediation systems. The methods employed include extractive gas-phase Fourier transform infrared (FTIR) spectrometry, passivated canister sampling for gas-chromatography-mass spectrometry (GC/MS), liquid impinger/adsorbent resin sampling for liquid chromatography-mass spectrometry (LC/MS), and others. We demonstrate that each of these methods is important to investigating air emissions of fluorine-containing compounds across a varied range of volatility and reactivity to identify and quantify compounds specific to the destruction technique and PFAS-containing matrix.

4.01.P-Tu-033 Moss biomonitoring in southeast Portland to verify the effectiveness of mitigation efforts

Dean Atkinson and Oluwaseyi Ebinoluwa Aderinboye, Portland State University

Epiphytic mosses are important indicators of atmospheric metal deposition and pollution. In this study, the cadmium concentration in *Orthotrichum lyellii* an urban growing epiphytic moss was determined around a previously identified Cd source in southeast Portland after mitigation efforts by the company. The results of this study were compared to a prior study by the United States Forest Service (USFS) published in 2016. Moss samples were collected from the previously sampled locations. Twenty-three moss samples were collected and analyzed using an inductively coupled plasma mass spectrometer. From the results obtained, maps were generated using ArcGIS Pro to represent the results spatially. Contamination factors were calculated to assess the level of pollution. The mean Cd concentration in moss was 0.39 mg/kg with a range of 0.10 to 1.72 mg/kg. The mean contamination factor for the affected sites was 2.6, with a maximum of 11.4. The results of this study show a 70% decrease in concentration and contamination of Cd in the moss samples, as expected from a decrease in Cd emissions following the installation of the pollution mitigation technology (baghouse).

4.01.P-Tu-034 Integrating silicone wristbands and polyurethane foam passive samplers for semivolatile organic compound monitoring in rural communities exposed to mixed-fuel combustion emissions

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Communities located near mixed-fuel combustion facilities face potential exposure to complex mixtures of air pollutants including volatile and semivolatile organic compounds (VOCs and SVOCs), inorganic gases, metals, and particles. We implemented a dual-method passive sampling approach to characterize VOC and SVOC exposure in two communities in Michigan's Upper Peninsula: Baraga, located near a wood, paper and tire-

derived fuels combustion facility, and Houghton, a background site. During late summer (July–August) and fall (October–November) 2024 sampling campaigns, we enrolled 17 participants, including 10 who participated in both sampling rounds. For each participant, three silicone wristbands were deployed for 7-day periods—one worn on the wrist, one placed inside the participant’s residence, and one placed outdoors of the residence. Polyurethane foam passive air samplers (PUF–PAS) deployed for 28 days were co-located with wristbands indoors and outdoors. Target SVOC analytes include polycyclic aromatic hydrocarbons, dioxins and furans, phthalates, flame retardants, and pesticides. NO₂, NO_x, and ozone were also monitored over 28 days using passive samplers, and PM_{2.5} was measured using low-cost sensors. This integrated sampling design enables comparison of personal and environmental exposures between residences and sample type. The influences of wind speed and direction and seasonal changes in home heating and ventilation on exposure to VOCs and SVOCs are assessed.

4.01.P-Tu-035 Assessing bioavailability and benthic bioaccumulation of per-/polyfluoroalkyl substances (PFASs) From marine sediments using ex-situ passive sampling techniques

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Sediments are a key route by which benthic organisms are exposed to per/polyfluoroalkyl substances (PFASs), potentially affecting organisms higher in the food web. However, quantifying the bioavailability of sediment-bound PFASs remains a complex task, as it is influenced by sediment properties, water chemistry, and contaminant sorption dynamics. Passive sampling devices (PSDs) are a promising technique to evaluate the bioavailable fraction of PFASs in sediment-porewater systems by simulating natural passive uptake mechanisms. This study investigates the use of polyacrylate-coated solid-phase microextraction (SPME) fibers as ex-situ PSDs in marine sediment-seawater environments, aiming to selectively extract bioavailable PFASs. We assess the capacity of PA-SPME fibers to capture a broad spectrum of PFASs, determine equilibration times, and calculate sampler-to-water partition coefficients (K_{sw}) under environmentally relevant conditions. The role of environmental parameters like organic matter content is examined to understand influence on PFAS bioavailability. Additionally, we compare the performance of the ex-situ sampler against tissue burdens in marine polychaetes exposed to similarly spiked sediments to evaluate predictive accuracy. Preliminary results demonstrate that equilibrium for all 15 PFASs is achieved within 48 hr in aqueous media, with K_{sw} values increasing with compound chain length. These findings demonstrate that PA-SPME fibers effectively accumulate both short- and long-chain PFASs. Results comparing PSD and benthic uptake from sediments have shown differential PFAS accumulation profiles between PSDs and marine polychaetes. There is a chain-length dependence observed for the accumulation of targets in both the worms and the PSDs. In worms, longer-chain PFAAs dominate the accumulation profile, whereas in the PSDs the largest contributions are from ether-containing, cyclic, and Cl-substituted isomers. PFAS accumulation in biota and passive samplers is governed by a combination of chain length-dependent hydrophobicity and matrix-selective sorption, where biological tissues preferentially accumulate long-chain perfluoroalkyl acids (PFAAs), while passive samplers exhibit enhanced affinity for more structurally complex or polar PFASs such as ether-containing and Cl-substituted isomers. This research enhances our understanding of PFAS behavior in marine environments and supports improved ecological risk assessment and management strategies.

4.01.V Advancing Science Through Current Approaches in Environmental Monitoring

4.01.V-01 Development of a simultaneous analysis of brominated flame retardants by using GC-MS/MS and temporal trend of the contamination recorded in a sediment core sample from Beppu Bay, southwest of Japan

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Marine sediment acts as a sink and reservoir for persistent organic pollutants (POPs). Dated marine sediment core samples, analyzed for their depositional age using varve-based chronology supported by lead isotopes (Pb-210), can be used to assess temporal trends in POPs pollution. Polybrominated diphenyl ethers (PBDEs) were previously used as flame retardants in textiles, plastics, electronic devices, home appliances and toys. However, they have been restricted by the Stockholm Convention since 2009 (tetra- to hepta-BDEs) and 2017 (deca-BDE). Bistribromophenoxyethane (BTBPE), an alternative to octa-BDE, has been detected in sediments around industrial areas in Korea and in river sediments in EU countries. However, few investigations have been reported on coastal sediments in Japan, and information on temporal trends in contamination is particularly lacking. In this study, we improved on the analytical method used in previous studies, establishing a new, simultaneous analytical technique for measuring PBDEs and four alternative brominated flame retardants (aBFRs), including BTBPE and decabromodiphenylethane (DBDPE), using a GC-MS/MS. We also conducted a temporal trend analysis of the environmental flux of PBDEs and aBFRs using sediment core samples collected from Beppu Bay in southern Japan. We confirmed the acceptable sensitivities of all the target compounds, including PBDEs and aBFRs such as BTBPE and DBDPE, in GC-MS/MS analysis (EI-MRM mode). We also confirmed their good recoveries in the recovery test of standard substances spiked into sediment samples and treated using a new clean-up method with Supelclean SPE (ENVI-CarbII/PSA). Analytical results of sediment core samples from Beppu Bay revealed the presence of BTBPE in sediment layers dating from the late 1990s to the early 2000s. This reflects the use of BTBPE as an alternative to octa-BDE flame retardants. Additionally, DBDPE, used by replacement for deca-BDE formulations, showed an increasing environmental load after the year 2000s. Despite the presence of BTBPE and the increasing concentration of DBDPE in recent sediment layers, the concentrations and fluxes of these aBFRs were several orders of magnitude lower than those of deca-BDE. This suggests deca-BDE formulations have continued to exert an environmental load in recent years. Further studies are necessary to clarify the distribution of aBFRs in the aquatic environment and the temporal trends of contamination.

4.01.V-02 The temporal trend of the POPs and new alternative flame-retardant contamination recorded in a sediment core sample from Beppu Bay, southwest of Japan

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Marine sediments acts as a sink and reservoir for persistent organic pollutants (POPs). Analyzing vertical distribution of POPs in the dated sediment core samples allows for evaluation of their temporal trends of contamination. The depocentre of the inner part of Beppu Bay in southwestern Japan has been proposed for a candidate site for the Anthropocene Global boundary Stratotype Section and Point (GSSP). Our previous studies of sediment core samples from Beppu Bay investigated temporal trends in POP contamination in sediment layers dating from the early 1950s to the early 2010s. However, trends from the 2010s to the present day, as well as those from before 1950, have yet to be clarified. In this study, we used newly collected sediment core samples from Beppu Bay for measuring PCBs and brominated flame retardants (BFRs), and evaluated the temporal trends of their contamination, including sedimentary layers dated from before the 1950s and from the 2010s onwards. The depositional age of each sediment layer sample was dated chronologically based varve-based chronology supported by lead isotopes (Pb-210). We measured the concentrations of all PCBs isomers by a GC- HRMS (EI-SIM mode), 37 congeners of polybrominated diphenyl ethels (PBDEs), and four new alternative brominated flame retardants (aBFRs) by a GC-MS/MS (EI-MRM mode) in the sediment samples. The analytical results of PCBs and PBDEs in the sediment core samples revealed temporal trends in the concentrations and fluxes of these compounds in relation to the regulations in Japan. Although the fluxes of major PCB congeners peaked in sediment layers dated to the 1960s, certain PCB congeners (e.g. CB-11, CB-47/48/75 and CB-51), which have been reported as occurring as by-products in pigments, paints and sealants, as well as being produced unintentionally in combustion processes, have shown an increasing trend in their fluxes in recent years. This indicates a possible continuous input of PCBs into the environment. In case of BFRs, BDE-209 is the dominant congener of PBDEs, and its flux showed continuous environmental load in recent

years. In addition, aBFRs such as Decabromodiphenylethane (DBDPE) revealed an significant increasing trend of the flux, suggesting continuous increasing environmental input in future. Continuous monitoring of POPs and related substances as well as their source apportionment are necessary for implementation of effective management on these compounds.

4.02.P-We Automation of Methods for Environmental Contaminants: Per- and Polyfluoroalkyl Substances (PFAS), Dioxins, Polychlorinated Biphenyls (PCBs), Pesticides and Beyond

4.02.P-We-039 StayClean™ ion source liquid chromatography tandem mass spectrometry for determination of over 30 antibiotics and veterinary drugs in milk

Sheng-Suan (Victor) Cai and Jacob Jalali, PerkinElmer Inc. (US)

The objective of this work was to develop methods for the analysis of antibiotics and veterinary drugs in milk listed by Food Safety and Standards Authority of India (FSSAI) using a Liquid Chromatography Tandem Mass spectrometry (LC-MS/MS) system with a StayClean™ ion source. During our method development, we found that a single extraction method by protein precipitation gave poor and irreproducible spiked recoveries for polar analytes due to varying water content in milk from batch to batch. To improve spiked recoveries and better method performance, two separate methods were developed for the analysis of over 30 analytes in milk due to their different polarities. For polar analytes, a mixed mode liquid chromatography (LC) method was used for analyte separation and a trichloroacetic acid/acetonitrile mixed solution was used for protein precipitation. For other less polar analytes, a reversed phase LC method was used for analyte separation and an acidified acetonitrile extraction was applied for sample preparation. For both methods, EDTA was used to reduce analyte-metal (i.e., calcium) ion complex formation and dispersive solid phase extraction (d-SPE) was performed to remove fatty matrix using end-capped C18 sorbent. To reduce matrix effects, matrix-matched calibration with isotopic mass dilution method was performed. Both methods were validated by spiking different concentrations of analytes in a milk sample matrix. The recoveries of most analytes are between 70 to 120%. The results demonstrated that all the analytes could be determined with limits of quantification (LOQs) below the tolerance limits set by FSSAI. In addition, StayClean™ ion source allows continuing injections of large amounts of “dirty” samples with 476ly no instrument cleaning and maintenance. An instrument robust test result dataset with continuing injections of 25,500 samples will be presented. This methodology can be extended to analysis of similar analytes in milk and milk products regulated by other governmental agencies.

4.02.P-We-040 Rapid leaching test method for soil and waste using online solid phase extraction/LC-QTOF

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In Japan, when measuring PFOS/PFOA in soil, the content is extracted with ammonia-containing methanol, and the leaching amount is extracted with water. When conducting a leaching test with water, the provisional measurement method in Japan requires 6 hr of shaking. In this study, we used the online solid-phase extraction/LC-QTOF method, which can analyze PFAS with high sensitivity in small volume sample, to confirm the relationship between shaking time and leaching concentration. In the soil elution test, the eluted concentration reached equilibrium within 1 hr after shaking began and remained nearly constant until 6 hr later. Additionally, it has been confirmed that for many compounds, more than 50% of the equilibrium concentration can be dissolved by simply adding water and stirring for 10 seconds. Next, when conducting leaching tests on waste materials such as incineration ash and sludge, it was confirmed that the leaching amount increased with the passage of shaking time for many compounds. On the other hand, in some waste materials such as carpets, the concentration of long-chain compounds (e.g. PFOS) was highest immediately after the start of the leaching

test and decreased over time. Since leaching behavior varies greatly depending on the type of soil and waste and the compound, it seems difficult to set a satisfactory leaching test time for all targets and compounds. Therefore, we think that in the official method, it would be better to change the elution test time for short-chain compounds and long-chain compounds, at least for waste materials. However, even for waste materials with large concentration differences, no concentration change of more than 5-fold was confirmed when comparing the results of short-term and long-term leaching, so we have decided to use the elution procedure in a short time in this rapid test method. High concentrations of PFAS have been detected in the leachate in many waste landfill sites in Japan, but which waste is the source of contamination is not clear. In the next step, we will try to identify the source of contamination by measuring various types of waste using this rapid leaching test method. While conventional methods require a lot of time and cost, this method allows for a large number of measurements to be taken with low effort and cost, making it a very effective way to identify the source of contamination. **Disclaimer/Disclosure:** This research was supported by the Environment Research and Technology Development Fund [3-2303(1) (3):JPMEERF20233003] of the Environmental Restoration and Conservation Agency (ERCA) of Japan.

4.02.P-We-041 Real-time visualization of PFAS binding using electrochemiluminescence and molecularly imprinted polymers

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants with potential health risks, making their rapid and selective detection a priority. This work presents a novel sensing strategy that combines electrochemiluminescence (ECL) with molecularly imprinted polymers (MIPs) to enable real-time visualization of PFAS binding. An indium tin oxide (ITO) electrode, optionally coated with platinum, is used as the sensing substrate. The MIP layer is imprinted for perfluorooctanoic acid (PFOA), and its selective binding is monitored through simultaneous changes in both ECL intensity and electrochemical current. This dual-mode detection links optical and electrochemical signals, providing more robust readouts and deeper insight into molecular interactions. The integration of ECL with MIP-based recognition supports the development of sensitive, portable, and field-deployable sensors for PFAS monitoring in environmental systems.

4.02.P-We-042 Routine EPA 1633 PFAS analysis with a novel slotted bandpass ion guide to improve signal response robustness

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There is a growing demand for PFAS analysis in environmental samples (both liquid and solid) with EPA 1633 becoming the foundation method for this type of analysis. This gives associated analytical method performance criteria and there is a need for a robust tandem quadrupole mass spectrometer to deal with the variety of challenging matrix types for this methodology. Even though EPA 1633 describes a solid phase extraction (SPE) stage for all sample types, these matrices, at the point of introduction into an LC-MS/MS system, are far from "clean". This work presents how a new novel slotted bandpass ion guide combined with a high sensitivity tandem quadrupole mass spectrometer can improve signal robustness and increase method reliability. The extraction and SPE method as detailed in EPA 1633 was followed for the sample extraction of domestic sludge (5g), landfill leachate (50mL) and environmental water (50mL). A weak anion exchange (WAX) SPE cartridge with graphitized carbon black (GCB) was used for clean-up of extracts. An aliquot of the eluent from the SPE cartridge was pH adjusted (pH<5) with formic acid and transferred to a polypropylene vial. This was injected onto a high-performance tandem quadrupole mass spectrometer, with compact design, enhanced negative ion detection and fitted with a novel slotted bandpass ion guide. Challenging environmental matrices were selected to test the novel slotted bandpass ion guide over one month of continuous operation, running the equivalent of 100 batches (20 samples per batch) of mixed sample types. The novel slotted bandpass ion guide filters out high m/z ions, preventing accumulation of these ions on the MS1 quadrupole rods. Minimum Level of Quantitation

(ML) for the first 8 batches was calculated for all analytes with 0.2 ng/L (PFHxA), 0.18 ng/L (PFBS), 0.40 ng/L (GenX), 0.20 ng/L (FOSA), 2 ng/L (NMeFOSE), 4 ng/L (3:3 FTCA) and 0.4 ng/L (PFMPA) being achieved for the various PFAS analyte classes. This was maintained over the 8 initial batches that were run. Ion ratio stability was calculated, and all values were within the required tolerance for the EPA 1633 method of $\pm 50\%$ of the mid-point calibration value of the calibration bracket run. This research demonstrates enhanced robustness of a tandem quadrupole mass spectrometer with a novel slotted bandpass ion guide for PFAS analysis.

Disclaimer/Disclosure: Authors are employees of Waters Corporation.

4.02.T Automation of Methods for Environmental Contaminants: Per- and Polyfluoroalkyl Substances (PFAS), Dioxins, Polychlorinated Biphenyls (PCBs), Pesticides and Beyond

4.02.T-01 Dual electrospray ionization/atmospheric pressure chemical ionization StayClean™ ion source LC-MS/MS for analysis of near 400 polar and non-polar pesticide residues in tea

Sheng-Suan (Victor) Cai and Jacob Jalali, PerkinElmer Inc. (US)

Pesticides residues are commonly found in teas, and demand for analytical detection is increasing as global tea consumption increases. We developed a robust analytical method that enables almost 400 both polar and non-polar pesticides to be analyzed under electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) utilizing a dual source liquid chromatography coupled with a tandem triple quadrupole mass spectrometer (LC-MS/MS). This method eliminates the need for gas chromatography tandem mass spectrometry (GC-MS/MS) analysis by analyzing a list of 50 pesticides using APCI. Experiments were carried out using an ultrahigh performance liquid chromatography tandem mass spectrometer (UHPLC-MS/MS) via a diverter valve. A simple solvent extraction sample prep procedure with internal standard dilution was employed. Matrix effects were evaluated using black tea, chamomile, and herbal blend. In total, 690 multi-reaction monitoring (MRM) transitions were monitored for 345 compounds in the ESI method, and 100 MRM transitions were monitored for 50 compounds in the APCI method. A single C18 column was used for both LC methods. In this work, we demonstrate how a practical simple solvent extraction can be utilized to achieve the analysis of a large multi residue panel of pesticides, with low limits of detection (with 319 of 395 analytes displaying limits of quantification LOQs of 5 ng/g or below), minimal matrix effects (94% of compounds with negligible percent signal suppression or enhancement in black tea), and excellent method performance (93% of compounds with accuracies within 70 to 120% and %RSD < 20). Dual Source design allows for APCI analysis on traditionally GC only compounds to be included in this LC pesticide panel, reducing instrument workload, and potentially eliminating the need for complementary GC-MS/MS protocol for multi residue pesticide quantification. StayClean™ patent technology allows direct injection of sample extract without cleanup and without frequent instrument cleaning and maintenance. Simple sample preparation and reliable instrumentation allows for a robust analytical solution with the potential to analyze hundreds of pesticides in many different matrices.

4.02.T-02 Dynamics of organophosphate esters in the atmospheric matrix of Great Lakes region using enhanced ionization technique

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Following the phase-out of polybrominated diphenyl ethers (PBDEs), organophosphate esters (OPEs) have seen increased use as flame retardants and plasticizers in a variety of products. Due to their non-covalent bonding to polymeric structures, they are prone to release into the environment. The goal of this study is to present the spatial and temporal trends OPEs across the Great Lakes sampling regions. In total, 41 OPEs were analyzed in gas and particulate phase samples collected in 2023 at the sites around the Great Lakes as part of the Integrated Atmospheric Deposition Network (IADN). These sites range from urban (i.e. Chicago and Cleveland, OH), to rural (Sturgeon Point, NY and Point Petre, ON, Canada), to remote (Eagle Harbor, MI, and Sleeping Bear Dunes, MI). Alkyl OPEs were the most abundant (60%), followed by chlorinated (15%) and aryl (25%) OPEs.

Sampling site characteristics significantly influenced OPE concentrations: one of remote sites, Eagle Harbor, had an average concentration of 137 pg/m³ (particle+ gas phase), whereas Chicago reached 665 pg/m³. Several OPE compounds, Isodecyl Diphenyl Phosphate (IDP, n=144), 4-tert-Butylphenyl diphenyl Phosphate (4tBPDPP, n=110), Tetrakis(2-Chloroethyl)dichloroisopentyl Disphosphate (V6, n=71), Resorcinol bis(Diphenyl Phosphate) (RDP, n=75) showed detection frequencies exceeding 50 % across all sampling sites, highlighting their emerging presence compared to previous atmospheric studies conducted by our laboratory. Additionally, OPEs show a declining trend from 2016 to 2023. The concentration of the chlorinated OPE TCEP in Eagle Harbor was 41 pg/m³ in the 2016 study, 9 pg/m³ in 2019 and 2 pg/m³ in the most recent study. In contrast, at the urban site of Chicago, TCEP concentration decreased from 334 pg/m³ in 2016 to 106 pg/m³ in 2019 and 12 pg/m³ in the latest study.

4.02.T-03 The FluoroMatch multimodal PFAS atlas; high-resolution libraries covering gas chromatography, liquid chromatography and ion mobility

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While certain Per- and Polyfluoroalkyl Substances (PFAS) are persistent, bioaccumulate, and have been linked to various health effects, little is known about the exposure to the wide range of emerging PFAS chemicals. Assessing these unknown PFAS is essential; whereas legacy PFAS in blood have been decreasing, total organic fluorine analysis shows an increasing number of unknown or uncommonly measured PFAS in blood over the last two decades. Multimodal approaches are needed along with associated spectral libraries to cover the vast unknown PFAS space; not all PFAS are amenable to one technique and current libraries for screening PFAS have been limited. We therefore introduce the PFAS atlas containing libraries for gas chromatography (GC) electron impact (EI) and positive chemical ionization (PCI) spectra, a large collision cross-section (CCS) compendium for ion mobility, and the biggest fragmentation library to date for liquid chromatography tandem mass spectrometry (LC-HRMS/MS) covering both positive and negative mode ionization. The Atlas contains over 14,000 PFAS entries spanning various techniques and includes both experimental and rule-based (< 5% false positive rate for annotation) spectra. Libraries were curated from literature, from available standards using a vast array of PFAS standards available from SynQuest, and from industrial effluent from 5 major PFAS chemical manufacturers. Libraries are openly available, and embedded within FluoroMatch Suite, which covers the entire PFAS non-targeted analysis workflow. Coverage of PFAS in an aqueous firefighting foam contaminated soil sample distributed by NIST (NIST C) was assessed using GC-HRMS (EI and PCI), LC-HRMS/MS, and LC-IM-HRMS. Results show that each technique provides valuable and complementary information, with minimal overlap in PFAS observed between LC and GC based techniques. Further development of PFAS methods using GC-HRMS presents the greatest opportunities as many unique PFAS features remain unidentifiable. **Disclaimer/Disclosure:** Research was funded in part by Agilent Technologies

4.02.T-04 Automation of reference PFAS and persistent organic pollutant methods: Case studies from the lab

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When reference methods are developed and validated for organic contaminants, the focus typically is on accessibility of the method to a “typical” laboratory. This emphasis means that reference methods are finalized with standard, easily available lab equipment, materials, and lean on existing approaches that most people have experience with. For example, standard methods for organic contaminants like dioxins are written with manual Soxhlet extractions and feature use of solvents like dichloromethane (DCM). Most laboratories performing higher numbers of samples find the use of large volumes of hazardous solvents, and cumbersome manual processes to limit scalability and productivity. With a larger variety of automation available, both for sample

extractions and further processing, there have been many initiatives to automate standard reference methods such as EPA 1613 for dioxins or EPA 1633 for PFAS. However, in the highly regulated world occupied by environmental laboratories, a high level of attention to detail needs to be paid to fit for use including validation, customization for lab performance and robustness. In this presentation, we will detail recent case studies in validating reference methods for automation, solvent use reduction and solvent substitution. Two examples, one involving the automation of a PFAS cleanup workflow, and one involving the automation of a dioxin and PCB cleanup workflow using multiple column chemistries including alumina, silica, carbon and more. Both these projects resulted in successful validation and implementation in the laboratory. Successes and potential pitfalls will be discussed, along with a methodical approach to laboratory automation.

4.02.T-05 The automated solid phase extraction of PFAS from various aqueous environmental samples using dual-phase and blended PFAS WAX and carbon S SPE cartridges for USEPA method 1633

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Key to enabling automated solid phase extraction (SPE) is the use of sorbents specifically designed and tested for PFAS analysis. In this study, five extraction protocols using PFAS WAX and Carbon S, designed to meet the quality control requirements of US EPA Method 1633A, were evaluated. The protocols included two-step extractions with single-phase SPE cartridges and loose carbon, as well as dual-phase layered and blended sorbent SPE cartridges. The blended sorbent configuration, containing 200 mg of PFAS WAX and 10 mg of Carbon S, demonstrated the fewest number of outliers and was selected for full method validation for aqueous sample matrices. To evaluate method effectiveness across different matrix types, PFAS analysis was carried out on groundwater samples from residential private wells, effluent from a wastewater storage lagoon, and a landfill monitoring well. Recovery of internal standards and matrix spike targets were well within acceptance limits. This study highlights the effectiveness of automated SPE coupled with PFAS-ready cartridges in improving laboratory efficiency and accuracy in PFAS analysis.

4.02.T-06 Bridging innovation and implementation: Fully automated PFAS analysis across diverse environmental matrices

Lilit Ispiryan¹ and Helmer Jonathan Korb², (1)Trajan Scientific and Medical, Germany, (2)iChrom Solutions Per- and polyfluoroalkyl substances (PFAS) remain a global environmental priority due to their persistence, bioaccumulation, and associated health risks. Accurate, efficient analysis is essential not only for research and exposure assessment but also for ensuring regulatory compliance and informed decision-making. In this presentation, we showcase the continued development and implementation of a fully automated analytical workflow for PFAS analysis in both liquid and solid environmental matrices, building upon our previously presented work. The system integrates solid-liquid extraction, dispersive carbon cleanup, and online SPE, seamlessly coupled directly to the LC-MS/MS. A modular robotic 215 automates sample preparation and transfer, enabling a streamlined, contamination-free workflow specifically designed for PFAS analysis. The 215 supports over 40 PFAS compounds and is based on leading regulatory methods, including U.S. EPA Method 1633, EPA Method 8327, and European standards (DIN 38407-42 and EN 17892:2024). This automation not only improves reproducibility and throughput but also significantly reduces solvent consumption, labour intensity, and operator variability. For laboratories, this translates to greater capacity and cost efficiency. For regulatory bodies, the enhanced precision and traceability support more reliable monitoring data. We will present performance data, discuss challenges in method harmonization across matrices, and offer practical insights into implementing high-fidelity automated workflows in both research and compliance-driven environments. These developments highlight the critical role of automation in future-proofing PFAS analysis—and environmental testing at large.

4.03.P-We Bioactivity-Driven Chemical Assessment: Enhancing Risk Evaluation of Complex Mixtures

4.03.P-We-037 Integrating advanced technologies in environmental mixture monitoring: Lessons learnt and ways forward

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Current regulatory monitoring programs are unfit to monitor the increasingly complex chemical burden on the environment. Recent decades have seen an increase in advanced analytical techniques aimed at understanding the emissions, dispersal, and effects of complex mixtures of known and unknown chemicals at variable and trace-level concentrations. Yet, the integration of these technologies into environmental monitoring frameworks is still in its infancy and requires evidence-based guidance. This presentation highlights multiple research projects aimed at providing such guidance. The applied techniques include in silico toxicity prediction, passive sampling, chemical (non)target analyses, and effect-based (mixture) toxicity assessment. Different combinations of these techniques were applied in case studies covering multiple matrices in air, marine, and freshwater environments, and covered: - Comparing predicted and measured in vivo toxicity of structurally similar persistent, mobile, and toxic chemicals and their derivatives. - Combining passive sampling and effect-based methods for bioactivity assessment of residential ambient air near industry and heavy traffic. - Chemical vs. effect-based methods to capture temporal variation in aquatic ecotoxicological risks. - Comparing lines of evidence from chemical target and nontarget and effect-based methods in a wastewater treatment train. - Investigating the distribution and ecotoxicological impacts of contaminants of emerging concern in coral reef environments. Despite the divergent case studies, universal lessons can be learnt from the application of the advanced analytical techniques. Although chemical target analyses provide insight into the presence of anthropogenic chemicals in the environment, they explain only a small fraction of observed ecotoxic effects. Chemical nontarget analyses tap into a wealth of information on chemical mixture diversity and complexity. Nonetheless, mixture toxicity prediction based on nontarget data is still in development, and bioassays provide complementary information, highlighting the need for integrated monitoring frameworks. In some cases, the different lines of evidence provide contrasting outcomes, demonstrating the need for better integration of the methods, such as in effect-directed analysis. Based on the findings, specific combinations tailored to specific monitoring aims can harness the power of these advanced technologies to safeguard environmental health.

4.04.P-We Connecting Plastics and Plastic-Associated Chemicals, From Analytical Methodologies to Ecotoxicology

4.04.P-We-086 Workflow for automated analysis of microplastics using QCL-based infrared microspectroscopy

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In recent years, measurement of microplastics has increased, owing to the growing awareness of their ubiquitous presence in the environment. The availability of technologies to easily measure microplastics has provided the means for the exponential increase in the number published research studies on microplastics. Automation is a key element to spectroscopic studies of microplastics, enabling non-spectroscopists a way to collect high-quality data without having significant experience in molecular spectroscopic equipment. Quantum cascade laser (QCL)-based systems can provide automation and spectral accuracy, putting advanced measurements in the hands of non-spectroscopists. Current QCL-based systems use pulsed, tunable lasers, enabling a way to scan, using discrete wavelengths, which can be used to quickly find microplastic particles, and then measure microplastics' dimensional properties and infrared spectra. Identifications are accomplished by comparing the measured spectra to spectral libraries. The typical spectral range for QCLs is in the mid-infrared fingerprint region from 1,800 – 975 cm⁻¹; this has been a critique of the technology. Addressing this assessment, this presentation will focus on data that validate the spectral range of QCLs for identification of microplastic particles and will also discuss the automated workflow of a QCL-based microplastic analyzer.

4.04.P-We-087 Ecological risk assessment of the Tijuana River Estuary through integrated chemical, transcriptomic and developmental phenotypes in Japanese medaka (*Oryzias latipes*) embryos

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The Tijuana River Estuary (TRE) is a critical transboundary ecosystem that supports rich biodiversity but is historically and increasingly polluted by urban and agricultural runoff, threatening both its wildlife and nearby human communities. Our previous studies have characterized chemical profiles of estuarine sediment and water, but the biological impact of the detected environmental pollutants is unclear. This study investigates the lethal and sublethal effects of sediment-associated pollutants on Japanese medaka (*Oryzias latipes*) embryogenesis by integrating chemical analyses with transcriptomic profiling, along with physiological endpoints such as heart rate and developmental staging. Medaka's tolerance of varying salinities is ideal for embryotoxicity tests under brackish estuarine conditions. Sediment close to the U.S.-Mexico border within the estuary is elutriated with 15 ppt saltwater and diluted to varying concentrations. Medaka embryos were exposed to concentrated and diluted elutriates under controlled laboratory conditions and collected for 3'-end Shallow RNA Sequencing and targeted chemical analysis via GC/MS and LC/MS at two critical timepoints during heart development: three days post-fertilization (dpf) — when the sinus venosus, atrium, ventricle, and bulbus arteriosus differentiate, and seven dpf — when the pericardial sac forms, marking the final stage of heart development. Survival, heart rate, heart differentiation, and developmental delays marked by morphological alterations were assessed across treatment groups. While a dose-dependent trend in survival was not consistent, a 46% mortality was observed among embryos (n=34) exposed to undiluted elutriates. The increase in the proportion of embryos exhibiting developmental delays positively correlated with more concentrated elutriate treatments. While heart rates decreased as concentration increased, suggesting adverse effects on cardiac physiology during early embryonic stages, no significant differences in heart differentiation morphology were observed among treatment groups. This study combines chemical and gene expression analyses to enhance understanding of sediment toxicity in the TRE and identify biomarkers of exposure to complex chemical mixtures during vertebrate embryogenesis. Our findings help inform about pollution impacts in the TRE and support efforts toward environmental monitoring, remediation, and policy change in this critically vulnerable transboundary watershed.

4.04.P-We-088 In silico approaches for assessing the biodegradability of water-soluble polymers: A critical review of current capabilities and future directions

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The increasing use of water-soluble polymers emphasizes the need for robust environmental safety assessments that align with the European Green Deal and the Chemical Strategy for Sustainability. Historically exempt from REACH registration, polymers are now being reevaluated under evolving regulatory frameworks. Nevertheless, experimental biodegradability testing (e.g., OECD 301/302) is resource-intensive and time-consuming, presenting challenges for high-throughput screening and regulatory compliance. In this context, silico methods provide a promising solution. This review critically examines the applicability of current in silico tools for predicting the biodegradability of water-soluble polymers. We evaluate four major modeling strategies: (1) QSAR models developed primarily for small molecules; (2) pathway prediction systems (PPS) simulating microbial degradation reactions; (3) polymer-specific QSAR models utilizing new encoding formats such as BigSMILES and graph neural networks; and (4) molecular dynamics (MD) simulations for mechanistic insights into degradation processes. Each approach is evaluated based on its conceptual foundation, current implementation, and relevance to polymer systems. We identify critical gaps in data infrastructure,

representation formats, and model interpretability that hinder regulatory adoption and implementation. To address these challenges, we recommend: (i) creating standardized biodegradation datasets for polymers; (ii) integrating of more degradation mechanisms into PPS tools; and (iii) encourage further development of QSAR models as the most straightforward to establish in the short term. This work serves as a roadmap for advancing polymer-aware in silico tools that facilitate safer material design and support informed regulatory decision-making. **Disclaimer/Disclosure:** This research was funded by ICCS.

4.04.P-We-089 Disintegration of biodegradable plastic bags in marine mesocosm conditions: The effects of time and temperature

Hannah Collins, Larissa Tabb, Bridget Holohan and J. Evan Ward, University of Connecticut

Plastic pollution in the marine environment is a persistent and ubiquitous issue. Biodegradable and compostable plastics may present a positive alternative to traditional non-biodegradable polymers for marine applications such as aquaculture. Mater-Bi (MB) is a starch-based polymer used in compostable films and plastic carrier bags and has been proven to completely biodegrade in a variety of laboratory conditions. However, degradation rates in the natural environment differ from static laboratory tests and depend on the specific physicochemical and environmental conditions of a particular location. This study examined the degradation of Mater-Bi in coastal marine conditions in a flow-through mesocosm system, in comparison to polyhydroxybutyrate (PHB), a well-known biopolymer, and high-density polyethylene (HDPE), a traditional plastic polymer. Mass and area loss were both used as proxies for disintegration across a nine-month time span. Results indicate that both Mater-Bi and polyhydroxybutyrate disintegrate when exposed to shallow water column and sediment conditions, losing on average 25 to 47% of area or mass, whereas high-density polyethylene showed no evidence of degradation. Loss of area and mass of Mater-Bi and polyhydroxybutyrate were especially pronounced, averaging 0.87%/ week after 30 weeks of submergence when water temperature increased to 20 °C. The disintegration of biodegradable plastics through microbial action over time and in warmer temperatures likely contributed to these results. Biodegradable plastics such as Mater-Bi may therefore have a lower environmental impact and persist in the marine environment for shorter periods of time than conventional non-biodegradable polymers.

4.04.P-We-090 Plastic additives and the regulation of chemicals of concern

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Synthetic plastic production and use has grown exponentially since plastics were initially developed in the early twentieth century. Plastics play a critical role across nearly every market sector, including packaging, construction, textiles, transportation, and electronics. Plastic additives and processing aids are employed to modify the characteristics of plastic polymers and ultimately achieve the performance characteristics required for these varied purposes. Some properties include thermal and chemical resistance, oxidation resistance, stability, low outgassing, and low coefficient of friction. Due to the range in performance characteristics, plastics have become ubiquitous. Chemical additives and processing aids, used in plastic production, may be released throughout plastics' life cycle and have been identified as Chemicals of Concern by regulatory mechanisms both domestically and internationally due the potential risk to human health and/or the environment. Many additives and processing aids used throughout the manufacture, use, and disposal of plastic are being assessed, regulated, and managed under the U.S. Toxic Substances Control Act (TSCA). Regulatory actions under TSCA may impact the supply and availability of key additive and processing aid chemicals needed for a product to meet technical requirements and/or performance to specifications. Viable alternatives may not exist for many of these chemicals. If alternatives exist, but are not drop-in replacements, changes to product design may be required. For the Department of Defense (DoD), additive and processing aid chemicals provide plastic with unique physical, thermal, and chemical properties essential for product performance and critical to mission readiness and national security. This presentation identifies chemicals used throughout the life cycle of mission critical plastic products that are impacted by TSCA and other regulatory drivers; identifies

the roles that additives and processing aids play along the plastic supply chain, and elucidates their critical functionalities for the DoD.

4.04.P-We-092 Monitoring spectral changes in common plastics under accelerated UV photodegradation

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Plastic debris in the environment undergoes physical and chemical transformations that can alter its detectability, environmental fate, and potential toxicity. Environmental degradation alters the properties of plastics, complicating the identification of polymers via spectroscopy. Existing databases mostly feature pristine polymers, overlooking degraded and commercial plastics that contain additives affecting spectral signatures. This limits accurate identification, which is essential for assessing ecological risks and guiding effective management strategies. This study aimed to monitor the early-stage degradation of five common plastic types under controlled UV conditions to evaluate spectroscopic detectability and material changes over short-term exposure periods. High-density polyethylene (HDPE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and 100% recycled PET (rPET) were exposed in triplicate to UV irradiation (65 W/m², 300–400 nm) at 60°C using an Atlas SUNTEST XXL+ chamber. Samples were measured, washed, weighed, and analyzed by IR/Raman spectroscopy before and after three exposure intervals to ensure consistent tracking. Preliminary Carbonyl index (CI) analysis revealed early oxidative changes in plastics exposed to UV for two days. PP showed a detectable increase in CI (0 to 0.20), while PS showed no carbonyl development. PET remained stable (CI ~15.8), while rPET showed a notable decrease in CI (23.3 to 15.8), likely due to prior oxidation from recycling. This suggests that unstable carbonyl-containing compounds may degrade or volatilize during early UV exposure, reducing the carbonyl signal before new oxidation products accumulate. White HDPE developed a new carbonyl band (CI 1.72), indicating UV sensitivity, while yellow HDPE showed minor changes. These results highlighted the varied early degradation responses of different plastics, with recycled and pigmented (white) materials showing greater reactivity. Surface cracks appeared in PET, while PP and PS showed faded colors. Early IR peak shifts and intensity loss were more pronounced in HDPE and PET. All samples exhibited slight mass loss. These findings suggest that accelerated UV exposure can effectively simulate short-term environmental degradation in laboratory settings. This study contributes to the refinement of analytical approaches for microplastic tracking and supports rapid methods for predicting the weathering behavior of plastics in the environment.

4.04.P-We-093 Assessing chemical and spectroscopic characteristics of trace organic compounds leached from cellulose acetate cigarette filters in water

Sarah Poletti, Natalie Mladenov, Eunha Hoh, Margaret Stack and George Youssef, San Diego State University

Cigarette butts, which consist of cellulose acetate microfibers in cigarette filters (CFs), wrapping papers, plastic additives, and residual tobacco, are among the most prevalent trash items polluting coasts globally, leaching toxic chemicals that threaten aquatic organisms. This study aimed to (1) determine how various filter components contribute to chemical leachates, (2) investigate how diffusion limitations affect leaching kinetics, and (3) assess how environmental conditions influence these dynamics. The study examined characteristic differences of unsmoked CFs, machine-smoked CFs, and raw cellulose acetate leachates by assessing changes in UV-vis absorbance and fluorescence spectroscopic properties, dissolved organic carbon (DOC), and total dissolved nitrogen (TDN). Experiments found that most compounds leached within one hr. DOC and fluorescence values plateaued, while TDN continued to rise after 2 weeks. Five distinct peaks were identified within the fluorescence signatures of CF leachates. Smoked CFs consistently released more DOC, TDN, and fluorescent compounds and were more aromatic than unsmoked, leaching on average 41.47 ± 4.91 mg/g DOC and 0.612 ± 0.383 mg/g TDN after 24 hr under dark conditions. By contrast, measurements of cellulose acetate leachates were negligible. Photoirradiation during leaching did not affect DOC or aromaticity but led to increased TDN concentrations. While it did not impact unsmoked CF leachate fluorescence, photoirradiation of

smoked CF leachates reduced fluorescence intensity of all peaks by more than half and removed the humic peak entirely. Aging of filters prior to leaching reduced fluorescence but did not affect DOC or TDN. The findings of this study demonstrate that cellulose acetate is not a significant contributor to the organic leachates from CFs. Leachate fluorescence and TDN are likely derived from tobacco-related compounds that adsorb to filters during smoking, whereas the majority of DOC likely originates from additives or plasticizers added to filters during manufacturing. Organic compound leaching from CFs was surface area-limited, and DOC leaching kinetics were modeled using a radial diffusion model for cylindrical microplastics. With an estimated 4.5 trillion cigarette butts littered annually, this could mean 23,656 tons of carbon and 348 tons of nitrogen entering aquatic ecosystems each year in leachate alone. **Disclaimer/Disclosure:** Project funded by the Tobacco-Related Disease Research Program. Primary author supported by CSU Council on Ocean Affairs, Science & Technology (COAST).

4.04.P-We-094 Eco-corona-based characterization of environmentally weathered microplastics using ultra-performance liquid chromatography, ATR-FTIR spectra and unsupervised machine learning algorithms

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The adsorption mechanisms and carrier effect in microplastics (MPs) is crucial to assessing their transport, fate, toxicity, and environmental risk. However, microplastic adsorption study in the natural environment is complicated, and it is often a daunting challenge to accurately assess the level of threat posed by microplastics in complex matrices due to various environmental constituents (eco-corona) adsorb on MPs' surface, including natural organic matter, eco-proteins, organic pollutants, metals, and microbe colonization. To improve our understanding of microplastic corona formation and characterization under natural conditions, MPs were extracted from particulate organic matter obtained from marine 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). With a total of 1400 samples were collected, comprising a hundred spectra for each group of three different polymer types (polyethylene, PE, polystyrene, PS, and polypropylene, PP) were analysed using unsupervised machine learning algorithm and spectral integration for carbohydrate: protein ratio for each treatment. High-resolution mass spectrometry (HSA-14C-HRMS) was used to quantify different types of organic pollutants on the surface of isolated microplastics. Unsupervised machine learning showed a great promising tool for the characterization of environmental MP with over 70% variance across all treatments. MP separation methods showed variation in types and amount the environmental constituents present on the surface of the microplastics. Based on the mass spectrometry characterization, different types of organic pollutants were identified and quantified (conc. ranged between $1.67 - 79.9 \pm 3.63$) including Caffeine, BP3, Lidocaine, Tonalide (AHTN), TBP, Galaxolide (HHCB), TCEP, Norethindrone, TPhP, TBEP, DINP, DIDP in the positive mode and BPA only in the negative mode. This study provides a comprehensive knowledge on the carrier tendency and adsorption of organic pollutants on microplastics in the real environment and give a novel insight into how environmentally weathered microplastics could be effectively identified and enhance our knowledge of microplastics eco-corona formation.

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4.04.P-We-095 A method for isolation and identification of fiberglass from sediment and water column samples

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Since the 1960's, glass reinforced plastics (fiberglass) have been the dominant construction material for recreational watercraft. Glass fibers can be released from the fiberglass resin as the material ages, as boats are repaired, or when derelict vessels are abandoned and break apart. Evidence is emerging of glass fiber ingestion

by bivalves and aquatic invertebrates. These particles have been shown to generate inflammatory response in digestive systems and gills, as well as swimming impairment in *Daphnia magna*. Isolation of these particles from environmental matrices is difficult due to their small size and similar density to natural sediments. Particle identification is also challenging as glass fibers are chemically similar to natural minerals and diatom fustules. Here we present the first method for extracting glass fibers from sediment and water column samples. Preliminary results show recovery of 96 ± 8 % of glass fibers in sediment and water samples. Additionally, we show that glass fibers can be accurately identified using a combination of visual morphological observation and FTIR spectral analysis. These methods can be applied to environmental samples to better assess the spatial distribution of glass fibers, and to assess the presence of glass fibers in organisms to better understand toxicological impacts.

4.04.P-We-096 Mass-based quantification of micro and nanoplastic particles from filter samples

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Micro and nanoplastics (MNPs) are an emerging contaminant that is found throughout the environment as well as in biota and human tissue. The detection of these particles is typically carried out by spectroscopic methods, such as infrared and Raman spectroscopy, which typically have a lower resolution of ~ 10 to $1 \mu\text{m}$. These techniques also provide particle counts, rather than mass based quantitative data. Moreover, chemical speciation of the polymers can be challenging. This limits our understanding of smaller MNPs and limits the applicability of particle count data for exposure sciences. Rather, mass-based analyses of MNPs provide quantitative MNP polymer data. Mass-based analyses are typically conducted using pyrolysis and thermal desorption coupled to gas chromatography mass spectrometry (GC-MS). Here we present a novel approach that integrates a thermal desorption and pyrolysis unit with a photoionization time of flight mass spectrometer (PI-TOF-MS). The pyrolysis unit (multiwavelength carbon analyzer) is continuously ramped at a rate of $\sim 20 \text{ K min}^{-1}$ resulting in the thermal decomposition of different plastic polymers with time. An aliquot of the evolved gas is analyzed in real-time by the PI-TOF-MS to determine chemical composition. The advanced PI-TOF-MS technology enables soft ionization and detection of the complex degradation products while preserving the molecular identity. To deconvolute the resulting mass spectrometer fingerprint patterns and quantify the mass of a polymer type in a complex mixture, we employed a multivariate curve resolution alternating least squares (MCR-ALS) approach. We applied this approach to an artificial mixture of different plastics, plastics with natural organic matter, and environmental samples.

4.04.P-We-097 Quantifying polystyrene nanoplastics in tissues: A silica gel chromatography-based approach with pyrolysis–GC/MS

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Nanoplastics (NPs) are of growing concern due to their ability to cross biological barriers, enhanced reactivity, and potentially greater toxicity compared to microplastics. Despite increasing awareness of their adverse health effects, the bioavailability and distribution of NPs within the body remain poorly understood. Quantifying NPs in biological samples such as animal tissues remains challenging, primarily due to complex matrix interferences and contamination risks. In this study, we present a simple yet robust method for quantifying polystyrene (PS) NPs in animal tissues, utilizing silica gel column chromatography for sample cleanup followed by pyrolysis–gas chromatography/mass spectrometry (Py-GC/MS). Tissue samples were homogenized in dichloromethane (DCM), subjected to alkaline digestion, and purified using disposable pipette columns packed with silica gel. PS quantification was based on monitoring styrene trimers, characteristic pyrolysis products. To minimize background contamination, all materials, including silica gel, were pre-heat-treated at 550°C for 4 hr prior to use. The method achieved recovery rates of 102.0% and 91.2% at spiking levels of 0.3 and $1.7 \mu\text{g g}^{-1}$, respectively, using liver samples from commercial chickens. The detection and quantification limits were

determined to be 3.0 ng and 7.8 ng, respectively. Validation with liver samples from Japanese quail orally exposed to 50-nm PS NPs demonstrated significantly elevated levels of PS NPs on days 1 and 3 post-exposure (33.8 ± 1.5 and 34.1 ± 5.2 ng g⁻¹) compared to controls ($p < 0.005$). This confirms the method's effectiveness in differentiating exposure levels and its applicability in biological exposure assessment. This silica gel-based cleanup procedure significantly improves the removal of biological matrix components, enabling accurate and reproducible quantification of PS NPs in lipid-rich tissues such as the liver. The method's simplicity, applicability irrespective of NP size and morphology, and minimal contamination make it a valuable tool for advancing NP toxicity assessments and monitoring environmental contamination in biological systems.

4.04.P-We-098 Background microplastics in research laboratory: Inevitable and the need for QA/QC for microplastic research

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Microplastic contamination across the biosphere has garnered widespread public attention, prompting a need to assess its risks. However, over 90% of published microplastic research ignored laboratory-based contamination, which can affect results. The present study surveyed 18 institutions (comprising 19 laboratories) across five continents to characterize microplastics in laboratory settings. Microplastics were detected in all laboratories with an average deposition rate of 4.09 MPs/filter/day or 0.87 MPs/cm²/day, which is higher than many MP concentrations reported in the literature. No differences were observed between laboratories that had previously worked with microplastics and those that had not. Laboratories in Asia had elevated deposition rates, which may be attributed to increased foot traffic. Results demonstrate that MPs are present in laboratories at a level that can artificially inflate MP concentrations in environmental samples, highlighting the need to apply quality control procedures to provide confident results for risk assessment and effective management of microplastic pollution.

4.04.P-We-099 Evaluation of cryogenic milling for producing environmentally relevant nanoplastic particles

Megan Dodge, **Kolette Miller**, **Caden Anderton**, **Bryan J. Harper** and **Stacey L Harper**, Oregon State University
Widespread plastic pollution, combined with analytical limitations, has contributed to significant uncertainty regarding the environmentally relevant concentrations and physicochemical properties of nanoplastics (particles <1 µm in size). To fully assess the ecotoxicity of nanoplastics, it is essential to develop methods that produce

particles with environmentally realistic sizes, shapes, and concentrations. Cryogenic milling is one approach that can generate both micro- and nanoplastic particles within hr; however, it remains unclear whether the resulting particles accurately represent those found in the environment. In this study, we compare nanoplastics produced by cryogenic milling with those generated through a base-catalyzed hydrolytic degradation process, using two common consumer plastic sources: polyethylene terephthalate (PET) plastic and PET-based polyester fabric. Nanoparticle Tracking Analysis (NTA) is employed to characterize the size distribution and concentration of particles produced by each method. To date, we have found that nanoparticle concentrations from 45-day hydrolytic degradation and cryogenic milling of PET and polyester fabric are similar, with differences within the standard error for each plastic type. Additionally, we found that PET particles have average sizes of 244 nm and 258 nm from hydrolytic degradation and cryogenic milling, respectively. For polyester fabric, the average particle sizes are 244 nm and 395 nm for the same processes. These findings are directly applicable to ecotoxicity studies, as particle size critically influences nanoplastic toxicity. Showing that particles produced by cryogenic milling are similar in size to those formed through hydrolytic degradation strengthens our confidence in using cryomilled particles for environmentally relevant ecotoxicity testing. Future work will involve 96-hr algal growth inhibition assays using *Raphidocelis subcapitata* to compare the ecotoxicity of particles generated by the two production methods, with the aim of quantifying the extent to which potential differences in particle shape and/or surface chemistry influence toxicity.

4.04.P-We-100 From fish to feathers: Investigating microplastic transfer from foraged fish to Marbled Murrelet chicks

Daisy Youmans, Susanne M Brander, James Rivers, Alexa de Rivera, Elizabeth Daly, Ryan Berry, Matthew Betts, Kim Nelson and Daniel Roby, Oregon State University

Marbled Murrelets are small seabirds found in the Pacific Northwest that are listed as endangered by the IUCN. They live in coastal forests and employ a feeding strategy of bringing single prey items back to their nests. Our study aims to identify potential trophic transfer between the preferred prey items of the adult murrelet to their chicks. To examine this, we are extracting microplastics from the fecal ring of the murrelet nest as well as extracting microplastics from stomach and muscle samples of pacific sand lance, white bait, and northern anchovy, some of the most-consumed prey species of the murrelet. Prior studies investigating fecal matter from chicks have used feces collected from fecal sacs or entire nests. As such, to our knowledge separating the fecal ring from the nest for extraction has not been done previously. To attempt extracting microplastics from this challenging matrix, we combined mechanical removal of feces from the nests to obtain a weight of 3 grams, followed by a commonly used potassium hydroxide digestion and density separation with Sodium Bromide. This was chosen over other saline solutions because its density increases the chance of dense particle recovery. Finally, a H₂SO₄ digestion is performed, a method developed for sediment extractions to obtain cleaner filters for particle characterization. To examine particle recovery, select samples were spiked with 300 and 600 um polyethylene spheres and 500 um length polypropylene fibers. 10 of each sphere and 30 of the fibers were introduced into the samples. Sphere recovery was 85-90%, while fiber recovery was around 20-30%. This low recovery rate for fibers is comparable to that from sediment samples being run by collaborating laboratories. Overall, 25 feces and 25 fish samples were processed. We found a wide representation of chemical identities, shapes, and colors in the samples. Finding similar particles in both the birds and fish indicates that there could be trophic transfer from the prey items to the murrelet chicks, this analysis is underway. The trophic transfer of microplastics is not separated between the domains of land and sea, and this means that the impact of microplastics on marine food webs can also affect terrestrial food webs by introducing additional microplastics. Further research is needed to shed light on the ways microparticles affect organism health and behavior, but through this research we have a better view of the marine-terrestrial transfer impacts.

4.04.T Connecting Plastics and Plastic-Associated Chemicals, From Analytical Methodologies to Ecotoxicology

4.04.T-01 Enhanced leaching of plastic additives via enzymatic processes

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Plastic additives have become a significant environmental concern due to their persistence, bioaccumulation potential, and toxicity. As they are not covalently bound to the plastic matrix, these chemicals can leach into the environment. However, the influence of environmental factors on additive leaching behavior remains insufficiently understood. Therefore, this study investigates the impact of enzymatic biotransformation on the leaching of additives from low-density polyethylene (LDPE). Leaching experiments were conducted using LDPE fibers containing 2-(3,5-di-tert-butyl-2-hydroxyphenyl)-5-(octyloxy)benzotriazole (UV-327), 2-(3,5-di-tert-butyl-2-hydroxyphenyl)-5-chlorobenzotriazole (UV-328), benzophenone-3 (BP-3), and 1-naphthol in enzyme mixtures. The leaching behavior of four chemicals from LDPE was evaluated in the presence of enzymatic transformation. A coupled diffusion–reaction model was then developed to describe additive release from cylindrical plastic into the enzyme solution. Model predictions and experimental data revealed that enzymatic reactions significantly enhanced the leaching of hydrophobic additives by converting them into more hydrophilic metabolites, thereby facilitating mass transfer across the aqueous boundary layer (ABL). In contrast, enzymatic reactions had a minimal effect on the leaching rates of less hydrophobic additives, as the reaction rate was negligible compared to the rapid mass transfer in the ABL or the diffusion-limited nature of the leaching process. While enzymatic transformation did not always increase instantaneous leaching rates, it helped maintain the concentration gradient by acting as a chemical sink, resulting in a greater total leached mass. These findings offer insight into the role of biofilm-associated enzymatic reactions in the leaching kinetics of plastic additives.

4.04.T-02 Unraveling microplastic–PFAS interactions: Mechanistic insights and implications for contaminant co-removal

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Microplastics (MPs) and polyfluoroalkyl substances (PFASs) have emerged as emerging contaminants, drawing global attention due to their persistence and extensive presence in ecosystems. Although there are comprehensive studies on the individual behavior of these pollutants, their synergistic interactions remain unexplored. Hence, there is a requirement for an in-depth investigation to understand their impacts on different environmental compartments. The aim of this study is to investigate the interactions between these two contaminants, providing insights that may guide future research. This study investigates the adsorption behavior of two PFASs (PFOA, and PFOS) on five of the most abundant types of MPs (PE, PP, PS, PA and PVC) in aquatic systems. The experiments were performed using environmentally relevant concentrations of PFAS (approximately 1000 ng/L) in surface water samples. The adsorption behavior of PFAS on MPs varies with the types of MPs. The adsorption of PFOA and PFOS onto MPs increased significantly within the first 1-2 weeks, stabilizing by weeks 3-4. Among the MPs, PE and PA exhibited the highest adsorption efficiencies, with PFOA adsorption reaching up to 89.30% on PE and 86.09% on PA (50 mg/L, week 4). In contrast, PVC had comparatively lower adsorption, with PFOA 80.82% (50 mg/L, week 4). PFOS showed higher adsorption than PFOA, with PFOS adsorption on PE reaching 94.17% (50 mg/L, week 4) and on PP reaching 92.96% (50 mg/L, week 4). Adsorption increased with concentration, with 50 mg/L samples showing significantly higher adsorption than 1 mg/L samples. Some MPs showed fluctuations after week 2, possibly due to desorption or surface saturation effects. This study reveals significant interactions between microplastics and PFAS, with adsorption strongly influenced by various factors including chemical and physical characteristics of microplastics and PFAS, characteristics of the medium, etc. These interactions can severely affect aquatic species, causing intestinal damage, oxidative stress, and disrupted reproductive systems in fish and other organisms. Despite these findings, significant knowledge gaps remain in understanding their combined toxicity and co-removal technologies. Finally, this study provides fundamental information for developing combined removal technologies and source abatement measures to effectively manage the associated environmental and health risks.

4.04.T-03 Sorption and desorption of marine phycotoxins in virgin and weathered microplastics

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Microplastics are ubiquitous in the aquatic environment, causing global concern due to their persistence and ability to harm marine life. They have garnered attention as possible vectors of organic contaminants to aquatic organisms and therefore humans. Several studies have shown that legacy persistent organic pollutants (POPs) and contaminants of emerging concern (CECs) adsorb to microplastics. However, the data on adsorption of biotoxins such as phycotoxins produced by harmful algae blooms (HABs), and the data on subsequent desorption of phycotoxins once ingested by an organism, are severely lacking. As HABs are worsening due to climate change and microplastic pollution is global, this issue is a research priority. In this study, three types of microplastics (polypropylene, polyethylene, and polyester), both virgin and weathered (in an artificial sunlight chamber), were exposed to marine phycotoxins (okadaic acid, domoic acid, and brevetoxin-2) in artificial seawater for 96 hr. Plastics that sorbed phycotoxins were placed in artificial gut fluid for 96 hr to replicate digestion of an aquatic organism. Concentrations of phycotoxins on the plastics, in seawater and in the gut fluid were determined via liquid chromatography tandem mass spectrometry (LC-MS/MS). Sorption and 'post-digestion' desorption rates were derived from these concentrations. This study determines whether, a) phycotoxins sorb to plastics, b) they subsequently desorb under simulated digestive conditions, and c) at what rate do they sorb/desorb. This information is key to understanding whether plastics are a vector for exposing aquatic organisms to high doses of toxins in a concentrated manner, or if plastic sorbent material could be a mechanism for phycotoxin remediation. This can help to inform risk assessments and better predict the fate of organic contaminants in aquatic environments and potentially offer new solutions to the growing issue of aquatic pollution.

4.04.T-04 Tracking the degradation and transformation of tire wear particle-derived chemicals under experimental and environmental conditions with fluorescence spectroscopy

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Tire wear particle (TWP) leachates contain numerous organic chemicals, including chemicals known to cause toxicity in a variety of aquatic species. Therefore, monitoring how TWP-derived degrade, persist, and transform under environmental and experimental conditions is important for advancing our understanding of the fate of these compounds in aquatic ecosystems. Given that many TWP-derived compounds contain chromophoric compounds that exhibit auto-fluorescence, we utilized fluorescence spectroscopy for rapid tracking of the behavior of TWP-derived fluorescent organic compounds. We obtained reference standards of previously identified compounds in the TWP leachates of passenger car tires, including N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), 6PPD-quinone, dicyclohexylamine (DCHA) and 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ), and measured the temporal variability of 3D fluorescence excitation emission matrix (EEM) spectra of each compound under 6 h of simulated sunlight, elevated temperature, and dark conditions. We found that the fluorescent peak of TMQ at 420 nm emission was rapidly removed by photoirradiation and a new photo-transformation product occurred at ~350 nm emission. Similarly, the single peak of 6PPD at 430 nm emission degraded rapidly in simulated sunlight, decreasing by >90 % within the first 0.5 h, and followed a double exponential decay curve ($k_L = 2.31 \text{ h}^{-1}$, $k_{SL} = 0.32 \text{ h}^{-1}$). A new and highly fluorescent photo-transformation product of 6PPD formed at 365 nm emission when 6PPD was photo-irradiated. While it was suspected that the new peak could be due to the formation of 6PPD-Q, neither 6PPD-Q dissolved in water nor 6PPD-Q dissolved in acetonitrile were fluorescent. Our ongoing nontargeted chemical analysis seeks to identify the photo-induced transformation product of 6PPD to inform future toxicity studies. Overall, our study revealed that TWP-derived compounds with emission peaks at higher wavelengths were more photo-labile than compounds with emission peaks at lower wavelengths. Our study also recognized that spectral overlap may occur in mixtures or natural water samples, due to simultaneous photobleaching of some compounds and photoformation of others. However, under controlled laboratory conditions and in dilute solutions, fluorescence

also was found to be a rapid technique for quantifying the photodegradation kinetics of known fluorescent compounds in the TWP leachates. **Disclaimer/Disclosure:** This work was supported by the National Science Foundation (CBET Project # 2327008) and California State University's Council on Ocean Affairs, Science, and Technology (CSU COAST) (COAST-SSINP-2020-001).

4.04.T-05 Microplastics spilled in the marine environment: Analysis of high molecular weight polycyclic aromatic hydrocarbons (PAHs) in pyroplastic

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In 2021 a fire on board the container ship M/V X-Press Pearl, followed by its sinking, resulted in the largest recorded release of microplastics (MPs) into the marine environment. Samples collected from the beaches of Sri Lanka following the disaster included relatively pristine polyethylene pieces, burnt nurdles and combustion remnants. Thorough characterization of PAHs of 302 Da and lower molecular weight in these samples revealed the highest levels of PAHs ever reported for marine plastic debris. However, the conditions of uncontrolled combustion combined with the many inputs into the fire led to ideal conditions for the creation of a host of additional combustion products of concern. The possible classification as hazardous waste has significant impact on the cost of disposal of the debris. The pyroplastic component of the spill presents additional challenges for MP analysis over and above those posed by MPs unaffected by fire. Existing analytical methods are limited in their ability to accurately assess PAH related toxicity. Furthermore, evidence indicates that specific high molecular weight (HMW) PAHs warrant further study. For instance, multiple dibenzopyrenes have toxic equivalent factors (TEFs) of 10 to 30 indicating substantially higher potential toxicity than benzo[a]pyrene. Because these compounds are less volatile and less likely to be water-soluble, they have the potential to serve as higher specificity, longer-lived markers of pyroplastics collected from marine environments as well. Evaluation of the ability of cyclic ion mobility mass spectrometry (cIMS) to separate sample components not chromatographically separated was performed. cIMS separation of related compound conformations based on individual compound properties of size, shape and charge state will be demonstrated with this data. Pattern analysis software identified heteroatom-containing PAHs including nitrogen-, sulfur-, and oxygen-substituted species in the samples. These findings improved the chemical characterization, particularly for analytes that are not compatible with traditional GC/MS techniques. Accurate mass measurement was performed on the compounds detected through pattern analysis, further refining the chemical profiles of the samples. This work enables future investigation into whether analytes originate from the MP materials themselves, are naturally occurring, or are synthetic substances absorbed during the MPs transport and residence in environment.

4.04.T-06 Single and mixtures toxicity of emerging (polystyrene microplastic) and persistent (PAHs and PCBs) pollutants and their sub-lethal adverse effects on embryo-larval zebrafish (*Danio rerio*)

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Persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), along with emerging contaminants like microplastics, pose potential risks to fish and human health. This study assessed the toxicity of PAHs, PCBs, and polystyrene (PS) microplastics exposure using embryo-larval life stages of zebrafish (*Danio rerio*). Exposure to PAH and PCB mixtures strongly induced cytochrome P450 (CYP450) biotransformation activity, indicated by elevated ethoxyresorufin-O-deethylase (EROD) activity, which corresponded with increased metabolic rate (MO₂). The observed MO₂ increase suggests an elevated demand for molecular oxygen, likely supporting enhanced CYP450-mediated mono-oxygenation reactions. Exposure to PS microplastics alone (10 µg L⁻¹) had no significant effects on measured biomarkers. However, co-exposure of PS microplastics with either phenanthrene (PHE) or PCB 81 increased

MO₂ by 70% and 57%, respectively, though these effects were not statistically significant. Additionally, a significant decrease in cardiac blood flow was observed in zebrafish co-exposed to PCB 18 + PS, PCB 81 + PS, and PCB 105 + PS, suggesting that the observed cardiovascular effects were primarily driven by PCBs rather than PS microplastics. These findings highlight the utility of in vivo EROD activity as a potential proxy for MO₂ measurements in assessing PAH and PCB exposure. Furthermore, they highlight the importance of including cardiac function biomarkers in the toxicity assessments of both persistent (PAHs, PCBs) and emerging (microplastics) contaminants.

4.05.P-Th Contaminant Property Measurement and Estimation: Methods and Applications

4.05.P-Th-097 Variability in site-specific pyrethroid Koc and Kdoc coefficients and implications for surface water quality monitoring

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Default organic carbon-water (Koc) and dissolved organic carbon-water (Kdoc) partition coefficients are widely used to monitor the bioavailability of pyrethroid insecticides in both the water column and sediments of aquatic systems. However, the variability of site-specific coefficients and their implications for surface water quality monitoring has not been thoroughly examined. In this study, Koc and Kdoc partition coefficients were experimentally measured for 6 pyrethroids (bifenthrin, cyfluthrin, cypermethrin, esfenvalerate, λ -cyhalothrin, and permethrin) in 19 sediment samples collected across California. The purpose of this study was to examine site-specific variability in pyrethroid coefficients and investigate the implications for surface water quality monitoring using California statewide pyrethroid and *Hyaella azteca* toxicity monitoring data. Measured log Koc values ranged from 5.33–7.11 and log Kdoc values ranged from 5.28–7.41, across all pyrethroids. The toxic units estimated using default and site-specific coefficients varied within a factor of 7 in reported sediment samples and 10 in reported water samples. Site-specific coefficients did not explain any additional variance in reported *H. azteca* survival for either 10-day sediment or 4-day water toxicity tests. The results of this study provide strong evidence that default pyrethroid coefficients can adequately capture site-specific considerations in aquatic health risk assessments. We recommend the use of the default coefficients derived in this study, which encompass 19 sediment types and, to the authors' knowledge, represent the most comprehensive database of experimental pyrethroid Koc and Kdoc coefficients to date.

4.05.P-Th-098 Database of measured and estimated rate constants for abiotic reduction of contaminants by natural and engineered materials

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Contaminants are subject to a variety of abiotic transformation reactions in the environment, including hydrolysis, photolysis, oxidation, and reduction. Abiotic reduction of organic contaminants mostly involves dehalogenation and nitro reduction, but reduction also is a major factor in the fate of metal(loid) oxyanions. Most of these transformations involve electron-transfer from ferrous iron or sulfide, but the specific donor can range from dissolved-complexed Fe(II) to nano Fe(0). The kinetics of these reactions have been studied extensively to understand their role in natural attenuation and remediation of contaminants. Most of these studies report pseudo-first-order observed rate constants, and those involving heterogeneous reductants usually report mass- and/or surface-area normalized rate constants. These rate constants often are subject to (correlation) analysis and modeling within the dataset from an individual study, or across a few studies of limited scope (e.g., dechlorination by zerovalent iron). However, more comprehensive analyses of these data (e.g., by machine learning methods) is limited by the many practical and fundamental challenges that arise when compiling and curating data from many diverse sources. We have been working to overcome these challenges through the compilation of a database that includes a large portion of the currently published kinetic data and their associated meta-data. We published the first version of the Abiotic Reduction Kinetics (ARK) database at zenodo.org in May 2025. Currently, ARK includes over 4000 rate constants for ~150 contaminants

from ~200 publications, with +100 types of meta-data that define the reaction conditions. There are many aspects of the process and results of assembling such a database that can be used to illuminate fundamental issues related to abiotic contaminant transformation under environmental conditions. One prominent example is the harmonization of rate constant representations to allow comparisons across systems without undue loss of precision. Another example is characterization of reducing systems (e.g. fresh vs. aged minerals $\pm$ Fe(II)aq) in ways that efficiently represent rate controlling factors. Advances on both these fronts are needed to enable correlation analysis—and ultimately quantitative structure-reactivity relationships—that describe this whole domain of reactivity.

4.05.T Contaminant Property Measurement and Estimation: Methods and Applications

4.05.T-01 Computational prediction from first principles of aqueous and non-aqueous pKa of PFAS and other chemicals using only molecular structures and COSMOtherm

Anthony Sigman-Lowery, Dominic M. Di Toro and Olga Dmytrenko, University of Delaware

Chemical acid dissociation constants (pKa values) are of great environmental relevance, yet the accurate prediction of pKa values, particularly in non-aqueous solvents, remains a significant challenge, especially for emerging contaminants such as PFAS with limited experimental data. Here, we present a first-principles computational method using COSMOtherm to predict the pKa values of organic acids in protic and non-protic solvents that relies only on molecular structures and the inclusion of an explicit water molecule to the neutral and charged chemical molecular structures for protic solvents. A dataset of experimental pKa values for organic acids in 16 solvents was compiled, and the corresponding Gibbs free energies of dissociation (ΔG_{diss}) were calculated in COSMOtherm. In non-protic solvents, the theoretical ΔG_{diss} values correlated strongly with experimental pKa values and possessed slopes near unity, indicating COSMOtherm was accurately modeling the solvation of these chemicals. However, in protic solvents the ΔG_{diss} – experimental pKa slopes deviated significantly from unity. The addition of an explicit water molecule at the site of deprotonation for both the neutral and charged chemical species resulted in a slope near unity between predicted ΔG_{diss} and experimental pKa. This proposed protocol enables pKa prediction across diverse chemicals and solvents, supporting the use of COSMOtherm in calculating the solvation energies of charged species and in predicting the physicochemical properties and partitioning behavior of emerging contaminants such as PFAS. **Disclaimer/Disclosure:** Supported by SERDP ER22-3175 Measuring and predicting toxicity of PFAS mixtures

4.05.T-02 Experimental pKa values of substituted and unsubstituted perfluoroalkyl sulfonamides via ^{19}F NMR

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Perfluoroalkyl sulfonamide-containing substances (FASAs) such as perfluorooctane sulfonamide, (FOSA) and perfluorobutyl sulfonamide, (FBSA), are a class of per- and polyfluoroalkyl substances, (PFAS), found throughout the environment in water, soil, and organisms. Their prevalence in many types of media is caused by PFAS containing a hydrophobic tail, and a hydrophilic headgroup. Research has shown that exposure to FASAs leads to negative health effects like nephrotoxicity and hepatotoxicity. Despite their prevalence and toxicity, little information exists on how FASAs move through the environment. One characteristic used to determine transport through the environment is the acid dissociation constant, pKa. The pKa informs when a molecule is predominantly charged, and impacts partitioning coefficients, aqueous solubility, and retention in sediment. Though the pKa is a powerful constant to know, few published pKa values for FASAs have been reported with a majority these values being modeled and not determined experimentally. Moreover, recent research has highlighted that variability in the determined pKa can exist between models. Variability based on the model used can lead to misunderstandings between researchers and highlights the need for experimentally determined pKa values. NMR was chosen due to ^{19}F being both NMR active and having high natural abundance. This

coupled with the ability of ^{19}F NMR to sense changes in the electronic environment of neighboring atoms means that NMR could be a powerful tool when studying PFAS. Previous research has shown that ^{19}F NMR can be used to determine the pKa of fluorine-containing molecules. However, no study has previously looked at using this technique to determine the pKa of PFAS or FASAs specifically. In this work, five FASAs (perfluorobutane sulfonamide (FBSA), perfluorooctane sulfonamide (FOSA), N-methyl (Me) substituted FBSA (MeFBSA) and FOSA (MeFOSA), and N-ethyl (Et) substituted FOSA (EtFOSA)) were analyzed by ^{19}F NMR. Once the pKa of the five FASAs were determined, analysis was done to understand the role that chain length and substitution about the nitrogen of the sulfonamide plays on the observed pKa. The experimentally determined pKa were then compared with three models, (COSMOtherm, VF, and Chemicalize), currently used to determine the pKa of PFAS. Discrepancies between the experimental and modeled values were seen in all cases, but some models were better at estimating the pKa than others.

4.05.T-03 Predicting PFAS partitioning using biomimetic chromatography and machine learning

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Biomimetic chromatography is a high-throughput method that is used to derive partition coefficients for biologically relevant phases, including phospholipid membranes and serum proteins. This makes biomimetic chromatography well-suited for evaluating partitioning of compounds of interest, such as of per- and polyfluoroalkyl substances (PFAS), present in commercial products and environmental samples. However, with over 4,700 PFAS listed in the global market (OECD list), experimentally measuring partition coefficients for all is impractical. To address this, this study aims to estimate partitioning behavior using a combination of biomimetic chromatography and machine learning. Specifically, our objectives are to 1) generate biomimetic chromatography data for marketed PFAS not previously characterized in the literature to estimate their partition coefficients, and, and 2) train a machine learning model using PFAS molecular structures and estimated coefficients to predict partitioning behavior for untested PFAS. To achieve our objectives, target PFAS will be selected from cluster analysis using structure similarity analysis across a large set (>20,000) of commercially available PFAS. In addition to target PFAS, suspect and nontarget PFAS from aqueous film forming foams and environmental (e.g., fish and water) samples will be analyzed. Partitioning coefficients will be measured for selected target/suspect/non-target PFAS and will constitute the dataset used to train our machine learning model. Partition coefficient predictions could facilitate modelling of in-vivo tissue distributions of PFAS to support more efficient ecological risk assessments and site-specific management decisions. Ultimately, this project provides a scalable and transferable framework to prioritize PFAS for further study and regulatory action, while advancing our understanding of their environmental fate.

4.05.T-04 Systematic evaluation of in vitro intestinal permeability measurement data across diverse chemicals: Insights for advancing new approach methods for chemical assessment

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In human exposure and toxicokinetics assessment, evaluating absorption, reabsorption, and excretion processes requires estimating a chemical's permeability across biological membranes, such as the intestinal epithelium. Intestinal transmembrane permeability is typically assessed using in vitro assays, including the cellular Caco-2 transwell assay and the non-cellular parallel artificial membrane permeability assay (PAMPA). In this presentation, we will share findings from our systematic investigation into longstanding assumptions about the relationship between Caco-2 and PAMPA permeabilities and their relationship with hydrophobicity. This analysis is based on the most comprehensive datasets to date that we collected and curated, including measured Caco-2 permeability (8,087 measurements for 5,760 chemicals), PAMPA permeability (2,364 measurements for

1,854 chemicals), as well as octanol-water partition ratios at physiological pH (19,567 measurements for 17,032). We confirm the widely held idea that hydrophobicity correlates with PAMPA permeability and that PAMPA permeability correlates with Caco-2 permeability. However, we find that such correlations are only moderate and hence insufficient to be used for reliable prediction or read-across purposes in chemical assessments. We find that the deviation between PAMPA and Caco-2 permeabilities is not primarily driven by active transport mechanisms. Furthermore, the lack of standardized PAMPA protocols makes literature reported permeability measurements highly sensitive to test conditions. Therefore, using PAMPA permeability data without considering the appropriateness of test conditions may result in misleading conclusions in chemical assessments. In addition, we reveal that a chemical's partitioning and permeability are not inherently interdependent, and therefore, permeability should be treated as a fundamental parameter, alongside partition ratios and reaction rate constants, in human exposure and toxicokinetics assessments.

4.05.T-05 Microplastics analysis and the infrared spectrum: Validating methodology with respect to spectral range

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Microplastic contamination is an emerging threat to environmental and ecological systems across the globe. Studies have shown that plastic particles cause harm to multiple types of organisms through exposure to toxic substances. Despite this, the full extent of the impact of microplastics remains unclear due to challenges in developing reliable methods for characterizing this broad class of micro-sized polymers. Validation of methodology is one key area of interest as it has been noted that deficiencies in this area can impact the comparability of studies and the data presented. Infrared (IR) spectroscopy is an established technique for characterizing microplastics. It identifies the molecular composition and structure of materials by measuring the absorption of IR radiation. The resulting absorption bands, which are due to intramolecular vibrational modes, help identify organic or mineral materials through comparison of spectra with a library of spectra from known polymers. IR spectral acquisition is typically performed between 750 and 4,000 cm^{-1} ; however, only specific regions are used for the identification of microplastics. These spectral ranges are selected based on considerations such as exclusion of regions with a low signal-to-noise ratio (S/N), substrate or filter limitations, or instrument manufacturer specifications. This study investigated the impact the choice of IR spectral range on the identification of microplastics using three data collection modes (FTIR-ATR, μ FTIR-FPA, QCL-IR) and multiple spectral ranges. In doing so, it suggests a methodology for the validation of the spectral matching process that is critical to this technique.

4.05.T-06 Modeling the degradation rates of plastics in the environment

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The production and use of plastics has led to growing problems associated with end-of-life (EOL) effects given that disposal remains highly inefficient, with recovery and recycling rates below 10% globally. This has led to the proliferation of micro and nano-scale plastics in many areas of the environment where their ease of biouptake, ability to sorb other pollutants and potential for chemical and mechanical transformation risk numerous ecological and human health impacts. To mitigate these impacts there has been a significant push toward the design and development of novel polymer materials with comparable functional properties to current standards but with significantly more favorable degradation characteristics. This effort, however, requires the ability to predict the environmental persistence, or degradation, of a given material early in the design phase, to minimize the costs associated with even small-scale production and subsequent long-term degradation experiments. To this end we have developed computer models to predict the end-of-life degradation properties of plastics in the environment using the molecular structure considerations as model inputs. These models are developed with both quantum chemistry (QC) and machine learning (ML) techniques as QC enables ab initio calculations of chemical properties from molecular structure and ML uncovers complex relationships between features and output. We quantify plastic degradation kinetics through the use of specific surface degradation

rates (SSDRs) which are size and shape normalized and thus allow for comparison across different types of commercial and laboratory plastic products. Initial results from a random forest (RF) regression of about 200 polymer degradation samples performed well with a root mean square error (RMSE) of 0.84 (log (um/yr)) across eight orders of magnitude in SSDR values. Additionally, an analysis of feature importance has revealed interesting and useful relationships between degradation rates and the thermal properties of polymer materials. Predicting the persistence of polymers in the environment is a critical step toward the design and development of new polymer materials with less environmentally harmful fate and effect characteristics. The tools that come from this work will allow for screening such novel material candidates early in the design phase, to limit potential false starts and environmental liabilities.

4.05.V Contaminant Property Measurement and Estimation: Methods and Applications

4.05.V-01 Generating Abraham ppLFRs parameters for solvent-water partitioning for neutral PFAS and other chemicals using only molecular structures and COSMOtherm

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For predicting partition coefficients using the Abraham poly parameter linear free energy relation (ppLFR); $\log K = sS + aA + bB + vV + lL + c$, a method is presented that can simultaneously estimate all the ppLFR parameters: the solute (chemical) SABVL and solvent-water (system) sabvlc parameters for neutral organic chemicals in general and for neutral PFASs in particular. The method uses the solvent-water partition coefficients computed using the COSMOtherm quantum chemistry solvation model. A large data set (N = 10962) of partition coefficients for 54 solvents and 203 chemicals including 78 fluorinated chemicals requires estimating the 933 solute parameters (SAB) and system (sabvlc) parameters using the 10962 logK partition coefficients. A nonlinear regression method has been successfully applied to this very large problem: fitting approximately 1000 parameters to 10,000 observations. The quality of the resulting fit is remarkable, root mean square error RMSE = 0.082. The resulting ppLFR is tested using observed partitioning data not used in the fitting. For partitioning into 21 solvents the RMSE ranged from 0.4 to 0.6 with log Kow, RMSE = 0.501. The fluorinated chemicals ppLFR predictions exhibit a deviation is related to the chemical volume V computed by COSMOtherm. With a correction based on the number of CF₂ groups in the chemical the resulting fit for fluorinated chemicals is reduced to RMSE = 0.275. This demonstrates the utility of reducing the COSMOtherm partition coefficients to a ppLFR. Defects can be corrected in the ppLFR parameters that relate to the chemical bonding and energy terms whereas no similar corrections can be made for the COSMOtherm methodology. The development of this ppLFR methodology is an important step toward developing a similar method for producing ppLFRs for anionic PFAS. **Disclaimer/Disclosure:** Supported by SERDP ER22-3175 Measuring and predicting toxicity of PFAS mixtures

4.06.P-Mo Drop by Drop: Natural and Anthropogenic Drinking-Water Contaminant Exposures and Human Health Risk

4.06.P-Mo-109 Per- and polyfluoroalkyl substances (PFAS) in Montana private wells: A case study utilizing a more sensitive PFAS method

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Exposure to per- and polyfluoroalkyl substances (PFAS) through drinking water is a global concern, but information on drinking water PFAS exposures in private-well dependent communities remains limited. Tapwater samples were collected by volunteer community members in three southwestern Montana counties from 59 private-well dependent residences and assessed for PFAS using two different analytical methods. No PFAS were detected in the initial sample analysis using analytical Method 1 (MDL range: 5 – 22.8 ng/L, for 34

PFAS). However, re-analysis employing a more sensitive and analytically rigorous protocol, Method 2, (MDL range: 0.2 – 1.0 ng/L, for 54 PFAS) detected at least one PFAS in 53% (31/59) of wells. Twelve PFAS were observed at least once, with PFOS, PFOA, PFBS, PFHxS, FOSA, PFHpA, and PFHxA observed most frequently and in 10 - 25% of samples. The number of individual PFAS observed in a single well ranged from 0 to 7 with cumulative concentrations (sum of PFAS detected) ranging from 0 to 22.2 ng/L. A greater number of compounds were generally detected in shallower wells and on smaller sized properties, consistent with higher prevalence in younger (shallower) groundwater in areas with higher intensity land use. Cumulative risk assessments were conducted by calculating Toxicity Quotient(s) (TQ) based on available human-health benchmarks, to evaluate potential human-health effects from exposure to multiple PFAS in private-well tapwater. Human health risks were driven primarily by PFOA and PFOS. Cumulative TQ (ΣTQ) ranged from 0.03 to >1 in wells where PFAS were detected, with elevated risk ($\Sigma TQ \geq 1$) predicted in 25% of these wells. These findings document the presence of PFAS in private-well drinking water in rural and minimally developed areas and demonstrate the critical importance of employing PFAS analytical methods with detection limits below existing regulations to more accurately assess corresponding drinking water human-health risk.

4.06.P-Mo-111 Fireworks-induced perchlorate contamination of surface water

Efe Jeffery Isukuru, Jessica La Grenade, Todd Anderson, and Andrew Jackson, Texas Tech University

Perchlorate (ClO_4^-), a component of fireworks used extensively during celebrations across the U.S., can reach surface waters through atmospheric deposition, raising concerns about the potential for localized contamination of drinking water sources. This study investigated the potential for fireworks-induced ClO_4^- contamination in a ~8,600 acre lake in the southeastern U.S., selected for its proximity to a commercial display and its role as a local drinking water source. ClO_4^- concentrations were determined using ion chromatography-tandem mass spectrometry (IC-MS/MS; EPA Method 6860). Total atmospheric deposition samples were collected at two distances from the display site during the display. In addition, we also determined ClO_4^- concentrations in commercially available fireworks. Deposition from fallout during the display period ranged from 2.1 to 30.4 $\mu\text{g}/\text{m}^2$ and decreased with distance from the display. ClO_4^- concentrations in market-purchased fireworks ranged from 0.18% to 2.4% by weight. During the fireworks display, approximately 70 explosions occurred every two minutes over a 26-minute display; a typical commercial display uses 500-2000 kg of fireworks. Based on the change in deposition with distance and the predicted area of fallout, we estimated that a total of ~90 grams of ClO_4^- could have impacted the lake, only considering the fallout during the display period itself. Assuming complete mixing, this mass would have resulted in a concentration of 125 ng/L. However, for smaller lakes with similar sized displays, the concentrations could be much higher. Our findings highlight the transient yet potentially significant contribution of fireworks to ClO_4^- concentrations, particularly for small or enclosed water bodies where dilution is limited.

4.06.P-Mo-112 Perchlorate deposition attributed to fireworks displays across the United States

Jessica La Grenade, Efe Jeffery Isukuru, Todd Anderson, and Andrew Jackson, Texas Tech University

Fireworks, which contain perchlorate (ClO_4^-), are used extensively during Fourth of July and New Years Eve celebrations across the United States. Perchlorate can persist in the environment and reach surface waters through atmospheric deposition, raising concerns about the contamination of drinking water sources. In this study, we investigated whether a relationship exists between perchlorate deposition and fireworks usage throughout the United States during the July 4th holiday. Relationships between perchlorate flux and population density were examined as well as whether consumer fireworks had a meaningful contribution to perchlorate deposition rates. 1,172 wet deposition samples were collected from 75 National Atmospheric Deposition Program (NADP) monitoring stations during June, July 4th, mid-July, and August throughout a 5-year period (2019 – 2023). ClO_4^- concentrations were analyzed using ion chromatography-tandem mass spectrometry (IC-MS/MS; EPA Method 6860). ClO_4^- flux was significantly affected by time of fireworks use ($p < 10^{-27}$), with levels peaking on July 4th, remaining elevated afterward, and declining by August. The average perchlorate flux from all NADP sites during the week of July 4th was 14 mg/ha/week compared to 4.6 mg/ha/week in June and

6.5 mg/ha/week in August. During the week of the 4th, sites classified as urban by the NADP (≥ 400 people/km²) were determined to have a higher perchlorate flux compared to suburban, rural, and isolated sites. This relationship was observed in various sampling locations including states with legal, illegal, and restricted consumer fireworks usage. Regardless of state firework laws, there was a widespread and persistent atmospheric ClO₄⁻ deposition following municipal firework displays. This study, along with dry deposition data from future work, will allow drinking water sources to be evaluated for risks of perchlorate contamination attributed to fireworks displays.

4.06.T Drop by Drop: Natural and Anthropogenic Drinking-Water Contaminant Exposures and Human Health Risk

4.06.T-01 Assessing risk from cyanotoxins in lake and tap water at Tenmile Lakes: Participatory approaches to freshwater CyanoHABs in rural Oregonian household source waters

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Global freshwater cyanobacterial harmful algal blooms (cyanoHABs) threaten human and animal health, alter ecosystems, and devastate local economies dependent upon surface waters for recreation, fisheries, and livelihoods. Cyanotoxin breakthrough at treatment plants forces municipal drinking water moratoriums and public health emergencies for urban populations, yet rural households reliant on impacted surface waters are largely unmonitored. Roughly 25% of the population at Lakeside, Oregon, draws water from Tenmile Lakes with reoccurring cyanoHABs. Through community based participatory research, we partnered with 10 spatially distributed lakeside households with self-installed and maintained potable treatment systems for longitudinal monitoring of intake lake and tap water from March 2023 to March 2025. Our collaborative goals were: 1) to identify cyanobacterial metabolites present in lake water across seasons, 2) to evaluate community and household risk based on federal and state recreational and drinking water guidelines for cyanotoxins, and 3) to evaluate whether treatment systems reduce levels in tap water below available guidelines. This multimethod research employed community-based research design, citizen science training, longitudinal water sampling and detection of cyanotoxins by HPLC-MS/MS and ELISA, and surveys and focus groups with community members. We identified significant levels of microcystins and anabaenopeptins in lake and tap water. While most samples did not exceed USEPA or Oregon Health Authority (OHA) drinking water guidelines, metabolite breakthrough was observed in all potable water systems with some tap samples exceeding general and vulnerable group guidelines. We encountered high levels of distrust of state and local public health agencies because of conflicting messaging, lack of transparency, and absence of hazards communication about blooms, as well as frustration regarding lack of information and best practices for rural household treatment. Preliminary data and discussion of results with community members suggest need to highlight importance of routine and frequent maintenance and flushing of potable water systems, frequent changing of filters, extra testing during blooms, and additional point of use filtration or bottled water during blooms for all individuals and year-round for vulnerable persons. Our findings highlight potential cumulative exposure to cyanobacterial metabolites in rural drinking water sources. **Disclaimer/Disclosure:** Funding was provided by the Natural Hazards Center at University of Colorado Boulder, Public Health Disaster Research Award P31 (2023)

4.06.T-02 Haloacetonitrile and trihalomethane concentrations at point of use in rural drinking water systems impacted by elevated bromide concentrations

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Rural communities are vulnerable to the formation of disinfectant byproducts (DBPs) due to degraded

infrastructure, financial constraints, and large distribution networks with low demand. Nitrogenous DBPs have raised increasing concern due to their toxicity and tendency to form when source waters have elevated concentrations of nitrogen and bromide. While regulated DBPs such as trihalomethanes (THMs) have been considered to be an indicator for the presence of unregulated DBPs, such as haloacetonitriles (HANs), differences in precursors, disinfectants used, and variability within the distribution network can enhance the formation of other types of DBPs, reducing the reliability of THMs as an indicator for toxicity. This study analyzed the spatial-temporal complexity (e.g., seasonality, climate variables, water age, source water quality) characterized by rural Appalachian drinking water distribution networks and its relationship to HAN and THM concentrations. Monthly tap water (n homes=38; n total samples=663) was sampled over three years across two counties for DBPs. Source waters (n=2) were analyzed for monthly concentrations of DBP precursors (e.g., organic carbon, nitrate, chloride, bromide). The 3-year average concentration of total THMs was 0.075 ± 0.036 mg/L (62% chloroform; 24% bromodichloromethane; 11% dibromochloromethane; 3% bromoform), with 24 households' annual average concentration exceeding the EPA maximum contaminant level (0.080 mg/L). Total THM varied seasonally, with greater concentrations from July to November, correlating with increased temperature, tap water conductivity, and source water bromide concentrations. The average total HANs concentration was 0.016 ± 0.007 mg/L (35% dibromoacetonitrile; 32% bromochloroacetonitrile; 23% dichloroacetonitrile; 10% trichloroacetonitrile) with far less seasonal variation compared to THMs. Continued analysis of the spatial-temporal trends in THMs and HANs throughout the distribution networks will further elucidate the complex relationship between tap water DBPs. Findings will support predictive models to help small drinking water utilities reduce their formation. **Disclaimer/Disclosure:** This project was supported by the National Institute of Environmental Health Sciences (NIEHS) grants P30ES026529 and R01ES032396. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS.

4.06.T-03 Assessing point-of-use carbon filters as monitoring devices for organic contaminants in drinking water

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Over 43 million people in the United States, including nearly half of rural households, obtain their drinking water from private wells, yet private wells are not federally regulated via the Safe Drinking Water Act, putting the health of rural communities at risk. Private wells can be impacted by diverse organic contaminants including per- and polyfluoroalkyl substances (PFAS), pharmaceuticals, and agrochemicals. Determining accurate concentrations of these organic contaminants in drinking water via standard sampling procedures (e.g., grab sampling) can be challenging due to temporal variation in inputs, such as seasonal variation in herbicide use, or the occurrence of contaminants at concentrations below detectable limits. In this study, we hypothesized that point-of-use (POU) carbon filters, which are used to strip contaminants from drinking water immediately prior to consumption, can also be used to provide time-integrated and volume-averaged information about contaminant levels in source water. To replicate the accumulation of contaminants during filter use in rural households, we first loaded selected organic compounds in synthetic groundwater onto faucet-mount POU filters and measured filter effluent to evaluate the retention of compounds on the POU filters (including those not commercially rated for filter removal). Following effective filter retention of the selection compounds, we then investigated the extraction of those compounds from the POU filters using liquid chromatography with tandem mass spectrometry (LC-MS/MS). We assessed multiple methods for extracting organic contaminants from POU filters, including sonication assisted extraction and accelerated solvent extraction, and investigated the impact of additional factors such as time or solvent type to achieve the highest compound recovery. By combining (1) the extracted contaminant mass from a used POU filter, (2) the method-determined recovery percentage of the individual contaminant, and (3) the cumulative volume of water treated by the POU filter during use, the time-averaged concentration of each contaminant in the drinking water throughout the filter lifespan can be calculated. This study is a key step in validating the use of POU filters to increase accessibility to safe drinking water from private wells in rural regions by simultaneously reducing contaminant levels in

drinking water and providing information about source drinking water quality. **Disclaimer/Disclosure:** This work is supported by the US National Science Foundation (2344232, ECO-CBET 2033714), and the Monticello College Foundation/Washington University in St. Louis (Spencer T. and Ann W. Olin Fellowship for Women in Graduate Study to Jean M. Brownell)

4.06.T-04 Wildfire impacts on drinking water quality assessed using nontarget screening

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Wildfires are growing in frequency, size and ferocity across the planet, driven significantly by human-induced climatic changes. Beyond the immediate threats to human life and health that occur during active wildfires, numerous threats can persist long after the fire is extinguished. One critical aspect of these threats relates to the public water systems supplying communities impacted by wildfires, especially those at the wildland-urban interface. Two important sources of water contamination following fires include: (i) transport of wildfire related ash, debris and other combustion byproducts into water systems depressurized by a combination of leaks from structures being destroyed and firefighters placing maximum water demands on the system and (ii) thermally induced changes to infrastructure including internal pipe linings, piping, storage reservoirs, and connected fittings of the distribution system. The likelihood and extent of contamination are influenced by factors such as temperature, timing of system depressurization and re-pressurization, post-fire flushing frequency and effectiveness, and type and age of piping materials and infrastructure. Contaminants entering the systems can include both the “usual suspects” of volatile and semi-volatile organic compounds (VOCs and SVOCs including ketones, benzene derivatives, and polycyclic aromatic hydrocarbons (PAHs)) along with less expected or even unknown compounds that may be formed by combustion of natural and human-made materials found in and around structures that have burned, with fire temperature dictating the formation of byproducts. This study focuses on water samples taken as public water systems were returned to service after devastating fires in Maui, Hawaii, USA during August 2023. After using standard analytical methods to ensure that regulated volatile and semi-volatile organic compounds did not exceed regulatory limits, 18 samples were collected for nontargeted analysis on two dates from varying points in the system. All water samples (1 L) were solid phase extracted (HLB) and the extracts were concentrated and analyzed using gas chromatography with quadrupole time-of-flight mass spectrometry. Data is explored using clustering algorithms, correlations with target VOCs and SVOCs, and comparisons with literature sources to provide insights into compounds of potential health concern that are not captured by standard target methods.

4.06.T-05 Statewide cumulative human health risk assessment of inorganics-contaminated groundwater wells, Montana, USA

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Across the United States, rural residents rely on unregulated and generally unmonitored private wells for drinking water, which may pose serious health risks due to unrecognized contaminants. We conducted the first assessment of the nature, degree, and spatial distribution of cumulative health risks from inorganic contaminants in groundwater wells across Montana. Our analysis included nearly 84,000 data points from 6,500+ wells, across 51 of our state's 98 watersheds, using a public groundwater database. We compared a drinking water screening level cumulative risk assessment (CRA) for inorganics based on the U.S. Environmental Protection Agency (EPA) protective health thresholds (Maximum Contaminant Level Goals, Health Advisories [MCLG-HAs]) to a CRA based on EPA public supply enforceable Maximum Contaminant Levels (MCLs). Based on median concentrations of 19 inorganics (antimony, arsenic, barium, beryllium, boron, cadmium, chromium, copper, fluoride, manganese, molybdenum, nickel, nitrate, lead, selenium, strontium, thallium, uranium, zinc), 75% of watersheds had MCLG-HA-based cumulative risk values > 1.0; arsenic and uranium contributed the most risk, followed by strontium, fluoride, manganese and boron. Hence, this screening level (Tier I) CRA

indicated widespread potential for unrecognized human health risk to private well users from inorganic contaminants considering both carcinogenic and non-carcinogenic risks. Sensitivity analysis showed that benchmarks applied (MCLG-HAs versus MCLs) exerted the largest control on results, with MCL-based assessments underestimating the health risks. Our findings identify priority regions for Tier 2 risk assessments to elucidate local sources and distributions of geogenic versus anthropomorphic contaminants. The methodology, including R code, is published in Hydroshare and could readily be applied in other regions. Our study is the first statewide assessment of cumulative health risk from groundwater that we are aware of, and results support increased statewide drinking water education and testing to reduce human health risks from consuming contaminated private well water. **Disclaimer/Disclosure:** This material is based upon work supported in part by the National Science Foundation EPSCoR Cooperative Agreement OIA-1757351. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Additional funding was provided by the Montana Institute on Ecosystems and the Montana Water Center grant #WA538, National Institutes of Water Resources, U.S. Geological Survey award #G21AP10628-02. PMB and KLS were funded by the U.S. Geological Survey through the Environmental Health Program of the Ecosystem Mission Area. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

4.06.T-06 Exposure to metal(loid)s and organochlorine pesticides via breastmilk in infants living in the Lake Atitlán watershed, Guatemala

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Guatemala has the highest child stunting prevalence in the Americas and is ranked sixth internationally. We examined the concentrations of toxic metal(loid)s and organochlorine pesticides (OCPs) in the breastmilk of Mayan women in the Lake Atitlán watershed to explore potential relationships with stunting prevalence to guide future research. We completed a cross-sectional study in 2022 (n=80 mother/infant dyads) with 20 mother/infant dyads from each of four communities: San Juan La Laguna, San Pedro La Laguna, Panajachel, and Santiago Atitlán. We collected a breastmilk sample from each mother and measured the length of each infant to assess stunting prevalence (<-2 SD length-for-age below WHO growth standards median). The mean length-for-age z-score (LAZ) was -1.39 (SD = 1.24). Infants from Panajachel had a higher prevalence of stunting ($\bar{x}$ = -2.06) than did infants from the other communities. A panel of 20 metal(loid)s were quantified using inductively coupled plasma-mass spectrometry (ICP-MS) and OCPs were analyzed using gas chromatography-mass spectrometry (GC-MS). The mean concentrations of As and Pb in breastmilk were above the WHO safety standards in Panajachel and Santiago Atitlán, and concentrations of toxic metal(loid)s overall were highest in Panajachel. For infants from Panajachel and Santiago Atitlán, reduced LAZ were associated with higher breastmilk concentrations of As, Ba, Be, and Pb (p=0.02-0.04). Water samples from these two communities exhibited greater concentrations of As and Ba (p=0.003–0.03). We detected all 18 OCPs in some breastmilk samples, and 11 had at least five samples above the LOD (2,4'-DDD, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, cis-heptachlor epoxide, HCB, heptachlor, pentachlorobenzene, trans-chlordane, trans-heptachlor epoxide, trans-nonachlor). Concentrations were the highest for 4,4'-DDE (mean = 60.6 ng/g lipid weight, maximum = 313.1 ng/g lipid weight). Pesticide concentrations in breastmilk were not associated with stunting prevalence; however, the concentration of 4,4'-DDT was marginally associated with lower Z-score values (p = 0.08). Significant associations between concentrations of As, Ba, Be, and Pb with low LAZ warrant a comprehensive assessment in their role in child growth and development. Stunting is a complex multifactorial problem; our results indicate that investigations should also evaluate environmental exposures in addition to the traditional causal factors.

4.06.V Drop by Drop: Natural and Anthropogenic Drinking-Water Contaminant Exposures and Human Health Risk

4.06.V-01 Predicting occurrence of per- and polyfluoroalkyl substances (PFAS) in groundwater at the depths of drinking water supplies

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A myriad of adverse human health effects have been associated with exposure to per- and polyfluoroalkyl substances (PFAS) resulting primarily from inhalation and ingestion of food and water. Large-scale sampling efforts are underway for drinking water at both federal and state levels within the United States to help identify contamination and evaluate risk to the public. However, analysis of PFAS is expensive, time consuming, and rarely includes private well owners or small drinking water utilities that are common in rural areas. Predictive models help overcome some of these challenges and can be used to identify areas of concern for PFAS in aquifers used for drinking water supplies and direct limited resources to these areas. Here we present a machine learning model that predicts occurrence of PFAS in groundwater at the depths of public and private drinking water supplies in the conterminous United States. More than 1,200 groundwater samples were used for model training. These samples were collected using consistent methods and analytical protocols at locations spanning a broad range of geographic regions. The sampling targeted either drinking water supplies within a principal aquifer or specific land uses in areas overlying drinking water aquifers. The total sum of 24 PFAS was converted to a binary dependent variable (detected or not detected) for modeling, as any detection indicates anthropogenic influence. We used only mappable factors to enable predictions across the conterminous United States, allowing predictions in unmonitored locations where water-quality data are unavailable. In order of importance, model variables retained in the final model were percent urban land use, well depth, percent clay in soil, estimated nitrogen loading from septic systems, average annual natural groundwater recharge, population density, depth to groundwater, and distance to the nearest site potentially containing, using, or emitting PFAS. We found that 71 - 95 million people in the United States potentially rely on groundwater-sourced drinking water contaminated with PFAS. This work provides information to water resource managers and private well owners about where PFAS in groundwater are predicted to occur, and also where PFAS are less likely to occur. This talk will discuss model development and results and will also briefly describe ongoing follow-up modeling efforts.

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4.07.A.T Environmental Contaminants in Animals: Exposure, Pharmacokinetics and Risk Assessment

4.07.A.T-01 Updates on the use of congener-specific bioaccumulation factors for evaluation of dioxin TEQ in wildlife diets

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Dioxins and furans comprise a group of 210 individual congeners, of which 17 are known to act via a shared mode of action and elicit “dioxin-like” toxicity. Exposure to these 17 congeners is typically expressed as dioxin TEQ, a calculated sum that accounts for the differential toxicity of each congener using toxic equivalence factors (TEFs) relative to 2,3,7,8-TCDD. While congener-specific toxicity assumptions are well-established for dioxin TEQ, congener-specific uptake assumptions are not commonly used in ecological risk assessment. Dioxin TEQ risk assessment has traditionally been conducted using bioaccumulation assumptions for a single congener, 2,3,7,8-TCDD, to evaluate potential exposure to dioxin TEQ in the diet. Congener-specific uptake factors have been developed previously but are not widely used or accepted by regulators. Focusing on

invertebrate uptake, the current study provides a review of the congener-specific approaches, including available congener-specific bioaccumulation factors (BAFs) as well as development of bioaccumulation equivalence factors (BEFs) for use with the traditional 2,3,7,8-TCDD-based BAFs. The congener-specific BAFs and BEFs are also updated herein to include data from more recent publications and a sensitivity analysis is provided based on multiple interpretations of the congener bioaccumulation data. Wildlife risk estimates are presented from a site in southern Mississippi to demonstrate the effect of incorporating congener-specific bioaccumulation assumptions when estimating dietary concentrations in terrestrial invertebrates. Using the multiple interpretations of the congener-specific BAFs at the example site consistently predicts lower uptake of dioxin TEQ in invertebrate dietary items than when estimated assuming uptake for all congeners is the same as for 2,3,7,8-TCDD.

4.07.A.T-02 Longitudinal comparisons of unsubstituted and alkylated PAHs in crayfish and predictive modeling from passive samplers at an urban Superfund site

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Monitoring chemical contaminants in industrial waterways is vital for environmental and human health. Resident signal crayfish, *Pacifastacus leniusculus*, were paired with low-density polyethylene (LDPE) passive sampling devices (PSDs) from sites within and outside Portland Harbor Superfund Megasite and compared to past crayfish and LDPE collected in 2003 and 2013 in the Willamette River, Oregon. Polycyclic aromatic hydrocarbons (PAHs) and alkylated derivatives (a-PAHs) were extracted and analyzed from crayfish tail, visceral tissues, and water-deployed LDPE using GC-MS/MS. Within the harbor, Σ PAH concentrations in crayfish viscera decreased between 2003 and 2013 but increased to 2003 levels in 2023, suggesting early remediation efforts initially reduced contamination, especially at a separate creosote Superfund site. Individual PAH distributions varied across sites and years, indicating changing sources. Upriver, crayfish Σ PAH concentrations increased each decade, and 2023 benzo[a]pyrene carcinogenic equivalents exceed 2003 and 2013 upriver and 2023 harbor levels. In 2023, Σ a-PAH concentrations were 20x and 15x higher than Σ PAHs in crayfish tails and viscera, emphasizing the need for further toxicological research. Crayfish visceral PAH and a-PAH concentrations were modeled using concentrations from water-deployed LDPE. Predicted crayfish concentrations were within a 1.8-fold median difference of measured concentrations. This model enables non-invasive, resource-efficient approaches for future shellfish monitoring. **Disclaimer/Disclosure:** This research was supported by the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH) and by the Superfund Research Program (SRP) in partnership with Oregon State University under award number P42ES016465. This content is solely the responsibility of the authors and does not represent the official views of the NIEHS or NIH. The Pacific Northwest National Laboratory is a multi-program laboratory operated by Battelle for the U.S. Department of Energy under contract DEAC05-76RL01830.

4.07.A.T-03 A Framework for understanding the impact of chemical pollution through avian sentinel species of the North American Great Lakes

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The North American Great Lakes have been subjected to pollution from decades of industry and manufacturing. Consequently, several bioaccumulative, synthetic organic pollutants are found in avian sentinel species throughout the region. While concentrations of pollution in these birds are widely reported, there is no unifying model to describe them. Therefore, we used data from approximately 350 avian blood serum samples to develop a simple model that can be used to assess their impact. These samples came from 199 Herring gulls (*Larus argentatus*) and 150 Bald eagles (*Haliaeetus leucocephalus*) sampled throughout the state of Michigan. They were analyzed for approximately 200 target analytes, including 21 polybrominated diphenyl ethers (PBDEs), 12

non-PBDE flame retardants, 85 polychlorinated biphenyls (PCBs), 17 organochlorine pesticides (OCPs), and 36 ionic PFAS. These samples were collected from 2016 to 2024, with over 90% of the samples collected from 2014 to 2024. In most samples, S36 PFAS made up the highest portion of the contaminant burden (41-74% in gull serum, and 48-71% in bald eagle serum). Generally, PBDEs and OCPs made up very little of the contaminant burden (8-13% in gull serum, and <1-13% in bald eagle serum). PCBs contributed the highest contaminant burden (50% in herring gull serum) in an area of known PCB contamination that is currently under remediation. To model the quality of the habitat based on these chemicals, parameters of the Canadian Water Quality Index (WQI) were modified. These modifications were essential to compare lipophilic (legacy) compounds to proteinophilic (PFAS) compounds, as well as compounds that do not have published toxicological data. This modified model was named the Sentinel Species Habitat Quality Index (SSHQI). The SSHQI can be used to communicate the burden of chemical pollution more easily to both regulators and the general public. However, the lack of published experimentally determined physicochemical properties and toxicological information for newer compounds, such as PFAS, limits the model's scope. For example, the theoretically calculated acidity constant (K_a) of some PFAS vary by orders of magnitude in different publications. To improve this model, more expansive toxicological data are needed for the various congeners, isomers, and molecules in each of the chemical classes measured.

4.07.A.T-04 Integrating novel passive sampling and archived biopsies of bottlenose dolphins (*Tursiops truncatus*) from San Diego Bay to identify molecular biomarkers of long-term exposures to complex chemicals

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Quantifying long-term effects of environmental contaminants on marine mammals' health is challenging in part due to individual spatiotemporal variation within and among wild populations. While sampling free-ranging marine mammals' tissues from natural populations may provide informative cross-sectional data, it is difficult to discern effects of environmental pollutant exposures from other variables such as diet and life history. A population of bottlenose dolphins (*Tursiops truncatus*) living in San Diego Bay (San Diego, California) allows for more precise quantification of environmental chronic chemical stress effects while controlling for critical confounders such as diet, health history, and healthcare. The dolphins housed in netted ocean enclosures and cared for by the U.S. Navy Marine Mammal Program in San Diego, California receive high quality healthcare, nutrition, and daily exercise, and are health-monitored and managed extensively throughout their lifetimes. This regiment provides a unique opportunity to study environmental effects under relatively controlled conditions. Industrial chemicals and persistent organic pollutants (POPs) historically present in San Diego were sampled using novel Composite Integrative Passive Sampler (CIPS); deployed for two two-week periods during dry and wet weather conditions at multiple locations within the Navy Dolphin housing facility, CIPS accumulated both polar and non-polar ocean contaminants. Targeted analysis of primary organic pollutants via GC/MS and LC/MS identified over 100 organic pollutants including polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pesticides, herbicides, pharmaceuticals, and plasticizers. In parallel, dolphin archived fatty tissues—blubber and liver from four pairs of mother-calf biopsies—were analyzed for chemical body-burdens quantified in CIPS analysis. Integration of environmental and tissue analytical chemistry helps further identify molecular biomarkers of life-time exposure to complex chemical mixtures. Many of the environmental organic compounds detected in dolphin blubber and liver suggest bioaccumulative potential and potentially long-term health effects. Assessment of a unique dolphin population in San Diego Bay, provides insights about long-term exposure effects to environmental contaminants on marine mammal health with broader implications for comprehensive marine ecological risk assessment modeling.

4.07.A.T-05 Pharmacokinetics of potential eco-friendlier anticoagulant rodenticides

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Anticoagulant rodenticides (ARs) are used on a global scale for the control of vertebrate pest species. Despite efforts to mitigate risk, there are numerous reports documenting AR exposure of non-target predatory wildlife, and depending on dose, adverse effects and mortality may occur. Widely used second-generation ARs (SGARs) are mixtures of cis- and trans-stereoisomers that are equipotent when tested in vitro (microsomal vitamin K epoxide reductase activity from susceptible rats) and in vivo (no-choice feeding trials in rodents). Remarkably, some stereoisomers are rapidly eliminated in vivo, resulting in residue patterns in exposed target rodents that differ from the AR bait formulations. Use of less persistent but equally potent SGAR isomers in baits could reduce risk to non-target predatory wildlife. We examined the toxicity of three concentrations (0.05, 0.1 and 0.5 ug/g) of two diets containing different brodifacoum (BROD) stereoisomers (Formulation A with the 2 least persistent stereoisomers in rats; Formulation B containing only the most persistent stereoisomer) using American kestrels (*Falco sparverius*; a model for raptorial birds) in a 7-day feeding trial. Relative to unexposed controls, kestrels ingesting 0.5 ug/g diet of the longer-lived BROD Formulation B exhibited extreme coagulopathy (prolonged prothrombin time and Russell's viper venom time), while the same dietary dose of the shorter-lived Formulation A had no effect on blood clotting time. Whole liver BROD residues in exposed kestrels were directly related to dietary dose, with retention of Formulation B being 2 to 7 times greater than Formulation A. Hepatic microsomal vitamin K epoxide reductase (VKOR) activity, the target enzyme inhibited by ARs, was inversely related to BROD dietary concentration. Estimation of the in vitro median inhibitory concentrations (IC₅₀) of BROD Formulations A and B are currently being evaluated. These findings support the notion that SGAR baits enriched with less persistent stereoisomers pose lower hazard to non-target wildlife, and may be an eco-friendlier alternative to current use SGAR formulations. **Disclaimer/Disclosure:** This project was funded by the U.S. Geological Survey through the Environmental Health Program (BR, JL) and the Biological Threats and Invasive Species Research Program (RE) of the Ecosystems Mission Area. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

4.07.A.T-06 Exposure pathways of microplastics in a local population of bottlenose dolphins (*Tursiops erebennus*)

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As long-lived coastal residents, bottlenose dolphins are ecosystem sentinels that can indicate ecological change and potential public health concerns. Estuarine populations of Tamanend's Bottlenose Dolphins (*Tursiops erebennus*) are apex predators at risk of ingesting contaminants that persist in the marine food web, such as microplastics. However, there are no long-term studies assessing microplastic exposure pathways in a wild population of bottlenose dolphins. The current study assessed microplastic ingestion and transfer pathways in a population of dolphins residing within the urbanized harbor of Charleston, South Carolina. Gastrointestinal tracts were collected during routine necropsies on dolphins that stranded in the Charleston Harbor from 2015-2024 (n=50). Stomach and intestine samples were dissected and processed for microplastics $\geq 125\mu\text{m}$ using both visual identification and vibrational spectroscopy. Additionally, the dolphins' preferred prey species (Atlantic Croaker, Silver Perch, Spot, Star Drum, and Striped Mullet, n=20 of each species) were sampled from the Charleston Harbor to estimate trophic transfer of microplastics to local seafood consumers- including adult dolphins and humans. Preliminary results indicate high levels of microplastic ingestion in dolphins (877 ± 161 , mean $\pm$ SE) and in fish (32 ± 5 , mean $\pm$ SE). Microplastic concentrations were higher in dolphin calves compared to adults, indicating that maternal transfer is a significant exposure pathway in bottlenose dolphins in addition to trophic transfer from contaminated prey. These results will provide ecologically-relevant exposure levels and transfer pathways of microplastics in the coastal region to inform management strategies that will best protect wildlife, ecological, and human health.

4.07.B.T Environmental Contaminants in Animals: Exposure, Pharmacokinetics and Risk Assessment

4.07.B.T-01 Changing composition of legacy and novel per- and polyfluoroalkyl substances (PFASs) in Hudson River fish at three timepoints (2001, 2006, 2014)

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Per/polyfluoroalkyl substances (PFASs) are highly persistent synthetic compounds extensively used in various commercial applications, leading to their widespread distribution in the environment. PFASs pose significant risks to aquatic ecosystems and human health. Current PFAS risk assessment strategies tend to focus on contemporary contamination, often overlooking the long-standing and historical presence of these compounds in environmental media. Additionally, focus has always been on well-known legacy PFASs like PFOS. However, this often overlooks historical contamination and fails to account for the rising presence of newer, less-studied PFASs—including short-chain compounds, ether-based alternatives, and other emerging structures. This study aims to fill that gap by examining how PFAS contamination profiles in fish from the upper Hudson River have evolved over time. By analyzing archived fish tissue samples from 2001, 2006, and 2014, we explore shifts in both the types and concentrations of PFASs, and how these changes reflect broader trends in chemical production and regulation. We hypothesized that levels of legacy PFASs, particularly long-chain perfluoroalkyl acids (PFAAs), have declined over the years, while the presence of short-chain, ether-containing, and other emerging PFASs has increased. We used high-resolution mass spectrometry (LC-HRMS) for both targeted quantification and suspect screening of PFASs in fish tissues using an expanded analyte list to capture a broader range of compounds. Results indicate a growing number of fluorinated features over the sampling years, suggesting increasing diversity of PFAS contamination. To enable cross-year comparisons, literature-based conversion factors were used to estimate liver concentrations from 2014 whole-body data, to compare with liver samples from 2001. Results show an increasing number and diversity of fluorinated compounds over time, indicating a growing complexity of PFAS contamination. The concentration of legacy PFASs appeared relatively stable between 2001 and 2014. However, paired serum and liver samples from 2001 revealed that serum contained a higher proportion of long-chain legacy PFASs, such as PFOS—highlighting tissue-specific differences in PFAS distribution. Together, these findings offer insights into the long-term dynamics of PFAS contamination in aquatic organisms and underscore the importance of including both legacy and emerging PFASs in future risk assessment efforts.

4.07.B.T-04 Evaluation of per- and polyfluoroalkyl substances (PFAS) in eggs of higher trophic level birds

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The goal of this ongoing study is to evaluate exposure of higher trophic level, water-associated birds, common loon (*Gavia immer*), double-crested cormorant (*Nannopterum auritum*), and osprey (*Pandion haliaetus*), to per- and polyfluoroalkyl substances (PFAS). In this study, PFAS have been measured in archived, abandoned eggs collected throughout Maine, encompassing heavily populated and remote areas, and analyzed using high-resolution liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QToF). To-date, analyses have been completed on 187, 15, and 32 loon, cormorant, and osprey eggs, respectively. PFOS was the most abundant compound in all eggs, comprising 55% of total PFAS in cormorant samples, 42% in osprey samples, and 32% in loon samples. PFOS exceeded a proposed egg toxicity reference value (TRV) of 92.4 ng/g wet weight in approximately 13% of samples in all species. To explore potential developmental effects, embryonic development in eggs was assessed using a scaled system of embryological stage and compared with PFAS concentrations to evaluate the relationship between PFAS concentration and development stage at abandonment. To-date, no relationship has been identified. A similar study was conducted in New Hampshire, with PFAS concentrations reported for 144 loon eggs. Comparisons of results for eggs from Maine and New Hampshire will be discussed. In addition, PFAS concentrations in eggs will be compared to nearby surface water, forage fish, and game fish concentrations in Maine. These comparisons aim to evaluate spatial patterns, characterize anthropogenic backgrounds and potential point sources, provide insight on PFAS bioaccumulation

levels, and explore likely exposure pathways for birds. We will use our findings to consider the application of laboratory-based TRVs to wild bird populations and guide further investigation of PFAS exposure and effects in birds. Results will aid in understanding PFAS contamination and accumulation within aquatic ecosystems, inform PFAS risk assessments, and provide insights for contaminated site investigations.

4.07.B.T-05 The derivation and evaluation of uncertainty of water quality values in mammalian wildlife exposed to PFOS in lotic and lentic systems

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Perfluoroalkyl acids (PFAAs) are synthetic fluorinated organic compounds that can be released into the environment during manufacturing process, from commercial products and applications, or indirectly via oxidation of precursor molecules containing perfluoroalkyl chains. While PFAAs have received increased attention in monitoring programs, approaches and methods that can be used to assess the potential impacts of PFAAs on avian or mammalian wildlife health are still being evaluated. One approach, the USEPA's Great Lakes Initiative (GLI) has been used to establish water-quality values for mink and mustelids but was developed for persistent organics such as PCBs and DDT and has prescribed methods for deriving trophic position specific bioaccumulation factors (BAF) that are used to derive water quality values for wildlife such as mustelids. Because this approach was established for neutral, persistent organic compounds the use of this approach for PFAAs can be uncertain related to the derivation of BAFs that may not be representative of conditions at location being evaluated. This is particularly true when GLI values are applied to lotic systems that are within the Great Lakes Basin. To investigate some of these uncertainties, benchmark dietary and liver PFOS concentrations were determined using data from a multigenerational rat study and a mink pharmacokinetic study. Bioaccumulation and biomagnification factors were derived from laboratory and field-based values that are representative of lotic and lentic systems and were used to extrapolate toxic doses to water values (WVs) for mink and otter. In addition, sediment and food web structure was also considered relative to their impact on the magnitude of BAFs values used to derive wildlife water values. The results indicated that the sediments as well as food web structure can have an impact of exposure and bioaccumulation of PFOS by mustelids and need to be taken into consideration when deriving water values, especially when considering values in both lentic and lotic systems. These findings will be discussed relative to current and historical PFOS concentrations measured in surface water as they relate to mustelids in the Great Lakes basin.

4.07.P-We Environmental Contaminants in Animals: Exposure, Pharmacokinetics and Risk Assessment

4.07.P-We-022 How well do QSAR models work for tropical pesticides? Insights from a systematic evaluation of 112 pesticides

Patricia Fai¹ and Li Li², (1)University of Bamenda, Cameroon, (2)School of Public Health, University of Nevada, Reno

Pesticides play a critical role in global food security but pose significant environmental and health risks, particularly in tropical regions where data gaps hinder accurate risk assessments. In this presentation, we will share our systematic evaluation of four widely used QSAR toolkits (IFSQSAR, EPI Suite, OPERA, and QSARINS-Chem) for their performance in predicting properties of 112 pesticide active ingredients commonly used in Cameroon, a tropical country in central Africa. We compiled and curated experimentally derived data of partitioning [octanol-water (KOW), octanol-air (KOA), air-water (KAW) or Henry's Law Constant, and organic carbon-water (KOC)], solubilities [solubility in water (SW), solubility in octanol (SO), and vapour pressure (VP)], and reactivity [microbial biodegradation half-life (HLbiodeg)]. Partitioning and solubility data were harmonized using thermodynamic corrections. The performance of the QSAR toolkits was evaluated against the curated and harmonized experimentally derived values. In general, the QSAR toolkits performed best for KOW and SW; however, higher uncertainty was found for KAW, KOA, VP, and HLbiodeg. We found that using consensus values from multiple QSARs improved prediction robustness. Our multimedia fate and exposure modeling further demonstrated that compared to uncertainty in partitioning properties, uncertainty in reactivity

parameters (e.g., HLbiodeg) played a more influential role in exposure estimates. The presentation highlights the scarcity of experimental data for key properties, especially for ionizable and large-molecule pesticides. These findings underscore the need for expanded experimental datasets to refine predictive models and support evidence-based pesticide regulation in tropical regions.

4.08.A.T Environmental Exposure to Per- and Polyfluoroalkyl Substances (PFAS) From Non-Traditional Matrices: Exploring the Where and How?

4.08.A.T-01 PFAS results aren't always answers: Data challenges across non-traditional matrices

Noelle DeStefano, Metiri Group

PFAS data can carry the illusion of certainty, but interpreting those results, especially in non-traditional matrices, is rarely straightforward. As PFAS monitoring expands beyond drinking water into materials like biosolids, food packaging, landfill leachate, and consumer goods, professionals are encountering new layers of complexity in sampling, method selection, and reporting. This presentation explores common issues that undermine confidence in PFAS results: mismatched methods for unconventional matrices, unclear detection limits, and inconsistent application of QA/QC criteria. Drawing from field experience across multiple sample types, it highlights how well-intentioned data can become misleading, especially when regulatory frameworks haven't caught up to analytical capabilities. These challenges directly affect how environmental exposures are understood and communicated, especially when the matrix in question falls outside standard risk frameworks. Beyond the lab, communication breakdowns are just as common. Reports full of flagged results and qualifiers are often misread or misunderstood entirely by regulators, consultants, and clients. In non-traditional matrices where guidance is sparse, those gaps lead to confusion, overreaction, or misplaced trust. Rather than offering a one-size-fits-all fix, this talk outlines practical strategies to avoid common missteps when generating or using PFAS data. Attendees will walk away with a clearer understanding of what makes a dataset defensible and how to talk about PFAS results without overstating what they mean.

4.08.A.T-02 Accepted first action method for targeted PFAS screening in foods addressing AOAC SMPR 2023.003

William Lipps¹, Toshiya Matsubara¹, Dominika Gruszecka¹, Nozomi Maeshima², Kota Ishioka² and Manami Kobayashi², (1)Shimadzu Scientific Instruments, Inc., (2)Shimadzu Corporation, Japan

PFAS have been well studied for environmental testing, as they've been used in consumer products, machining, and firefighting foams, steadily leaching into our environment for more than 70 years. Unlike environmental samples, food samples are regulated through the FDA or USDA. While the recent EPA 1633A does include procedures for sampling fish and animal tissue, this method is not dedicated to screening PFAS in foods meant for human consumption. AOAC published SMPR 2023.003 asking the public to present a method to test for 30 PFAS from 11 food matrices, among them fish and animal tissue. As one of 4 submitting entities to this SMPR, we present an accepted first action method addressing the public need for PFAS testing in food. In this method we will present a general procedure of QuEChERS followed by SPE to prepare the sample before introduction to an LCMS, in this case the 8060NX triple quadrupole instrument. To address ecological and time concerns faced by analytical laboratories, the method is designed to reduce unnecessary sample preparation steps, and eliminates QuEChERS and concentration steps where unnecessary. Our method reached the limits of detection required by SMPR 2023.003 for all food categories for all PFAS compounds, often reaching limits lower than required.

4.08.A.T-03 Non-invasive detection of PFAS in wildlife: Method development and application to elephants and primates in Central and Southern Africa

Yaw Edu Essandoh, Chunjie Xia, Kevin Romanak, Daniella Chusyd, Michael Wasserman and Marta Venier, Indiana University, Bloomington

Per- and polyfluoroalkyl substances (PFAS) are widely used in industrial and consumer products and are

recognized as persistent environmental contaminants with global distribution. Despite increasing concern, data on PFAS exposure in terrestrial wildlife remain limited. This study presents a non-invasive method for detecting PFAS in wildlife feces, developed using solid-phase extraction (SPE) with weak anion exchange Strata PFAS (GCB/WAX) cartridges and quantified using liquid chromatography-mass spectrometry (LC-MS). The method was applied to fecal samples from elephants in Zambia (n=13) and primates in Uganda (n=18), reported here for the first time. Overall we detected 24 PFAS and the Σ 24 PFAS levels detected in Olive baboons (*Papio anubis*) in Uganda (mean: 6.41 ng/g) were approximately three orders of magnitude higher than those in African elephants (*Loxodonta africana*) (mean: 3.78 ng/g). In elephants, the short-chain compound perfluoropentanoic acid (PFPeA) was the dominant contributor, accounting for 55% of the Σ 24 PFAS burden (3.91 ng/g). For baboons, perfluorobutanoic acid (PFBA) was the most predominant compound, accounting for 27% of Σ 24PFAS (1.69 ng/g). Although the overall concentration was higher in primates, overall PFAS levels in both species were relatively low compared to values reported in highly contaminated environments. These findings show that analyzing fecal samples is a useful and practical way to monitor PFAS exposure, especially in remote or hard-to-access areas. This approach helps improve our understanding of how these contaminants are spread and the risks they may pose to wildlife in terrestrial ecosystems. These results also show that PFAS are present in wildlife in protected parks and remote areas, confirming their status as ubiquitous chemicals.

4.08.A.T-04 Rapid detection and analysis of per- and polyfluoroalkyl substances in consumer products and environmental matrices

Esmee Belzer and Graham F. Peaslee, University of Notre Dame

Per- and polyfluoroalkyl substances (PFAS) are a class of over 14,000 environmentally persistent organic chemicals, many of which have known adverse effects on human and environmental health. Measuring PFAS can be particularly challenging because they are found in a wide variety of matrices, each of which may require novel method development to ensure accurate and precise detection. Additionally, PFAS analysis can be costly and time-consuming because of the need for specialized analytical instrumentation and labor-intensive sample preparation. We have developed a combination of total fluorine and targeted PFAS methods to rapidly screen for intentional PFAS use in a variety of consumer products and related environmental matrices. Specifically, particle-induced gamma ray emission (PIGE) spectroscopy is a total fluorine screening method which requires minimal sample preparation and data acquisition time. PIGE has been implemented in a wide range of matrices to triage samples, some of which will later undergo targeted analysis such as liquid chromatography tandem mass spectrometry (LC-MS/MS). PIGE and follow-up LC-MS/MS analyses are used to detect and measure PFAS throughout the life cycle of consumer products, from consumer use to end of life, and finally to environmental degradation. Examples of PFAS detection in paper, textiles, plastics, and personal care products will be presented alongside method development for the rapid screening of related environmental matrices including water, sediment, dust, and compost. The long-term environmental implications of PFAS use in consumer products will be evaluated. These measurements of PFAS and subsequent publication in peer-reviewed literature will be discussed as a driver for science policy and consumer market transformation.

4.08.A.T-05 Analysis of target PFAS compounds and total organic fluorine in personal hygiene products

Chris Gleba, Weidong Li, Jordan Tower, Brenton Thomas Murphy and Franco Pala, Battelle

PFAS (Per- and polyfluoroalkyl substances) are pervasive environmental contaminants that can be found in numerous locations around the globe. Often referred to as "forever chemicals," these substances are characterized by their remarkable persistence in both the environment and biological systems, owing to the exceptionally strong carbon-fluorine bonds that compose their chemical structure. With increasing awareness of their potential risks to both environmental integrity and human health, there is a mounting concern regarding the presence and concentrations of PFAS in everyday personal hygiene products. This research undertakes a comprehensive examination of 40 target PFAS compounds, alongside an analysis of total organic fluorine (TOF) levels, in a variety of commonly used items such as toilet paper, adult and baby diapers, tampons, and other absorbent products. Utilizing a modified version of the EPA Method 1633, this study determines the

concentrations of specific PFAS compounds. In addition, combustion ion chromatography (CIC) is employed for the analysis of total organic fluorine. The data gathered from this research sheds light on the specific concentrations of PFAS compounds detected in the analyzed personal hygiene items and highlights the differences in levels compared to non-targeted total organic fluorine compounds. Furthermore, by integrating this analytical data with market insights, the study estimates the overall contribution of PFAS and total organic fluorine compounds to landfill waste generated from the disposal of these products. This information is designed to assist scientists, regulatory bodies, and utility companies in modeling the transport and eventual fate of these fluorinated pollutants as they move from households to landfills, emphasizing the importance of understanding the implications of these substances in our daily lives.

4.08.A.T-06 Per- and poly fluorinated materials and concrete: Is encapsulation effective?

Michael J Deible¹, Logan Tyler Miller², Michael Baker¹ and Shih Hung Yang¹, (1)RJ Lee Group, (2)Shimadzu Aqueous Film Forming Foam (AFFF) exposures frequently occur on large concrete surfaces such as airport runways. These exposures can cause PFAS to adsorb to the concrete, migrate through it to the ground water, or be released to the surrounding environment over time due to concrete weathering. PFAS leaching behavior is also of interest where cementitious encapsulation of environmental contaminants before landfilling has been used as a cost-effective measure for safe disposal. In either scenario, PFAS leaching out of the concrete is a concern. To demonstrate the ability to measure PFAS in a concrete matrix, several types of concrete mixes were prepared using water contaminated with a fluorotelomer sulfonate based AFFF. After casting, cores were milled, extracted, and analyzed for PFAS content using Liquid Chromatography Mass Spectrometry (LCMS). Encapsulation experiments were conducted using a single concrete mix to cast cores with the addition of a PFAS adsorbent (e.g., Granulated Activated Carbon). To understand the material properties of the fortified concrete and understand mechanisms of leachability of the PFAS, all cores were petrographically characterized with Scanning Electron Microscopy (SEM) and extracted and analyzed via LCMS. To evaluate the impacts of weathering, one core was further sectioned and coated with an epoxy based concrete sealant. Coated and non-coated portions were exposed to weathering conditions to determine the amount of PFAS that leaches from the concrete to the surrounding environment to evaluate the efficacy of concrete sealants in protection of the surrounding environment. Conclusions from this work confirm the analytical extractability of PFAS from contaminated concrete. Additionally, this work reports on the leachability of PFAS from encapsulated contaminated concrete with the addition of binders or sealants.

4.08.B.T Environmental Exposure to Per- and Polyfluoroalkyl Substances (PFAS) From Non-Traditional Matrices: Exploring the Where and How?

4.08.B.T-01 Per- and polyfluoroalkyl substances and total organic fluorine in field-collected light non-aqueous phase liquids from US military sites

Mireia Roig-Paul¹, Chris Heron² and Jennifer Field¹, (1)Oregon State University, (2)Jacobs Consulting Per- and polyfluoroalkyl substances (PFAS) and light non-aqueous phase liquid (LNAPL) co-occur in the subsurface at military installations due to firefighting training, landfilling wastes, and storing/transferring fuels. A total of 14 LNAPL and groundwater pairs were analyzed for PFAS in order to report PFAS concentrations and to compute field-observed partition coefficients. Optimized analytical methods and workflows were utilized for target, suspect, and nontarget PFAS. Nuclear magnetic resonance (¹⁹F NMR) was utilized to quantify total organic fluorine in LNAPL and groundwater phases in order to determine total organic fluorine partition coefficients. Target analyses revealed a range of PFAS concentrations from <LOD to over 9,000,000 ng/L in LNAPL. LNAPL-groundwater PFAS partition coefficients (KNAPL) ranged from 0 to over 1500, with larger partition coefficients correlating with longer PFAS retention times, which are a proxy for hydrophobicity. Total organic fluorine concentrations were significantly higher than the sum of target PFAS concentrations. Suspect screening is underway to identify remaining PFAS that contribute to total organic fluorine levels. Total organic fluorine partition coefficients ranging from 0.006 to 0.45 indicate that the bulk of total organic fluorine will

preferentially into the groundwater over time. Therefore, LNAPLs at field sites may act as long-term sources of PFAS to groundwater.

4.08.B.T-02 Investigating PFAS content and leaching potential of automotive shredder residue

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Automotive shredder residue (ASR), the heterogeneous waste remaining after recycling vehicles and other metal-rich materials, has emerged as a potential long-term source of per- and polyfluoroalkyl substances (PFAS). However, the types, concentrations, and leachability of PFAS in ASR remain poorly understood. This study addresses key knowledge gaps by quantifying both targeted PFAS and extractable organic fluorine (EOF) in ASR solids using methanol, as well as assessing the water-leachable fraction of PFAS. We analyzed nine (9) large-mass ASR solid samples (5 kg–8 kg) for 40 targeted PFAS (EPA Method 1633) and 15 smaller ASR solid samples (~ 5 g) for 33 targeted PFAS (Wisconsin DNR List) and EOF using EPA Method 537M.

Corresponding water-leachate measurements were conducted on the smaller samples. Results showed that total extractable concentrations of the 40 targeted PFAS in the large-mass samples ranged from 10–40 ng/g.

Concentrations of specific PFAS compounds such as PFBS, PFOA, and PFOS ranged between 1 and 5 ng/g, while concentrations of other detected PFAS were below 1 ng/g. In the small-mass ASR samples, EOF measurements revealed that over 99% of organic fluorine was not accounted for by targeted PFAS analysis (i.e., 33 PFAS compounds), underscoring the limitations of targeted methods in capturing the full extent of organic fluorine in ASR. Synthetic Precipitation Leaching Procedure (SPLP) (EPA Method 1312) tests indicated that 7–44% (mean: $23 \pm 13\%$) of targeted PFAS in ASR solids were released under the synthetic precipitation conditions. For context, municipal solid waste and construction & demolition waste typically leach 10% and 12%, respectively, of targeted PFAS mass. These results highlight the potential leachability of ASR-derived PFAS in stormwater and landfill environments. Reported total targeted PFAS in ASR has ranged from 3–5,100 ng/g across studies, but comparisons are limited by differing extraction methods, target analyte lists, and source materials. As PFAS-containing materials such as fluoropolymers are increasingly used in vehicles to improve performance and reduce emissions, the environmental implications regarding the handling and management of PFAS in ASR will likely grow. Our findings, representing the first side-by-side comparison of solid-phase and leachable targeted PFAS and EOF in ASR can guide the development of best management practices and regulatory frameworks for effective administration of ASR-associated PFAS.

4.08.B.T-03 Does the disposal of PFAS-containing special wastes impact leachate PFAS concentrations?

Vie Villafuerte Patino¹ and Morton Barlaz², (1)Cornell University, (2)North Carolina State University

The presence of per- and polyfluoroalkyl substances (PFAS) in landfill leachate is well documented as many products known to contain PFAS, including textiles, carpet, and food packaging, are disposed of in municipal solid waste (MSW) landfills. Under current MSW landfill regulations, landfills may also accept non-hazardous industrial solid wastes ("special wastes"), but there is no information about how these may impact PFAS concentrations in leachate. This study aimed to quantify the extent to which the addition of PFAS-containing special wastes to MSW contribute to PFAS concentrations in leachate through laboratory-scale experiments and field data analysis. Laboratory-scale reactors simulating the chemical and biological matrix of a landfill were filled with MSW alone and MSW plus a PFAS-containing special waste for comparison. Six special wastes were tested: biosolids, soils contaminated with firefighting foam, auto shredder residue (ASR), reverse osmosis (RO) concentrate, and both spent granular activated carbon (GAC) and ion exchange (IX) resin generated from the treatment of landfill leachate. In addition to assessing PFAS release from the special waste, the relative mass of PFAS stored in the solid waste after methanogenic decomposition was measured. Leachate from all special waste reactors showed elevated PFAS levels compared to MSW-only reactors. Soil A released nearly three orders of magnitude more PFAS than the MSW-only, while GAC, IX, soil B, liquid RO, and two ASRs released 18 - 64 times more. Solidified RO and biosolids released 2 - 6 times more PFAS. PFAS storage in solids varied, with more than 50% stored in MSW, Soil B, and IX resin; ~37% in RO blocks; and 4 - 19% in others. Field data from both public and privately owned landfills, categorized by their acceptance of MSW with or without

industrial waste, was analyzed for selected PFAS including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). Landfills accepting industrial waste exhibited higher PFOS and PFOA concentrations in leachate relative to landfills that only accepted MSW, though no significant difference was found between landfills accepting less or more than 10% of industrial waste. Findings indicate that the disposal of non-MSW wastes containing PFAS increases PFAS concentrations and alters PFAS signatures in landfill leachate. This information can enable landfill operators to make informed decisions on waste acceptance strategies.

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4.08.B.T-05 Evaluation of the relationship between waste type, landfill liners and PFAS in groundwater at Minnesota landfills

Erin Bulson¹ and Jim Eidem², (1)WM, (2)Barr Engineering Co.

The Minnesota Landfill Coalition PFAS Group conducted a comprehensive study to advance the understanding of the relationship between landfills, waste type, and the occurrence of per- and polyfluoroalkyl substances (PFAS) in groundwater. PFAS are a family of fluorinated chemicals used across numerous industries and incorporated into countless products and building materials over the last 70 years. The purpose of the work was to evaluate the occurrence and concentrations of PFAS in groundwater samples collected from monitoring wells at participating landfills, which included consideration for different waste matrices. The study involved the collection of groundwater samples from 48 permitted landfills throughout Minnesota. Each monitoring well was categorized by hydraulic positioning relative to landfilled waste, upgradient land use, waste type, and landfill liner presence. The samples were analyzed using United States Environmental Protection Agency (USEPA) Method 537.1 (modified) for PFAS. The results showed that most participating landfills effectively manage solid waste, with no significant Minnesota state regulated PFAS releases in groundwater samples collected downgradient of lined municipal solid waste (MSW) landfills constructed with RCRA Subtitle D liners. PFAS concentrations met promulgated groundwater standards in all samples collected downgradient of lined construction and demolition debris (C&D) landfills and at approximately half of the legacy closed (unlined) MSW and unlined C&D landfills. One or more regulated PFAS were detected in groundwater upgradient of 76% of the landfills, and upgradient PFAS in groundwater were most prevalent in areas of industrial and agricultural land use. The study concluded that landfill liners effectively manage solid waste containing PFAS, and the presence and concentration of PFAS above regulatory criteria in downgradient groundwater correlate more with landfill bottom liner status than the landfill waste type. The findings of this study provide valuable insights for improving landfill management practices and monitoring programs. The results can be applied to enhance regulatory frameworks and environmental protection efforts related to PFAS.

4.08.P-Mo Environmental Exposure to Per- and Polyfluoroalkyl Substances (PFAS) From Non-Traditional Matrices: Exploring the Where and How?

4.08.P-Mo-037 Per- and polyfluoroalkyl substances in fish species in the Pacific Northwest

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The goal of this study was to investigate the presence and distribution of per- and polyfluoroalkyl substances (PFAS) in fish in the Pacific Northwest. Several PFAS bioaccumulate in aquatic organisms and fish, posing potential health threats to piscivorous wildlife and humans. Ocean-going parasitic life stage of Pacific lamprey (*Entosphenus tridentatus*) were collected along the Oregon and Washington coastline. Multiple fish species (4 total) including adult Chinook salmon (*Oncorhynchus tshawytscha*) were also collected from the Bonneville Pool on the Columbia River. Our objectives were to: 1) quantify the differences in PFAS composition in fish tissues from different fish species, 2) consider whether the findings can contribute to a fish consumption health advisory based on the PFAS concentrations in fish tissues, and 3) evaluate potential risk to piscivorous wildlife using the USEPA's draft ambient water quality criteria. We screened for 50 target PFAS in 82 fish samples

from 5 species. To date, the 31 Pacific lamprey samples have been analyzed while the Bonneville Pool samples are still under analysis. In the lamprey samples, 8 target PFAS compounds were detected ranging in concentrations from <limit of detection (LOD) – 33.2 ng/g. Compounds detected included carboxylic acids (PFOA, PFNA, PFDA, PFUdA), the sulfonate PFOS, and three electrofluorination-based compounds (FBSA, FHxSA, FOSA). Fluorotelomer-based compounds were not detected. FBSA and FHxSA had the highest concentrations detected (33.2 ng/g and 17.1 ng/g, respectively), while PFOS was the compound most widely detected (quantified in 21 out of 31 samples) at concentrations ranging 0.264 – 4.38 ng/g. Several of the measured concentrations of PFOS and PFNA exceeded the Oregon Health Authority's screening values under their Fish Advisory Program. Laboratory analyses of the fish collected from Bonneville Pool are anticipated to be completed in summer 2025. Pacific lamprey and Chinook salmon are culturally and ecologically important fish species. This study raises concerns about consumption of fish containing PFAS and the potential effects on human health. Previously, PFOS was the only compound for which toxicity information was available. In 2024, the US EPA added PFDA, PFHxS, PFNA, and PFOA to the list of contaminants to monitor for fish advisories. This work highlights the need for more research on the toxicological effects of PFAS.

4.08.P-Mo-038 The Effects of environmental matrices on organofluorine mineralization and products of incomplete destruction from the pyrolysis of perfluorooctanoic acid (PFOA)

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Due to their persistence, per- and polyfluoroalkyl substances (PFAS) have been found to contaminate the environment extensively and globally. Deteriorated water quality has become the number one issue in terms of PFAS contamination, inducing a concentrated research effort in developing remedial technologies to remove PFAS from water. Various environmental matrices like soils, sediment, and sewage sludge have also become contaminated with PFAS at significant levels. Thermal treatment can be applied to soil sites contaminated with PFAS as well as PFAS-contaminated sewage sludge for remediation and to produce biochar for agricultural application. However, how the presence of environmental matrices influences the thermal degradation of PFAS is not fully understood. This research investigates the influence of varying levels of metals, minerals, and organic carbon in soil samples on the thermal degradation of perfluorooctanoic acid (PFOA). We characterized the products of incomplete destruction and measured mineralized fluoride in both gas and condensed phases from the pyrolysis of PFOA in (1) low-level Cr (VI) contaminated soil, (2) estuarine sediment, and (3) domestic sludge. Gas chromatography-mass spectrometry (GC-MS) analysis shows that many products of incomplete destruction in the form of volatile organofluorines (VOFs) can be formed even at extremely high temperatures (800 °C). Matrices with higher organic carbon result in more hydrogen substituted perfluorocarbons, like trifluoromethane. The domestic sludge on its own produces a significant amount of hydrocarbons, but when pyrolyzed with PFOA, hydrocarbons like benzene can become fluorinated to produce a variety of fluorinated aromatics and other VOFs. In addition, the pyrolysis of PFOA in these matrices can suppress the formation of highly toxic perfluoroisobutene. Gas-phase mineralization to inorganic fluoride was significantly suppressed with the domestic sludge due to the formation of water-insoluble mineral fluorides in the condensed phase. This research evaluates the efficacy of thermal treatment on PFAS-contaminated solid matrices and informs the need for improved engineering controls to prevent emissions of thermal treatment byproducts, many of which are known greenhouse gases. **Disclaimer/Disclosure:** This research was supported in part by the UCR OASIS Internal Funding Award and the USDA National Institute of Food and Agriculture, Hatch (accession no. 7008753, project no. CA-R-ENS-5214-H).

4.08.P-Mo-039 Evaluating TD-GC-MS/MS for measuring PFAS in air

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Per and polyfluoroalkyl substances (PFAS) continue to dominate environmental and public health news. As we

learn more about their ubiquitous presence in every aspect of life and work analytical chemists continue to adapt monitoring techniques to new matrices and new priority species. Whilst toxicological data on many volatile PFAS is limited they are able to transform into compounds with known adverse health effects such as perfluorocarboxylic acids (PFCAs) making robust monitoring a priority. Volatile PFAS is a broad term covering many species, from fluorotelomer alcohols (FTOHs) to freons, this can mean extra cost for labs when trying to target different compound classes. Each compound class can have different sources and reasons for requiring monitoring: understanding emissions, consumer product testing, atmospheric monitoring, indoor air quality, vapour intrusion, occupational health monitoring and ensuring PFAS destruction. These multitude of different sample types can also pose a barrier to broadening research or offering more services if a technique cannot easily be applied to other matrices. Beyond the practicalities of sampling, concentration ranges for PFAS in different sample types can vary hugely. TD-GC-MS/MS offers a flexible approach to sampling different matrices and analysing multiple target species whilst negating the influence of background. With accessories for collecting samples from air, materials, and industrial sources the technique offers a powerful, practical solution to monitoring PFAS from multiple sample types. In this presentation data will be presented on the performance of the technique overall including detection limits, robustness and repeatability. Analysis of real samples will show how multiple sources can be monitored.

4.08.V Environmental Exposure to Per- and Polyfluoroalkyl Substances (PFAS) From Non-Traditional Matrices: Exploring the Where and How?

4.08.V-01 Nonpolymeric PFAS and side-chain fluorinated polymers in Chinese and US fluorosurfactants used for consumer products

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Nonpolymeric PFAS were previously investigated in fluorosurfactants (FS) used in aqueous film-forming foams. However, there is limited information on nonpolymeric PFAS in FS products used in other applications. The occurrence of side-chain fluorinated polymers (SCFPs) in FS products is another important knowledge gap, precluding further environmental and human exposure assessment. Here, we characterized nonpolymeric PFAS and SCFPs in 35 FS products collected from Chinese retailers, with products originating from manufacturers of China and the United States. Comprehensive PFAS profiling was achieved using different analytical methods, including combined 1D- and 2D-dimensional Diffusion-Ordered Spectroscopy (2D-DOSY) fluorine nuclear magnetic resonance (19F NMR), gel permeation chromatography (GPC), target and suspect screening by liquid chromatography-high resolution mass spectrometry (LC-HRMS), target analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS), and total oxidizable precursor (TOP) assay. Three FS products contained low to nondetectable levels of total organofluorine (TOF) or PFAS, while the other 32 FS products exhibited high concentrations of TOF/precursors/PFAS. In the latter FS products, the TOF contents varied from 1.2 to 774 mg/g ww and 12 different types of PFAS, including 4 types of 6:2 fluorotelomer (FT) nonpolymers, 4 types of 8:2 FT nonpolymers, 1 type of aromatic-CF3 nonpolymer, 2 types of 6:2 FT SCFPs, and 1 type of PBSF SCFP, were identified. Consistent with 19F NMR results, the GPC method further confirmed nonpolymeric or polymeric PFAS in select FS products. LC-HRMS/LC-MS/MS clarified the identity of 3 types of 6:2 FT nonpolymers as 6:2 FTS, 6:2 diPAP, and 6:2 FTAB, which explained up to 63-120 % of TOF in some FS products, while the TOP assay revealed the presence of high levels of unknown precursors (up to >99% of TOF) in most FS products. 19F-NMR, LC-HRMS/LC-MS/MS, and TOP assay commonly indicated the prevalence of regulated long-chain PFCAs or long-chain precursors in some FS products, vs. short-chain PFCAs or short-chain precursors in other products. FS products used for consumer products are important pools of SCFPs and nonpolymeric PFAS and could thus represent direct and indirect sources of both short- and long-chain PFAAs to the environment.

4.09.P-Th Environmental Fate: Elucidating the Mechanisms and Kinetics of Chemical Transformation Products

4.09.P-Th-102 Determining degradation rates and potential transformation products using OECD 302B methodology with concurrent GPC analysis: Case study with modified celluloses

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In previous studies, a method for analyzing molecular weight changes for various types of modified cellulose polymers over the course of a 302B using GPC assessment was presented. Through this assessment, molecular weight and chemical alterations could be observed that would not be identified with the traditional OECD 302B measurements alone. This study aims to focus on a single cellulose type, hydroxyethyl cellulose (HEC) with varying degrees of molecular substitution (MS) to determine the effect MS has on the percent degradation result, the molecular weight distribution, and degradation rate constant over the course of a 60-day OECD 302B assay. Six grades of HEC, with MS ranging from 0.5 – 1.7 MS, were tested in an OECD 302B. All samples tested were found to be classified as “primary, inherently biodegradable”. The three HEC grades with “low” MS were found to have a different pattern of molecular weight changes compared to those at “high” MS, such as a further decrease in molecular weight (MW) narrower MW distribution. The lower MS materials were calculated to have a two-phase degradation rate constant of 0.5-1.66/day in phase 1, and 0/day in phase 2. The higher MS materials were calculated to have a two-phase or three-phase degradation rate constant of 0.36-0.42/day in phase 1, 0.02-0.03/day in phase 2, and 0.01/day in phase 3. This acts as a case study to evaluate the: (1) usage of concurrent GPC monitoring during an OECD biodegradation screening assay to be able to determine the differences small changes in physio-chemical properties (i.e, MS) have on the biodegradation profile, (2) ability to calculate degradation rate constants without using higher tier testing methods, and (3) identify potential transformation products for further toxicity/biodegradation evaluation.

4.09.P-Th-103 Evaluating electron beam irradiation for the degradation of per- and polyfluoroalkyl substances in aqueous matrices using targeted and non-targeted analytical approaches

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Per- and polyfluoroalkyl substances (PFAS) are a class of persistent organic pollutants that resist conventional water treatment methods due to their strong carbon-fluorine bonds. Electron beam (e-beam) irradiation has emerged as a promising advanced oxidation/reduction process for PFAS degradation in water. However, the degradation pathways and formation of transformation products are not well understood. This study investigates the degradation of selected PFAS in aqueous solutions, including both real water matrices (groundwater, reverse osmosis membrane concentrate, firefighting training water, and landfill leachate) and multi-PFAS solute spiked deionized water to simulate environmentally relevant concentrations. Samples were subjected to different e-beam irradiation doses. Prior to irradiation, sample conditions were adjusted to reflect a range of parameters that could influence degradation, including pH and the initial concentration of parent compounds. PFAS degradation and transformation were analyzed before and after irradiation using target analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) following the EPA method 1633, and non-targeted analysis (NTA) using an Orbitrap Exploris 480 high-resolution mass spectrometer for comprehensive screening of transformation products. Feature extraction and filtering were conducted using Compound Discoverer 3.3 software and publicly available PFAS databases. The results showed a decrease in parent PFAS concentrations, accompanied by the formation of transformation products, -some identified as potential perfluoroalkyl carboxylic and sulfonic acid derivatives. Distinct degradation patterns were observed across dose levels, suggesting dose-dependent transformation pathways. The Total Oxidizable Precursor (TOP) assay was also performed to assess the presence of PFAS precursors that could be converted into detectable perfluoroalkyl acids under oxidative conditions. These results provided a complementary perspective on total PFAS burden

and supported the interpretation of degradation efficiency and potential precursor transformation. These findings enhance our understanding of how e-beam treatment can degrade PFAS from aqueous matrices and highlight the importance of combining targeted and non-targeted analyses to track degradation and transformation. This work ultimately supports the development of more effective remediation strategies for contaminated water systems.

4.09.P-Th-104 Improved assessment of soil nonextractable residues of the pyrethroid insecticide cyphenothrin

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The environmental fate of the pyrethroid insecticide cyphenothrin in soil was investigated, focusing on its degradation pathways and the characterisation of non-extractable residues (NER). NER are chemicals that remain in soil after extensive extraction and are traditionally considered to have limited environmental relevance in terms of their ecotoxicology/persistence. However, growing awareness of environmental protection has driven the development of analytical procedures for NER, highlighting the demand for their classification and structural determination to achieve advanced chemical risk management. Under this circumstance, previous studies have shown a high accumulation of radioactivity in soil NER originating from ^{14}C -labeled cyphenothrin after application, however their detailed characterisation has not yet been available. To address this issue, we employed ^{14}C -cyphenothrin labeled at the 1-position of the cyclopropane ring to monitor its degradation and transformation in aerobic soil over 120 days. Our findings revealed significant mineralization of cyphenothrin to CO_2 , suggesting effective microbial degradation in soil. The half-lives of cyphenothrin ranged from 19.0 to 47.4 days, indicating low soil persistence. Furthermore, we successfully differentiated the types of NER, with the majority being classified as nonhazardous biogenic NER (bio-NER). Detailed quantification of bio-NER (^{14}C amino acids) indicated the significant involvement of the tricarboxylic acid cycle and the pyruvate pathway in the bio-NER formation of the chrysanthemic moiety of cyphenothrin. In conclusion, cyphenothrin released into soil rapidly disappeared forming limited metabolites, and was eventually mineralised to CO_2 or incorporated into nonhazardous bio-NER via microbial degradation/assimilation. This finding enhances our understanding of the terrestrial fate of the chrysanthemic acid moiety of pyrethroids and facilitates risk management and regulatory assessment of biocidal products.

4.09.P-Th-105 PhotoFate 2.0 enables high-throughput analysis of indirect photochemical degradation: A case study of N-heterocycles

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Indirect photochemistry in sunlit surface waters can allow degradation of environmental contaminants that are recalcitrant to other transformation processes. However, the many reactive species and photoactive precursors involved in this process make comprehensively evaluating the mechanism and importance of indirect photochemistry for a substance an arduous process. This is further exacerbated by the varied approaches used by research groups around the world, which makes comparing across studies difficult. Here we present PhotoFate 2.0, a fast and comprehensive technique that allows for simultaneous measurement of full range of environmentally relevant half-lives with respect to indirect photochemistry, transformation product analysis, and estimation of second-order rate constants with key photochemically produced reactive intermediates. This approach starts with photochemical experiments in a series of synthetic surface waters that are representative of the compositions of real bodies of water globally. The data from these experiments are then fed into an explicit kinetic model which can deconvolute the contributions of the various reactive species in each of these solutions. We demonstrate the application of this tool to measure the role of indirect photochemistry in the degradation of a series of small nitrogen-containing aromatic heterocycles, which are important building blocks and in some cases transformation products of many pharmaceuticals and pesticides. PhotoFate 2.0 is a significant step towards including indirect photochemistry in environmental risk assessments by allowing for rapid, reproducible measurements of indirect photochemical fate.

4.09.P-Th-106 3M expansion of the analysis of PFAS in soils and sediments

Danielle Webb, 3M Science

3M internal analytical methods developed to measure PFAS in soils and sediment have undergone continuous expansion to include an ever-increasing number of PFAS analytes. Currently, these internal methods exceed the PFAS analyte lists in standardized test methods and include PFAS that recover poorly from some soils (e.g., clay, silt, loam, sand). By identifying the critical soil properties that can impact extraction efficiency, we have been successful in expanding the PFAS analysis capabilities of our methods which are used to support critical studies. The performance of the 3M soil and sediment analysis method in a variety of types of soils will be discussed.

4.09.P-Th-107 Is SPE clean-up needed for PFAS analysis in tiny biological tissues following EPA method 1633

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The US Environmental Protection Agency (US EPA) has developed Method 1633, for targeted analysis of 40 per- and polyfluoroalkyl substances (PFAS) analytes in various matrices including biological tissues however a minimum sample size of 1 g is suggested. Ecotoxicological investigations often require analysis of PFAS in much smaller amounts of tissue such as macroinvertebrates and specific fish organs. Our present effort aims to modify EPA Method 1633 for measuring 40 PFAS from tissue amounts that are 100 times smaller, with the expectation that tissue analyses may not suffer from significant matrix interferences when the amount of matrix is substantially less. A model organism for freshwater ecotoxicology, *Daphnia magna* was investigated for method validation. Extraction of 100 mg of rehydrated *Daphnia* reference material was performed after addition of extracted mass-labeled internal standards (EIS) followed by Solid Phase Extraction (SPE), solvent evaporation, and addition of a second set of labeled internal standards (NIS). An aliquot of final extract was analyzed by LC/MS/MS (Thermo Scientific Vanquish LC/ TSQ ALTIS mass spectrometer) using multiple reaction monitoring (MRM) mode. The corresponding recoveries for mass-labeled internal standards (EIS) ranged from 7 % to 350 %, and method detection limits (MDLs) were comparable to the EPA 1633 Method's cited interlaboratory MDLs for 1 gram of tissues. However, the recoveries of D7-NMeFOSE (23%), D3-NMeFOSA (32%), D9-NEtFOSE (7%) and D5-NEtFOSA (16%) were relatively lower and had poor reproducibility. The modified method was further updated to eliminate the SPE cleanup, and 100 mg of rehydrated *Daphnia* reference material was extracted using methanolic potassium hydroxide (0.05 M M-KOH) and acetonitrile, followed by solvent evaporation to dryness and reconstitution in solvent for final analysis using LC/MS/MS. EIS recoveries were significantly improved as compared to the SPE-based approach in particular for D5-NEtFOSA (71%), D5-NetFOSAA (80%) , D7-NmeFOSE (49%) and D9-NetFOSE (83%). Additional efforts focus on extending protocol for measuring PFAS in algae, plasma, and fathead minnow organs including liver, gonads, muscle, brain, and whole juvenile fish.

4.09.P-Th-108 Automated multi-contaminant LC-MS/MS workflow for diverse contaminants: PFAS and algae toxins

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The demand for efficient, cost-effective, and sustainable analytical workflows continues to grow in laboratories dedicated to R&D, regulatory testing and emergency response. There is an opportunity for method consolidation and automation using LC-MS/MS in environmental laboratories that will enable seamless routine testing and emergency response (during natural disasters, spills, intentional contamination...) with an automated workflow using a single LCMS system. In this work, PFAS and cyanotoxins, a class of aquatic toxins that may be released during Harmful Algae Blooms, were selected to optimize an automated workflow that targets two diverse contaminant classes of concern in aqueous samples, because of their relevance in surface and drinking

water and varied physico-chemical properties. Following standardized methods to demonstrate the performance of the workflow, we quantified 18 PFAS compounds (EPA 537.1), cylindrospermopsin and anatoxin-a (EPA 545), and six microcystins plus nodularin (Method 544). Automated method switching was implemented with minimal rinsing steps, enabling efficient transitions between analyzing targets in both positive and negative electrospray ionization modes with different chromatography conditions while preserving analytical sensitivity and accuracy. Chromatographic separations were optimized individually for each contaminant group: PFAS (18 min), cylindrospermopsin/anatoxin-a (8 min), and microcystins (8 min). A total of 294 sequential injections were performed to assess ion suppression due to solvent contamination and to evaluate calibration stability across multiple switching events. All analytes achieved calibration curve R^2 values >0.99 , with %RSD values below 15% and accuracies within $\pm 30\%$, in line with commonly accepted QA/QC requirements. By enabling multiple methods for diverse classes of analytes to be executed on a single instrument 215 with automated switching, this workflow demonstrated here enhances efficiency and responsiveness without compromising data quality. This workflow highlights a practical, automated solution that improves laboratory throughput, reduces resource consumption, and enhances readiness for both routine monitoring and environmental emergencies.

4.09.T Environmental Fate: Elucidating the Mechanisms and Kinetics of Chemical Transformation Products

4.09.T-01 Bio-stimulation of nitrotriazolone (NTO) and 2,4-dinitroanisole (DNAN) removal by adding natural organic matter to freshwater sediments

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Insensitive high explosive compounds, NTO and DNAN were introduced to replace traditional explosives due to their ability to tolerate unplanned stimuli during storage and handling. Given the reported toxicity of these compounds to different species in the environment, it is important to investigate attenuation mechanisms and kinetics of these compounds via environmental-field approaches. In this study, we compared natural attenuation of NTO and DNAN in freshwater silt to bio-stimulated compound removal and mineralization by adding natural organic matter to the sediment, in an aquaria scale set up that closely mimic the environmental conditions and concentrations. We added two types of organic matter sources: leaf detritus (high C:N) and algal detritus (low C:N) into the sediments and 50 L aquaria containing amended sediments were spiked with isotopically labelled NTO (^{15}N) and DNAN (^{13}C) as a single pulse input. Time series water and sediment samples were analyzed for parent and transformation products for three weeks. Both compounds were rapidly removed from the water and removal rates were strongly correlated to the % organic carbon content in sediment. NTO showed complete mineralization to inorganic nitrogen with the addition of leaf litter (13%) and algal detritus (60%) while no significant mineralization of DNAN to inorganic carbon is reported in any of the treatments. However, significant amount of ^{13}C isotopic tracer derived from DNAN was found in sediments in both leaf litter (75%) and algae (48%) treatments, suggesting majority of DNAN lost from the water column might be adsorbed onto the sediments. Overall, organic matter is a great stimulator for removing NTO and DNAN from the surface water in freshwater aquatic environments and NTO removal translates into efficient complete mineralization. This study provides valuable insights for managing contaminated sites economically using high organic rich systems such as constructed wetlands and retention ponds. **Disclaimer/Disclosure:** We acknowledge the funding source of the present study as the Department of Defense, Strategic Environmental Research and Development Program, USA under Project ID ER-19-1054.

4.09.T-02 Mechanistic insights into photochemical transformation of nitro- and amino-substituted aromatic contaminants via stable isotope and product analysis

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Nitro- and amino-substituted aromatic compounds – such as nitrotoluenes, chloronitrobenzenes, chloroanilines, and the agrochemical propanil – are common environmental contaminants that pose risks to ecosystems and human health. This study investigates their transformation under light exposure in both natural and engineered systems, comparing direct photolysis under simulated sunlight with titanium dioxide-catalyzed photolysis under UV-A irradiation. The aim is to characterize degradation pathways, identify resulting products, and assess whether photochemical remediation results in effective breakdown or the formation of persistent or toxic intermediates. To this end, we employed compound-specific isotope analysis (CSIA), a technique that provides mechanistic insight into contaminant degradation by detecting subtle shifts in stable isotope ratios during the cleavage of chemical bonds. CSIA was combined with transformation product identification via high-resolution mass spectrometry to resolve both degradation pathways and product identities. This integrated approach enables us to link isotope effects to specific bond-breaking steps and intermediates, providing a powerful means to distinguish between competing photochemical mechanisms in complex systems. Initial carbon isotope fractionation results for dichloroaniline (DCA) isomers reveal distinct mechanistic differences. Direct photolysis of 2,5- and 3,4-DCA caused pronounced inverse carbon isotope fractionation, with enrichment factors of 4.8 ± 0.4 ‰ and 6.2 ± 0.2 ‰, respectively, indicating rate-limiting, photoinduced homolytic cleavage of the carbon–chlorine and/or carbon–amino bonds. In contrast, photocatalytic treatment produced markedly different patterns: 3,4-DCA showed a slight inverse fractionation (0.9 ± 0.2 ‰), while 2,5-DCA exhibited normal fractionation (-1.8 ± 0.1 ‰). These patterns suggest alternative, complex degradation pathways under catalytic conditions, likely involving radical-mediated multistep processes that mask typical isotope effects. A comprehensive dataset on both carbon and nitrogen isotope fractionation across all target analytes, combined with transformation product profiles, will be presented. Together, these results enhance our mechanistic understanding of contaminant transformation in both natural and engineered systems and support improved strategies for environmental assessment and water treatment optimization.

4.09.T-03 Stable isotope fractionation associated with anaerobic dechlorination of 2,3- dichloroaniline and 2-chloroaniline by dehalobacter

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Compound-specific isotope analysis (CSIA) is a powerful tool to investigate the in situ transformation of organic contaminants by measuring their stable isotope signatures. Chlorinated anilines, a group of persistent groundwater contaminants, are known to undergo reductive dechlorination by Dehalobacter under anaerobic conditions. However, their associated isotope fractionation has not yet been characterized, limiting the application of CSIA for evaluating in situ dechlorination of chlorinated anilines. Here, we experimentally determined stable isotope effects associated with dechlorination of 2,3-dichloroaniline (2,3-DCA) and 2-chloroaniline (2-CA) using a mixed well-characterized enrichment culture dominated by Dehalobacter. During the dechlorination of 2,3-DCA to primarily 2-CA, a substantial normal carbon isotope effect was observed. The remaining 2,3-DCA became enriched in ¹³C, shifting from -31.9 ± 0.1 ‰ to -15.0 ± 0.3 ‰ after 85% conversion, corresponding to a carbon enrichment factor (εC) of -8.1 ± 0.4 ‰. Previous work in our group showed negligible stable carbon isotope fractionation during aerobic microbial degradation of 2,3-DCA. This work therefore demonstrates that carbon CSIA can be used to distinguish between aerobic and anaerobic microbial degradation in the field. We also found that anaerobic 2-CA dechlorination to aniline resulted in a significant enrichment in ¹³C, yielding εC = -8.4 ± 0.5 ‰. These results indicate cleavage of the C–Cl bond and suggest a nucleophilic attack during Dehalobacter-driven dechlorination. Furthermore, we applied the measured isotope enrichment factors to identify and quantify the anaerobic dechlorination of chlorinated anilines at a heavily contaminated industrial site.

4.09.T-04 Non-targeted analysis to understand PFAS profiles and transformations in the liquid and solid waste streams from 3 Canadian WWTPs

Million Woudneh, Bharat Chandramouli, Connor Taggart and Coreen Hamilton, SGS AXYS Analytical Services Ltd., Canada

Municipal and industrial waste are a significant source of PFAS entry into the environment. With concerns around widespread PFAS contamination due to land application of biosolids for example, PFAS in waste streams have been monitored extensively. However, the focus of these investigations has been specific target PFAS, typically stable/terminal end-products such as PFOS, PFOA, other perfluoroalkyl acids, and select precursors such as legacy C8 sulfonamides. While we know precursors transform into perfluoroalkyl acids through wastewater treatment, far less is known about the uncharacterized precursors responsible for this transformation, and the completeness of these transformations through treatment. Non-target analysis (NTA) using high-resolution accurate mass (HRAM) methodologies are a promising tool to fill these knowledge gaps. To investigate PFAS profiles and potential transformations during wastewater treatment, influent, effluent, and biosolid samples were collected from three municipal wastewater treatment plants (WWTPs). To capture changes in PFAS profiles driven by physical, chemical, and biological processes, the selected WWTPs included primary, secondary, and tertiary treatment technologies. To ensure broad PFAS coverage, sample extractions were performed at two different pH levels. Identification of PFAS and their transformation products followed a non-targeted analysis (NTA) workflow using a high-resolution LC-Orbitrap-MS instrument operated in both positive and negative electrospray ionization modes. For quality control (QC), 90 PFAS standards belonging to various chemical functional groups were spiked into quality control (QC) samples. Additionally, 34 isotopically labeled surrogate standards, also covering diverse chemical classes, were spiked into all samples and QC samples to monitor analyte recoveries. Preliminary results indicate that the workflow developed has sufficient sensitivity to detect PFAS at low ng/L levels for the Schymanski Confidence Level 1 PFAS characterized, those with known standards. Initial comparisons with corresponding measurements on unit-resolution LC-MS/MS data show broad method compatibility with some interferences observed in the LC-MSMS method that was removed using the HRAM analysis. The NTA experiments in monitoring and identifying PFAS and transformation products are ongoing and will be discussed. **Disclaimer/Disclosure:** The project is funded by SGS AXYS Analytical Services.

4.09.T-05 Environmental fate, transformation and toxicity of quinone outside inhibitor fungicides

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Quinone outside inhibitor (QoI) fungicides, including azoxystrobin, pyraclostrobin and kresoxim-methyl are widely used in agriculture, landscaping and as additives in mold-resistant drywall. Due to their widespread use, they are commonly detected in soil, sediment, and surface waters, however, their environmental fate and transformation pathways are understudied. We explore the fate and transformation of the QoIs in natural and engineered aquatic systems. Two QoIs, namely kresoxim-methyl and trifloxystrobin, undergo base-mediated hydrolysis under common water treatment conditions (i.e. lime-soda softening), and five QoIs, azoxystrobin, pyraclostrobin, fenamidone, kresoxim-methyl and dimoxystrobin exhibit reactivity toward free chlorine, a common oxidant in disinfection processes. Transformation kinetics were observed by pH and chloride ion concentration, highlighting the importance of investigating chemical speciation when determining transformation rates and product formation. Transformation products were isolated using semi-preparative HPLC, and structurally characterized by NMR spectroscopy and LC-MS/MS. Four distinct reactive sites were observed, yielding products with altered physicochemical properties (i.e. hydrolysis products yielded more hydrophilic products while chlorinated products were more hydrophobic species). Notably, several transformation products retained key functionalities responsible for their fungicidal activity, suggesting the potential for conserved bioactivity in transformation products. Ongoing work is focused on evaluating the

ecotoxicological profiles of these products and exploring additional processes such as photolysis that may be important fate pathways in aquatic and soil systems. **Disclaimer/Disclosure:** This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. 1945994. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation

4.09.T-06 Aquatic environmental fate of the rubber additive diphenyl guanidine (DPG)

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Diphenyl guanidine (DPG), a rubber vulcanization accelerator widely used in the tire and rubber industry, has recently emerged as an environmental contaminant of concern. While attention has focused on phenylenediamines like 6PPD and their toxic transformation products, DPG has been detected at relatively high concentrations in diverse environmental matrices including surface waters, drinking water, urban air, and indoor dust, often exceeding levels of well studied contaminants such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Despite its abundance, little is known about the environmental fate, transformation pathways, and toxicity of DPG. This study aims to address critical knowledge gaps by investigating the direct and indirect photochemical degradation pathways and kinetics of DPG in aqueous systems. In order to evaluate the aqueous environmental fate of DPG under sunlight exposure, we conducted controlled photolysis experiments using a photoreactor. Aqueous solutions of DPG at acidic, basic, and neutral pH levels were exposed to UV-B light, and sampling was done over a 48-hr period. High-performance liquid chromatography with a UV detector was used for quantitative analysis. The experimental data confirmed that DPG does not undergo direct photolysis around pH 4 or 7 in environmentally relevant buffer systems, indicating the ability of DPG to persist in the environment under direct sunlight. DPG has a pKa of 10.12, and at pH 4 and 7, it is expected to be in protonated form. However, at pH 10, when DPG is partially protonated as the pH is close to the pKa, it undergoes direct photolysis with a half-life of $26.5 (\pm 1.5)$ h. Using the chemical actinometer p-nitroacetophenone/pyridine, the quantum yield of this reaction was determined to be $5.6 (\pm 0.3) \times 10^{-5}$. High-resolution mass spectrometry identified phenyl guanidine as a transformation product of this reaction. The indirect photochemistry was also explored using both hydrogen peroxide as an OH radical precursor and dissolved organic matter. Preliminary results indicate that the indirect photolysis of DPG happens at a faster rate than the direct photolysis. These findings have implications for understanding the environmental persistence and risks associated with DPG as a rubber-related chemical.

4.10.A.T Environmental Forensics

4.10.A.T-01 Associating the exposure of individuals with PAH sources based on isotopic carbon ratios collected with passive sampling devices

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Historically, investigating an exposure source in previous literature has been done by identifying individual chemicals and their concentration characteristics. However, this technique can be influenced by chemical degradation and weathering over time. Stable isotopic ratios are not subject to this and have been used in geologic and geoauthenticity studies to identify source material. We are creating an isotopic source library for carbon isotope data from archived passive sampling extracts with contributions from one source and common sources of PAHs such as natural gas, vehicle exhaust, wildfires, superfund sites, etc. Carbon ratios from each source should be unique to each other, and act as an identifier for exposure sources. Isotope ratios don't degrade over time in the same way PAH concentrations can and aren't affected by weathering a sample might encounter in the environment. Passive sampler data from archived studies as well as available standard reference materials were analyzed for present PAHs via GC/MS/MS. Initial sample analysis was performed on an Agilent 7890 GC with a 7000 QQQ MS. Then a tertiary reference material was created for PAH specific identification isotopic analysis and analyzed using a continuous flow analyte specific GC-IRMS specifically a thermoscientific 1310

trace gas chromatogram with a thermoscientific Delta Q isotope ratio mass spectrometer. The GC-IRMS reference gas was calibrated to the Vienna Pee Dee Belemnite scale using secondary reference materials obtained from Indiana University. Calibration of the reference gas resulted in measured deltas being within 3% of the known delta for PAH compounds and 5% overall. Collections of additional PAH source material are currently ongoing with passive sampling devices and will be analyzed after extraction. Preliminary results from the analysis of NIST SRM 1991, a mixed petroleum and coal tar creosote standard, and a collected sample from creosote-impacted surface water in Northern Oregon near the Portland Harbor Superfund site show respective isotopic signatures within 1% of each other. Continued analysis of samples from the same source is ongoing to assess precision. Additionally, analysis of silicone wristband samplers is underway to assess the effectiveness of isotopic ratios in personal exposure extracts to identify specific exposure sources. The ability to pinpoint exposure sources from passive sampling extracts could inform risk mitigation as well as remediation procedures. **Disclaimer/Disclosure:** Funding was provided by the National Institute of Environmental Health Sciences with grant numbers T32ES007060, P42ES016465, and P30ES030287. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

4.10.A.T-02 Emissions, dispersion and concentrations of airborne polychlorinated biphenyls (PCBs) near the Portland Harbor (Oregon) Superfund Site

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The Portland Harbor Community Coalition (PHCC) reached out to the Iowa Superfund Research Program at the University of Iowa to assess the presence of airborne PCBs around the City of Portland, Oregon. The main goal was to identify and characterize potential airborne PCBs affecting the community's airshed. Portland Harbor holds historical and cultural significance and is one of the most complex Superfund sites designated by the United States Environmental Protection Agency (EPA), due to its long history of shipping, industrial and commercial activity, and its strategic location on the Willamette River. To address PHCC's concerns, we first used polyurethane foam passive air samplers (PUF-PAS), which were deployed over a six-week period at 28 locations, to capture and measure airborne PCBs. Second, using water PCB concentration data provided by the EPA, we estimated water-to-air emissions from the Superfund sites. Finally, we used these emissions as inputs to predict their dispersion and concentrations using the EPA's regulatory modeling system, AERMOD. Measured PCB concentrations ranged from 70 to 860 pg m⁻³ with an average of 380 ± 220 pg m⁻³. Estimated PCB emissions averaged 450 ± 120 ng m² day⁻¹ and our predicted airborne PCBs ranged from 5 to 100 pg m⁻³. PCB site profiles were compared to reported profiles for Aroclors, and we found most to be similar to 1016 (cos q = 0.74 + 0.15) and 1242 (cos q = 0.78 + 0.14). PCB air samples, when compared to water sample profiles, show no similarity (cos q = 0.34). The profile of PCBs in the water is distinctly non-Aroclor. While the air samples are enriched in PCBs 4, 8, 11, 18+30, and 52, the water profiles have more PCBs 44+47+65, 45+51, and 68, which are byproducts of silicone rubber manufacturing. This could mean that the source of PCBs in the air is not from the water but from various sources surrounding the PH.

4.10.A.T-03 A second significant source of 2,3, 7,8-tetrachlorodibenzo-p-dioxin in the Lower Passaic River, NJ

Michael Bock, Lauren Brown and Jennifer Jones, Verdantas LLC

The presence of elevated levels of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in sediments of the Lower Passaic River (LPR) in New Jersey has been well documented since the 1980s. Previous work has focused on the hypothesis that a single source, the former Diamond Alkali plant at river mile (RM) 3.5, is responsible for virtually all 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) in the LPR, with these dioxins theoretically transported upstream by hydrodynamic processes. However, several studies have presented evidence of a second source of 2,3,7,8-TCDD. Garvey et al 2011

identified an anomaly in the ratio of DDT and 2,3,7,8-TCDD in sediments deposited in the vicinity of the former Givaudan Clifton facility (RM 12), the largest historical manufacturer of Hexachlorophene (HCP) in North America. We analyzed sediment chemistry data from the same high resolution dated cores used in the Garvey analysis. We combined these data with information on the timing of chemical production at Givaudan and Diamond, wastewater infrastructure and CSO bypass events, and historical river flow and hydrodynamic models. Multivariate analysis of the dioxin and PCB data were combined with these other lines of evidence to evaluate the history of 2,3,7,8-TCDD contamination and transport. The initial increase in 2,3,7,8-TCDD observed in the late 1950-early 1960s in sediments between RM 10 and 12 corresponds to increasing TCP usage (the presumed source of 2,3,7,8-TCDD) at both facilities. During this time, river flows were significantly higher than those observed during the drought in the late 1960s, with the predicted upstream extent of particulate transport of material originating at Diamond unlikely to reach RM 10-12. Therefore, contaminants released at the former Diamond facility likely would not have been transported far enough upstream to account for the initial increase in 2,3,7,8-TCDD at RM 12. Diversions of the municipal sewer waste streams to the Passaic River through a large system bypass at RM 11.5 were much higher during this period due to system maintenance, resulting in the discharge of wastewater originating at Givaudan to the river. Furthermore, the chemical signatures in sediments at RM 10-12 do not match those associated with contamination from the Diamond facility. These results provide further evidence of the presence of a second significant source of 2,3,7,8-TCDD to the LPR. **Disclaimer/Disclosure:** This work was funded by Occidental Chemical Corporation

4.10.A.T-04 A Chemical fingerprinting method to identify sources of plastic pollution

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Ultimately, controlling plastic pollution relies on identifying sources for source-control efforts. We tested the hypothesis that the identity of specific plastic polymers can be determined using their chemical fingerprints obtained using non-target analysis and a Random Forest machine-learning algorithm to distinguish and classify those polymers. To test the hypothesis, we first obtained fingerprints of 14 pristine types of plastics based on analysis of their extracts by High-Performance Liquid Chromatography coupled with Quantitative Time of Flight Mass Spectrometry (HPLC-QToF-MS). Second, we obtained HPLC-QToF-MS features from two different mixtures of those pristine plastics. The machine-learning algorithm could identify the fingerprints of those 14 pristine plastics, but could only distinguish about one-third of the plastics in the mixtures, such as tire wear particles and polyurethane (PU) foam, but not polystyrene foam. We also tested the hypothesis that this protocol could identify plastics subject to weathering (UV radiation) and sampling processing of digestion and density separation. The results showed reasonable identification of "treated" plastics with the exception of PU foam. We conclude that this analytical protocol is promising for plastic polymer identification as a step towards source identification.

4.10.A.T-05 Sediment dating in environmental investigations: A review

Jessie Kneeland, Gradient

Sediment dating uses chemical markers and artifacts in sediments to estimate the deposition timing of different sediment or soil depth layers. Sediment age can be important in the context of cost allocation at contaminated sediment sites to establish the timing of release and deposition of specific contaminants. That timing can then be connected back to the owners or operators at sites that may bear responsibility for investigation and remediation costs. In this presentation, we will review the variety of geochronological tools that can be used in cost allocation and environmental forensics. Isotopes produced during atmospheric testing of nuclear weapons, such as lead-210 (Pb-210) and cesium-137 (Cs-137), can be a marker of the time that testing peaked around the early 1960's. Beryllium-7 (Be-7) is produced in the atmosphere and has a half-life less than two months, so its presence indicates recently deposited sediment. Many anthropogenically-produced chemicals have a known

timeframe of initial production and use, so their presence in sediments can provide a maximum age. Serendipitous finds such as newspapers or consumer products with unique packaging can provide a tie point to date the sediment layers around them. From these various chemostratigraphic methods and age tie points, the investigator can construct an age model that relates depth in the sediment with age of deposition. We will discuss limitations and uncertainties to consider when creating such an age model and then reconstructing a contaminant release timeframe for contaminant concentrations measured in those same sediments or soils. We will conclude with recommendations for best practices when using geochronological data and sediment time markers for cost allocation and environmental forensics purposes.

4.10.A.T-06 Urban background investigation of per- and polyfluoroalkyl substances (PFAS) in Chicago area surface waters: A forensic approach

Brian Drollette¹, **Chris Brueck¹** and **Gordon Getzinger²**, (1)*Ramboll Americas Engineering Solutions, Inc.*, (2)*Loyola University Chicago*

Urban areas, characterized by dense populations and diverse industrial activities, often exhibit elevated concentrations of contaminants in surface water compared to rural areas. Defining the urban background contaminant load is important for establishing discharge benchmarks, enabling identification of sources, informing regulatory frameworks, guiding remediation and risk assessment, and contextualizing environmental monitoring. Per- and polyfluoroalkyl substances (PFAS) are a class of chemical often found in both rural and urban environments from diffuse regional or global background aerial deposition, local non-point sources including stormwater runoff, and point sources including effluent discharges. The growing regulatory scrutiny of PFAS combined with ever-lowering environmental screening levels obviates the need for characterization of their urban background. This study sought to fill knowledge gaps about the presence and distribution of PFAS in the greater Chicago area surface watershed. The greater Chicago area is the third largest metropolitan area in the U.S. with a population of nearly 10 million and a rich history of industrialization. The Chicago River has a unique history of engineering which, after its flow reversal in 1900, funnels source water from Lake Michigan to the Mississippi River. The unique hydrodynamics make it a prime watershed to study the cumulative loading of contaminants, including PFAS, in an urban environment. In a partnership with researchers from Loyola University Chicago and Ramboll, a multi-season sampling program of select surface water bodies in Chicago and in Lake Michigan revealed patterns in PFAS forensic signatures reflective of the complex urban environment. Spatially paired samples showed signatures consistent with watershed hydrodynamics, point-sources (e.g., wastewater treatment plant outfalls), and non-point sources (e.g., combined sewer overflows). The results appeared consistent with the evolution of predominant use of different PFAS moieties (i.e., from longer-chain to shorter-chain) over time. Multivariate statistical analyses including Principal Component Analysis and Hierarchical Cluster Analysis provided an objective differentiation of samples collected with distance from the Lake Michigan headwaters. This study highlights the existing burden of PFAS to urban surface waters, emphasizing the need to characterize urban background PFAS for regulatory compliance and policy development.

4.10.B.T Environmental Forensics

4.10.B.T-01 A geospatial analysis of integrated personal and environmental passive sampling in St. Helens, OR to identify exposure sources

Alison Clark¹, **Emily Bonner¹**, **Lisa Bramer²**, **Diana Rohlman¹**, **Katrina M Waters²**, **Lane Tidwell¹** and **Kim Anderson¹**, (1)*Oregon State University*, (2)*Pacific Northwest National Laboratory*

The exposome is the sum of exposures that a person may encounter over a lifetime. This includes both environmental and personal exposures. Defining the role of each in an individual's exposome is valuable to informing exposure assessments. We've built onto a previous aspect of this study investigating demographic and behavior impacts on personal exposure. This study combines both personal and environmental passive

sampler data in a repeated measures design to investigate the relationship in personal exposure to outdoor air and localized PAH sources over time and by location. Over the period of one year, a total 46 participants wore silicone wristbands for one week every other month to collect a time-averaged personal exposure measure. 27 of these participants also placed air samplers at their homes during their wristband sampler period to create paired data points. These samplers were returned monthly to the laboratory by mail and analyzed via GC/MS/MS for 64 parent and alkylated PAHs. Personal wristband exposures varied little within a single participant but varied more widely between participants regardless of location, implying that a personalized and more stable indoor environment was the main influence on personal exposure. When compared to paired air samplers, changes in outdoor air did not often affect personal exposures at the same locations. One participant that sampled with only a wristband showed significantly higher concentrations of PAHs than other participants. This may be due to a nearby crematorium. Interpolation by ordinary kriging revealed a uniform landscape even around known sources of PAHs, such as a highway and several gas stations. The exception to this was an area near a legacy creosote contamination site. Both air and wristband samplers nearby this site showed higher PAH concentrations over most sampling months than all other samplers. While overall changes in personal exposure to PAHs did not correlate with changes in PAHs in outdoor air in the collected samplers, participants who lived within 0.5 km of known sources of PAHs had higher concentrations than other participants in at least 1 of the 4 sampling periods. It is possible that other samplers were outside of any nearby emission source's PAH plume. This suggests that known PAH emission sources are more localized in effect, and that the contribution to the exposome of most participants is from personal indoor exposures. **Disclaimer/Disclosure:** Funding for this research was provided by the National Institute of Environmental Health Sciences, National Institutes of Health grant numbers T32ES007060, P42ES016465, and P30ES030287. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Kim A. Anderson and Diana Rohlman, authors of this research, disclose a financial interest in MyExposome, Inc., which markets products related to the research being reported. The terms of this arrangement have been reviewed and approved by OSU in accordance with its policy on research conflicts of interest. The authors have no other disclosures of either financial or non-financial nature.

4.10.B.T-02 Implementation of silicone-based passive emissions samplers (Si-PESs) to evaluate primary and tertiary emission sources of polychlorinated biphenyls (PCBs) in a Vermont school

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Polychlorinated biphenyls (PCBs) are known to migrate from building materials into the air where they can be inhaled by building occupants. We designed and conducted a study to examine primary emissions of PCBs from Aroclor-containing surfaces and tertiary emissions that result from deposition and subsequent re-emission of PCBs from other surfaces in a school. The relative scale of emissions from primary and tertiary sources in the same building is not well understood yet could have major financial implications for schools during remediation. To determine the main sources of PCBs in the air of a Vermont school, we used polyurethane-based passive air (n=19) and emissions (n=26) samplers. We also designed a novel silicone-based passive emissions sampler (Si-PES, n=23) to compare emissions from primary sources and the materials adjacent to them. The Si-PES unit uses a small strip of silicone to collect PCBs that is surrounded by an aluminum housing and separated from the surface of interest by an air gap to ensure that only volatilized PCBs are collected. PCBs extracted from the silicone or polyurethane of each sampler were quantified using high resolution gas chromatography with tandem mass spectrometry. Fireproof coating was identified as a primary source of PCB emissions in this school. Several Si-PES units placed on two walls adjacent to fireproofing coated steel beams measured decreasing emissions with distance from the beams, although the rate of this decrease varied greatly. Emissions samples were grouped based on whether they were taken directly on the fireproof coating (primary emissions), adjacent to the coating (proximate tertiary emissions), or further than 10 cm from the coating (tertiary emissions). These three groups were found to be significantly different from each other. These results indicate that the proximate tertiary emissions sources that were adjacent to the fireproofing spray should be

remediated in addition to the fireproofing coated beams. The Aroclor signal from each of the eight material types sampled was consistent, resembling Aroclor 1254 according to cosine theta similarity analysis. The Aroclor signal was not found to be altered between primary and tertiary sources despite years of PCB deposition and re-emission.

4.10.B.T-03 Contribution of previously unidentified non-aroclor PCB congeners to sediment impacts

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Source identification and separation at PCB-based sediment cleanup sites is a costly and complicated endeavor, primarily due to the widespread use and release of PCBs, but also due to the complications inherent in source identification of chemicals manufactured as mixtures of individual congeners. Cleanup levels for PCB sites vary widely but tend to be much lower at sediment sites. The potential for misallocation increases due to the combination of lower cleanup levels and multiple sources. This work identifies polychlorinated terphenyls (PCTs) manufactured by Monsanto for products such as hot melt adhesives as additional sources of inadvertent PCBs that contribute to the need for sediment remediation. PCT Aroclors included undistilled products (e.g., Aroclor 5060) and distilled products (e.g., Aroclor 5432, 5442, and 5460) that were manufactured using the same polyphenol feedstock and equipment used for PCB Aroclor manufacturing. Documents confirm that PCTs contained from 5-10% by weight of PCBs. Production of PCTs represented about 10-15% of PCB production, such that the potential contribution of PCBs from PCTs is significant. Samples of PCT Aroclors 5432, 5442, and 5460 were obtained from a variety of sources. The samples were analyzed for: (1) PCBs by GC/ECD Method 8082; (2) PCTs by modified Method 8082; and (3) PCB congeners Method 1668A. Total PCB values were calculated and congener profiles were compared using standard methods. The resulting congener profiles are intended to serve as reference profiles - such as those developed by Frame (1996) - to elucidate and improve PCB congener source identification and separation work by environmental practitioners.

4.10.B.T-04 Per- and polyfluoroalkyl substances (PFAS) signatures in New England background soil data

Brian Drollette, **Jaana Pietari**, **Rajib Mozumder** and **Heidi Pickard**, *Ramboll Americas Engineering Solutions, Inc.*

Understanding the background concentrations of chemicals present in the environment may be important for delineating the extent of contamination from identified or suspected releases and evaluating remediation liabilities. Per- and polyfluoroalkyl substances (PFAS) were recently measured in statewide surveys of surface soils in Vermont (VT), New Hampshire (NH), Maine (ME), and Massachusetts (MA) to understand anthropogenic background concentrations of PFAS. The objective of our study was to evaluate whether PFAS detected in the background studies from VT, NH, ME, and MA were compositionally similar, and therefore, affected by similar, diffuse PFAS sources. We compared the PFAS signatures in surface soils (0-6 inches) across the four background datasets using multivariate statistical analyses techniques. Principal Components Analysis (PCA), Hierarchical Cluster Analysis, and K-medoids Cluster Analysis were used to identify compositional differences in PFAS signatures. Further, we explored whether incorporating Total Oxidizable Precursor (TOP) assay results into PCA would improve our forensic interpretation. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were the predominant PFAS, detected in almost 90% of the samples in the combined PFAS background dataset. Our preliminary PCA results show that the first four principal components (PCs) explain approximately 80% of the variance in the background data. PFOA, PFOS and perfluorobutanoic acid (PFBA) were the primary PFAS influencing the first three PCs, although PFBA was particularly relevant to the NH and ME background data. Considerable overlap of the background PFAS profiles across the states suggests similar widespread diffuse source(s) of PFAS affecting the New England region. However, there are noticeable compositional differences within the background data, suggesting that in certain areas, the predominant diffuse PFAS sources are mixed with localized sources of PFAS. Further, inclusion of the TOP assay results to our analyses suggests that background PFAS sources were impacted by certain PFAS precursors.

4.10.B.T-05 historic origins and long-term trends of polycyclic aromatic hydrocarbons in the Great Lakes atmosphere

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Atmospheric concentrations of 16 polycyclic aromatic hydrocarbons (PAHs) were measured at six Integrated Atmospheric Deposition Network (IADN) stations located near the shores of the North American Great Lakes. Samples were collected every 12 days from 1996 to 2023 and analyzed in both vapor and particle phases. Positive matrix factorization (PMF) was applied to apportion PAH sources over a nearly three-decade period. Five distinct source profiles were resolved via PMF modeling. Source assignments were supported by Spearman correlations between factor contributions and meteorological parameters. Factor 1 was characterized as petrogenic (e.g., fresh oil, fuel emissions, asphalt, and volatilization), enriched in low-molecular-weight PAHs, and elevated under warm and low wind conditions. The remaining four factors were attributed to pyrogenic sources: factor 2 is related to mixed coal and biomass combustion, factor 3 to coal combustion and heating with wind-driven transport. Factor 4 is related to vehicular emissions (diesel and gasoline combustion), and factor 5 to softwood biomass burning, with retene as a main tracer. Spatially, PAH concentrations showed an urban-to-remote gradient (e.g., $\sum 16\text{PAH} = 50,511 \text{ pg/m}^3$ in Chicago vs $< 2,000 \text{ pg/m}^3$ at remote sites). Temporal analysis revealed compound-specific halving times ranging from 7.7 (dibenz(a,h)anthracene at Point Petre) to 40.6 (chrysene at all sites) years, with PMF-derived source halving times ranging from 8.9 (factor 4 at Chicago) to 52.1 (factor 1 at Sleeping Bear Dunes) years. While urban sites exhibited consistent declines across most sources, several combustion-related factors displayed signs of persistence at remote sites such as Sleeping Bear Dunes and Eagle Harbor. These findings highlight the spatial heterogeneity and source-specific conditions of historic atmospheric PAHs in the Great Lakes region and highlight the value of long-term monitoring for assessing emission control effectiveness and regional atmospheric transport patterns.

4.10.B.T-06 from data-rich to decision-ready: Mike-MAP for efficient source attribution from whole oil chromatograms

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Whole oil analysis is a chromatographic method, typically using flame ionization detection (FID), that captures solvent-extractable organics without requiring mass spectrometry or analytical standards. It is widely used for its low cost and simplicity, especially in differentiating petroleum products such as diesel and condensate. However, it produces an unresolved complex mixture (UCM) that complicates interpretation in weathered or mixed environmental samples. Traditional forensic methods integrate signal intensity over defined carbon ranges, which can be effective for pure products but often fail under real-world conditions due to matrix effects and source mixing. As an untargeted technique, whole oil analysis also generates large datasets, with each sample often exceeding one gigabyte. This study introduces an open-source workflow to extract meaningful signal from the UCM and improve source attribution and allocation. Using OpenChrom for chromatographic preprocessing and R for dimensionality reduction, the workflow isolates relevant chemical patterns while filtering out noise and baseline features. A novel algorithm, Mike-Map, was developed to address the stochastic limitations of traditional dimensionality reduction. Mike-Map iteratively applies matrix factorization to minimize residuals in the predictive matrix, resulting in more stable and accurate chemical signatures. Mike-Map's performance was benchmarked against standard matrix factorization methods, including Non-Negative Matrix Factorization (NMF), Alternating Least Squares (ALS), and Polytopic Vector Analysis (PVA). It consistently produced lower residuals and more interpretable end-members. These were compared to reference oils using cosine theta similarity for source identification. A training dataset of 100 synthetic mixtures—built from reference oils with added noise to simulate environmental variation—was used to test performance. Accuracy was evaluated using root mean square error (RMSE) and cosine similarity, with Mike-Map outperforming all other methods in both attribution and reproducibility. This low-cost, open-source workflow enables scalable forensic analysis of complex petroleum mixtures. Though it lacks compound-level identification, it aligns with non-targeted workflows focused on comparative source allocation. The method is

licensed and ready for real-world use, offering significant time and cost savings without sacrificing interpretability.

4.10.P-We Environmental Forensics

4.10.P-We-082 Modeled global impacts of chlorine oxidation and temperature dependence on the atmospheric lifetime and concentrations of volatile methyl siloxanes

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Volatile Methyl Siloxanes (VMS) are a class of organosilicon compounds widely used in consumer items (such as personal care products) and manufacturing. These chemicals are highly volatile and are released to the atmosphere in large quantities where they have relatively long atmospheric lifetimes. The use of these chemicals has already been restricted in the European Union which has also drafted a proposal to add them to the Stockholm convention. However, some have argued that it is inappropriate to regulate these chemicals (particularly under the Stockholm convention) because there is insufficient evidence that VMS emissions from populated regions are transported and deposited in remote areas in significant quantities. In this study, we describe the addition of three VMS (D4,D5, and D6) to the Community Earth System Model (CESM) and the use of this model to quantify the degree to which VMS emissions from populated regions undergo long range transport and deposition. We begin by calculating and examining the scale of global VMS emissions based on comparisons with previous field measurements and per capita emissions rates. We then present calculated concentrations, lifetimes, and deposition rates of these compounds with a particular focus on the Arctic and other remote regions. In addition, we show the effects of chlorine oxidation, temperature dependent reaction rates, and short-lived halogen species (which have not been included in previous modeling efforts) on these values to quantify the size and direction of potential biases in previous work. Finally, we present a detailed comparison of the concentration predictions from our model and measurements from the literature to assess the accuracy and precision of our model.

4.10.P-We-083 Tracking PCB sources in a high-energy, low sediment river

William Hobbs, Washington State Department of Ecology

Polychlorinated biphenyls (PCBs) are legacy industrial contaminants that are highly persistent and bioaccumulative in the environment. Here, I present a detailed source assessment study for PCBs in the Wenatchee River located in eastern Washington State, USA. Two chemically-distinct PCB sources are clearly defined using multiple environmental media – water (semi-permeable membrane devices), biofilms, invertebrates and fish tissues. Upper trophic-level fish tissues are imprinted with a chemical signature indicative of geographic residence within the river. Sampling of environmental media has taken place over varying hydrologic regimes, yielding very different PCB loads and concentrations over time. I will present results on the pathways of PCBs to the river biota: (1) detailed groundwater-surface water interactions at one source location, and (2) porewater-sediment interactions and the diffusion of bioavailable PCBs at the other source location.

4.10.P-We-084 Unraveling non-source related changes in PFAS profiles

Michael Bock, Jennifer Jones and Lauren Brown, Verdantas LLC

Statistical fingerprinting is routinely applied to chemical mixtures such as PCDD/Fs, PCBs, and PAHs, using methods that are widely published. These methods provide a means to explore sources and receptors at contaminated sites. However, there are serious challenges associated with the application of traditional fingerprinting methods to PFAS. Multiple processes can be responsible for changes in the PFAS profiles that

can complicate the application and interpretation of statistical fingerprinting. Understanding these processes and how they manifest as changes in the profiles is critical in understanding and identifying PFAS sources and linking these sources to environmental impacts. These processes can include (1) changes in the mixture of PFAS used in response to changing market conditions or participation in the PFAS stewardship program, (2) the transformation of precursors to PFAS due to degradation processes, (3) changes in the relative concentrations of different PFAS in a groundwater plume as it migrates due to differences in the retardation rates, and (4) the unique properties of surfactants and their ability to self-aggregate into micelles and other structures that interact with surfaces and other compounds in solution, affecting PFAS fate and transport. We used publicly available data and simple simulations to show how different processes can result in changes in PFAS profiles and developed strategies to help identify the underlying processes when interpreting the results. Chief among these are: 1) developing a hypothesis and alternative hypotheses that address impacts from processes that can alter profiles and 2) providing a framework for robust hypothesis testing. We also characterize how these different processes manifest themselves in PFAS profiles. We demonstrate that traditional forensics statistical analysis methods, such as cluster analysis, principal components analysis and receptor models; and less common methods, such as UMAP, can provide useful lines of evidence for differentiating between different processes and evaluating hypotheses. Although these processes present complications, the careful application of our approach provides a means to test for the presence of these processes and develop an understanding of sources and receptors.

4.10.P-We-085 Extraction and analysis of airborne PCBs from indoor and outdoor environments from a rural school in India: A case study

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Polychlorinated Biphenyls (PCBs) have been detected in school air through multiple studies; posing a health risk to teachers, staff and especially children due to their higher intake of pollutants through air medium. This study is a case study that quantified and analyzed airborne PCBs from indoor and outdoor environments from a rural school in India. The sampling location was a school in a village in Haryana, India. A total of 23 samples were collected using polyurethane foam passive air samplers (PUF-PAS) in January of 2020 and analyzed for the whole suite of 209 congeners. PCBs were extracted from the samplers and analyzed using triple quadrupole gas chromatography mass spectrometry. The samples were spiked with ¹³C labeled surrogate standards for Quality Assurance and Quality Control of the data that resulted in an average recovery of 94%. Moreover, the ratio between average concentration from samples against method and field blanks were 5:1. The LOQs calculated from the blanks for each congener at a 99th percentile were 0.28 to 1.23 ng/m³. The average concentration of PCBs in outdoor (n=4) air was 0.86 ng/m³ and for indoor (n=13) air was 0.77 ng/m³. Lowest chlorinated PCBs were most abundant in all the samples while PCB 11, a non-Aroclor PCB, was detected in the highest amount throughout the school. **Disclaimer/Disclosure:** This study was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH) under award number P42ES013661. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

4.11.T Fate and Effects of Metals: Biogeochemical Perspective

4.11.T-01 Nitrogen cycle under stress: Effects of arsenic species and cadmium in soybean (Glycine max (L.) Merrill) cultivation

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This study assessed the impact of arsenic (As) and cadmium (Cd) on the nitrogen cycle in agricultural soils

during soybean (*Glycine max* (L.) Merrill) cultivation. Soybean fixes atmospheric nitrogen through symbiosis with *Rhizobium* and contributes to soil nitrogen availability. Understanding how heavy metal(loid)s disrupts these pathways is essential for managing soil fertility and crop productivity in heavy metal(loid)s contaminated areas. As species and Cd were applied individually under controlled conditions. Metal uptake, nitrogen speciation in soil porewater (NO_3^- , NO_2^- , NH_4^+), nitrogen fixation (via acetylene reduction assay), and the accumulation of molybdenum (Mo) and iron (Fe) in nodules were analyzed. Arsenite (As(III)) was oxidized to arsenate (As(V)) by manganese oxides and accumulated mainly in roots and nodules, with a bioconcentration factor (BCF) >1. Cd showed limited accumulation (BCF <1). As treatment increased nitrification by up to 1.35%, with elevated NO_2^- in porewater. In contrast, Cd reduced nitrification by up to 72%. Nitrogen fixation was more strongly inhibited by As. As(V), structurally similar to molybdate (MoO_4^{2-}), interfered with Mo transport and reduced its uptake, limiting nitrogenase activity. Fe accumulation was also suppressed due to antagonism with As(V), further weakening nitrogenase. As a result, nitrogen fixation decreased by up to 79%. Cd had minor effects at low levels but caused up to 48% inhibition at high concentrations, likely due to plant stress rather than direct cofactor interference. These results show that As species and Cd impair the nitrogen cycle through distinct mechanisms: As disrupts nitrogenase via cofactor limitation, while Cd inhibits nitrification by biochemical interference. The magnitude and nature of their effects differ even at the same concentrations, reflecting differences in uptake, speciation, and plant sensitivity. This study highlights how As species and Cd affect the nitrogen cycle in contaminated soils and provides a foundation for developing remediation strategies to restore nitrogen functionality in agricultural systems. **Disclaimer/Disclosure:** This work was supported by the National Research Foundation (NRF) grant funded by the Korea government (MSIT)(RS-2024-00405420). This work was also supported by Knowledge-based environmental service Program funded by Ministry of Environment.

4.11.T-02 Assessing the effects of wildfire on sulfur oxidation states in dissolved organic matter sourced from two different soil types

Jeff Murray and Brett Poulin, University of California, Davis

Over the past several decades wildfires have increased in both frequency and intensity affecting soil nutrients, metal concentrations, and dissolved organic matter (DOM) in the environment post-fire. This study examined the effects of wildfire on key functional groups in DOM, with a focus on dissolved organic sulfur. The proportion of reduced sulfur within DOM is expected to have a direct effect on the fate, transport, and bioavailability of trace metals such as copper, zinc, lead and mercury in aquatic ecosystems adjacent to burn areas. This study utilized laboratory soil heating experiments (temperatures: ambient, 75 °C, 125 °C, 200 °C, and 300 °C) – soils were then rewet and DOM isolated by solid phase extraction, and X-ray absorbance spectroscopy was used to identify the changes to sulfur oxidation state in the DOM. We have compared the effect of wildfire on two different soil types, an organic matter rich peat soil and a low organic matter, mineral soil. Preliminary data suggests a significant effect ($p < 0.05$) of temperature on the release of dissolved organic carbon (DOC), and inorganic sulfate. Peat soils exhibited a five-fold increase in DOC released when heated at 200 °C compared to ambient temperature (52.16 (± 2.17) mgC/L and 7.35 (± 1.79) mgC/L respectively), with the largest change in DOC occurring between 125 °C and 200 °C. In addition, there was a significant ($p < 0.001$) increase in the sulfate released from both soils heated at 200 °C with an average of 14.7(± 1.8) mg/L and 6.25 (± 0.50) mg/L for the peat soil and mineral soil respectively, when compared to measurements below detection limit at ambient temperatures. We interpret this large release of sulfate from both soils as the complete oxidation of organic sulfur to inorganic sulfate in turn decreasing the proportion of reduced sulfur in DOM, changing the binding affinity of DOM to trace metals in the aquatic environment. Heating effects on DOM sulfur oxidation state as well as major and trace element released from the soils will also be presented. The aim for this work is to improve our understanding of how these fundamental geochemistry principles are altered by wildfire filling crucial knowledge gaps and aiding in response and rehabilitation efforts post-fire.

4.11.T-03 The occurrence and bioavailability of colloidal particles in a mining-affected river

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Metal and metalloid contamination is described in terms of particulate and “dissolved” size fractions as a proxy for bioavailability to aquatic organisms. The dissolved fraction is considered bioavailable ions and is based on what can pass through a filter (commonly 0.45 µm). However, the dissolved fraction includes some small colloidal particles (1 – 1000 nm). These colloidal particles span both operationally defined size fractions. Colloidal particles are present in rivers worldwide, can sorb and transport contaminants, and have been shown to be bioavailable to benthic macroinvertebrates in laboratory studies. Though ubiquitous, colloidal particles’ relative concentration and influence on metal and metalloid accumulation in river food webs are poorly described. To describe the presence of colloidal particles we collected water samples monthly from the Clark Fork river, Montana, and analyzed the concentrations of Al, As, Cd, Cu, Fe, Mn, P, Pb, Se, and Zn in the “Truly Dissolved” (< 1 nm), Colloidal (1 – 700 nm), and Suspended Particulate Matter (SPM, > 700 nm) size fractions. We observed colloidal particles containing all of our focal elements, with concentrations of colloidal particles containing Cu and As up to 8.5 and 9.3 ppb, respectively. To investigate how the presence of colloidal particles affects the accumulation of metals and metalloids in stream food webs, we conducted a mesocosm experiment in which we exposed assemblages of periphyton and benthic macroinvertebrates to size-fractionated water from the mine-waste-contaminated Clark Fork River. However, water samples from the mesocosm experiment indicated the de novo formation and dissolution of colloidal particles after size separation. Mesocosm results show periphyton in the Colloidal Treatment accumulated more As, Cd, Cu, Pb, Se, and Zn than the Ambient Reference or other treatments, yet accumulation in Baetidae, Ephemerellidae, Heptageniidae, Hydropsyche, and Perlodidae was not significantly different between treatments. The limited accumulation in invertebrates might be due to a relatively short 15-day experiment length or uptake and excretion kinetics. Our data support the idea that colloidal particles in rivers could be common and contribute to metal accumulation in periphyton at the base of the riverine food webs.

4.12.A.T From Tread to Watershed: How Tire Wear Particles and Chemicals Affect Aquatic and Terrestrial Ecosystems

4.12.A.T-01 Target and non-target GC/ and LC/MS analyses reveal distinct chemical signatures of "fresh" and "weathered" pavements made of recycled tires

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According to the U.S. Tire Manufacturers Association (2024), approximately 73 million tons of the U.S. end-of-life tires end up as ground rubber, used for example for sports surfaces, asphalt, or as loose mulch. In this work, we identified and quantified particles and chemicals released from crumb-rubber parking lot pavements. Specifically, we compared the concentrations and types of particles and chemical signatures from a freshly-made permeable rubber pavement to those coming from “weathered” impermeable rubber pavers collected from a 10-yr old parking lot. The pavement samples underwent a standardized Hamburg Wheel Track test, which is commonly used in the transportation industry to test the pavement resistance to rutting under simulated traffic conditions. In this lab tests, we found that the recycled rubber pavements made of high contents of rubber released more particles than our controls (i.e., asphalt and concrete pavements). We also reported the presence of epoxy resins and ethylene-vinyl acetate in the particles released from the rubber parking lot pavement, suggesting they were signatures of binder used in the pavement. We collected simulated runoff from the induced degradation of our parking lot pavement specimens. Using authentic standards, we confirmed the presence of tire-wear associated chemicals, including benzothiazole, 2-hydroxybenzothiazole, 2-(methylthio)benzothiazole, 1,3-diphenylguanidine, hexa(methoxymethyl) melamine, and 6-PPD-quinone in

significantly larger amounts than the concrete and asphalt pavements. Non-targeted LC-MS/MS analysis identified additional rubber-related compounds, including the well-known vulcanization accelerators 1,3-benzothiazole, 2-aminobenzothiazole, 2-mercaptobenzothiazole and 1,3-dicyclohexylurea, as well as other additives such as diethyl phthalate, methyldicyclohexylamine and polypropylene glycols (n7–n11). Non-targeted GC/MS analysis identified several volatile and semi-volatile compounds (match score >90%), including methylpyrrolidone, dimethylformamide, nonane, and cyclohexane derivatives, which are commonly used to enhance the binding properties of rubber in pavement formulations. These results support the hypothesis that pavements made of a large proportion of recycled tires, such as the parking lot pavement materials tested here, and likely playgrounds and other rubber surfaces, are at potential risk of releasing toxic chemicals to stormwater.

4.12.A.T-02 Identification and behavior of compounds leached from tire tread particles under simulated sunlight exposure using nontargeted chemical analysis

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Tire tread particles are microplastics (< 5 mm) and leach organic chemicals into aquatic environments. It is important to understand the behavior of tire wear compounds in sunlight-exposed waters in terms of their persistence, removal, and transformation. In this study, we assessed the chemical composition of TTP leachates produced over 1 or 6 days in either dark or photoirradiated conditions using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry for nontargeted analysis (NTA). In total, 546 chromatographic features were detected across in the 1-6 days leachates, and clustering showed unique chemical profiles based on photoirradiation during leaching. Of the 546 features, 71 were considered tentatively identified based on mass spectral and retention time matching. Benzothiazole was the most abundant chemical in all conditions but decreased significantly from 1- to 6-days of leaching in both light conditions. 6PPD was reduced significantly in photoirradiated leachates but was not removed from dark leachates. To further understand the behavior of leached compounds over time, we leached TTP in ultrapure water for 72 h, filtered out the particles, and then irradiated the filtered leachate for an additional 72 h or kept leachate in dark conditions. Compared to the initial leachates at 0 h, additional photoirradiation resulted in ~12% decrease in dissolved organic carbon and ~30% removal chromatographic features. A decrease in total chemical abundance determined by NTA was observed, with normalized peak areas decreasing by 36.4% from 0 to 72 h. Fifty-three compounds were tentatively identifiable based on mass spectral matching and among them, 19 compounds were photo-labile, 27 were photo-resistant, and 7 were photo-transformation products. 6PPD and 1,2-dihydro-2,2,4-trimethyl-quinoline (TMQ) were among the photo-labile compounds that were completely removed. Overall, this research expands upon existing literature defining TTPs as toxic microplastics in the environment, and we show novel chemical data that demonstrates how leaching time and photoirradiation in the natural environment may impact toxicity.

4.12.A.T-03 Detecting tire-derived chemicals in Puget Sound tire reefs

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In the 1960s and 1970s, used tires were often used to construct artificial tire reefs, with some estimates suggesting that over one million tires were dumped into the Salish Sea by the 1980s. We now recognize that tires are a known source of rubber-derived chemicals, including 6PPD and its transformation product 6PPD-quinone (6PPDQ), that have been identified as highly toxic to coho salmon and other aquatic species. Therefore, tire reefs may be a legacy source of various tire wear chemicals to Puget Sound marine environments. Using environmental mass spectrometry techniques, this project investigated the relative pollution potential of these subsurface tire reefs to the Puget Sound and the composition of tire-derived chemicals they contain. At three tire reefs in the Puget Sound, a suite of tire-derived chemicals were measured

in whole tires along with adjacent sediment and water. Complementary laboratory experiments were conducted by submerging tire fragments in seawater for controlled time intervals to assess chemical leaching over time. Our results demonstrate the legacy chemical contributions of tire reefs and show that tire rubber retains some chemical additives, including 6PPD, even after decades underwater. This work provides new insight into the fate of toxic tire-wear chemicals from tire reef installations in Puget Sound.

4.12.A.T-04 Occurrence and leaching potential of tire rubber-derived compounds from Vancouver road dust

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Tire and road wear particles (TRWPs) can be transported from roadways into surrounding ecosystems, carrying with them harmful chemicals such as 6PPD and 6PPD-quinone. However, the magnitude of chemical emissions from roadways is difficult to estimate due to complex interactions among multiple variables. For instance, the generation of TRWPs varies as a function of driving conditions, vehicle types, and other factors. The potential for chemicals to leach from TRWPs may further be affected by conditions such as temperature and ground-level ozone concentrations. Together, these variables contribute to large uncertainties in estimates of TRWP and tire rubber additive emissions from roadways. To address these knowledge gaps, we measured TRWPs along with 6PPD and 6PPD-quinone concentrations near their source—roadways. We conducted roadside dust sampling over a one-year period across five different locations: a high-speed road, the on- and off-ramps on a high-grade bridge, a busy traffic intersection, and a lower traffic-volume residential street. We measured water-leachable 6PPD and 6PPD-quinone concentrations, and we are identifying and quantifying TRWPs in these samples. These efforts aim to deepen our understanding of the environmental occurrence and fate of 6PPD and 6PPD-quinone by investigating: (i) seasonal trends of 6PPD and 6PPD-quinone concentrations in road dust, (ii) the relationships between environmental conditions and 6PPD-quinone concentrations, and (iii) the effects of road type and traffic on the TRWP morphology and 6PPD and 6PPD-quinone concentrations in road dust. Preliminary results showed that the highest leachable concentrations of 6PPD and 6PPD-quinone were 15.3 ± 7.6 $\mu\text{g/g}$ and 2.6 ± 1.2 $\mu\text{g/g}$, respectively, in per gram road dust samples collected in the spring, while the lowest concentrations were observed in samples collected in winter. Concentrations of 6PPD-quinone in leachates appear to correlate with temperature during the antecedent dry period, but not with ground-level ozone concentrations. Additionally, the road dust from the intersection and the residential street contained similar concentrations, which were generally higher than those at the sampling points on the bridge or the high-speed road. Our findings provide new insights into TRWP generation and the potential release of tire rubber additives, thereby improving emissions estimates for TRWPs and tire-related chemicals and informing efforts to mitigate tire-derived pollution.

4.12.A.T-05 6PPDQ and other tire-wear chemicals in springtime snowbanks of Washington State, USA

Jeffrey Perala-Dewey and Edward P. Kolodziej, University of Washington, Tacoma

Tire wear chemicals present in roadway runoff threaten aquatic organisms, particularly 6PPD-quinone (6PPDQ), a transformation product of the ubiquitous tire preservative 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine) that is highly toxic to coho salmon and other fish species. While current research efforts on tire-wear chemicals typically focus on urban stormwater, melting roadway snow that contains tire-wear chemicals and tire particles may represent a significant localized and storm-independent source of these chemicals to aquatic ecosystems. Springtime melting of snowbanks may also lead to exposure of sensitive fish species at critical life stages at times when stormwater flows are absent (i.e. sunny spring days). We measured a suite of tire-wear derived chemicals, including 6PPDQ, in late-season aged snowbanks along high-traffic routes in mountain passes of the Cascade Mountains in Washington State. We detected tire-wear chemicals at variable concentrations in all snowbank samples, including 6PPD and 6PPDQ, as well as other p-phenylenediamines, benzotriazoles, benzothiazoles, and other roadway contaminants. These results provide a first look into the roles of melting snow of the Pacific Northwest as a transient source of tire-wear chemicals. To better understand the fate of tire-wear chemicals in roadside snow throughout winter and spring seasons, we characterized the

concentrations and compositions of tire-wear chemicals in snow samples relative to other environmental sources, including roadway runoff.

4.12.A.T-06 A Combined field and geospatial method to prioritize stormwater outfalls based on loadings of 6PPD-Quinone and other toxic road runoff compounds

Tim Frederick Mainguy Rodgers, Simon Drew, Gopal Sharma, Zoe Salinas and Rachel Scholes, University of British Columbia, Canada

Runoff from roadways and other impervious surfaces carries “toxic road runoff compounds” (TRRCs) including tire-associated chemicals, through stormwater infrastructure to outfalls that discharge to receiving bodies. A key challenge is to identify which stormwater outfalls contribute most to elevated TRRC levels in receiving waters. Prioritizing outfalls is difficult because concentrations of stormwater derived contaminants vary greatly across a storm, and between sub-catchments. To enable identification of priority outfalls, we developed a combined geospatial analysis and field sampling method incorporating estimates of TRRC releases from vehicles, urban drainage systems, and spatially resolved sediment sampling. We hypothesized that since many TRRCs are hydrophobic, trends in TRRC concentrations in sediment fines collected from stream hyporheic zones along a stream profile would provide evidence for which stormwater outfalls were contributing most to loadings. Our aims were to assess: 1) whether sediment TRRC concentrations were consistently elevated downstream of specific outfalls relative to other locations, 2) if elevated sediment TRRC concentrations corresponded with outfalls that increased water TRRC concentrations during rain events, and 3) whether relative sediment TRRC concentrations correlated with geospatially predicted loadings. This presentation will discuss the performance of our combined prioritization method, including how we addressed challenges due to gaps in geospatial data, inter-event variability in sediment TRRC profiles, and substantial variability in sediment characteristics along urban streams. Preliminary results from 8 sediment TRRC profiles across 2 catchments in British Columbia indicate that changes in sediment concentrations may indicate upstream sources. However, sediment concentration profiles alone may not be sufficient, in part due to apparent transport of sediment between locations. By combining evidence from sediment TRRC profiles with geospatial loading estimates, we identified outfalls likely associated with high loadings. Time-resolved in-stream TRRC concentrations measured up and downstream of these outfalls indicate that the combined geospatial and sediment data may be used to indicate outfalls that likely contribute high loadings of TRRCs to streams. If effective, this approach could reduce the need for intensive rain sampling during rain events, which can be costly or impossible for many stormwater managers.

4.12.B.T From Tread to Watershed: How Tire Wear Particles and Chemicals Affect Aquatic and Terrestrial Ecosystems

4.12.B.T-03 Quinone formation profiles and additive toxicity of para-phenylenediamine derivatives in Coho Salmon

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6PPD-quinone, a toxic transformation product of the tire rubber antiozonant 6PPD, has been identified as a causative agent of large-scale mortality events in coho salmon (*Oncorhynchus kisutch*) in urban watersheds. Our work evaluated the acute toxicity of alternative PPD antiozonants, specifically: 6PPD, IPPD, and 7PPD, and their respective oxidation products. Juvenile coho salmon were statically exposed for 24 hr to graded chemical stock concentrations to each ozonated PPD and pure PPD-quinone forms. Mortality data were fitted with logistic models to generate dose-response curves and estimate median lethal concentrations (LC50s). Dose-response comparisons revealed that ozonated 6PPD and pure 6PPD-quinone exhibited nearly identical LC50 values (63.6 ng/L vs. 65.5 ng/L). This supported the finding that 6PPD-quinone was the primary driver of acute toxicity. However, ozonated IPPD proved more toxic than IPPD-quinone (641.7 ng/L vs. 1074.1 ng/L),

with mortality likely influenced by the presence of small amounts of 6PPD ozonated to 6PPD-quinone in the commercial IPPD standard. For 7PPD, both the ozonated form and its pure quinone exhibited high LC50 values (4857 ng/L vs. 5702 ng/L) with shallow dose-response curves, indicating low overall toxicity and minimal contribution from secondary byproducts. To evaluate the potential for additive effects among quinones, salmon were exposed to a series of equipotent chemical mixtures containing 6PPD-quinone, IPPD-quinone, and 7PPD-quinone. Mixture concentrations were formulated using LC10-LC90 values derived from individual dose-response curves. While individual trials showed variability in mortality patterns, the pooled logistic trend ($R^2 = 0.675$) revealed a consistent dose-dependent increase in mortality, supporting the additive toxicity from diverse quinones. We also assessed the influence of environmental exposure history by comparing stream-reared and well-water fish exposed to 6PPD-quinone and IPPD-quinone. Stream-reared fish showed mortality only at the highest 6PPD-quinone concentration (40%) and no response to IPPD-quinone, while well-water fish displayed dose-dependent responses to both. These results suggest that prior pollutant exposure may confer a degree of physiological tolerance or conditioning effects in coho salmon. While quinone formation is the driver of toxicity in ozonated mixtures of PPDs, chemical mixtures, impurity profiles, and environmental history can influence survival and tolerance.

4.12.B.T-04 Leveraging multi-omics analyses to explore the toxicity of urban road runoff contaminants in Salmonid species

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Components of road runoff have been correlated to adverse health outcomes in fish and aquatic invertebrates residing in urban bodies of water. Recently, a transformation product of the antioxidant chemical added to car tires, N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD-quinone, 6PPD-q), was found to induce urban stormwater mortality syndrome in salmonids. Exposure to 6PPD-q has the potential to increase the sensitivity of salmonids to other co-occurring contaminants commonly detected in surface waters, such as the polycyclic aromatic hydrocarbon 9,10-Anthraquinone (AQ). Liquid chromatography mass spectrometry-based metabolomics and transcriptomics were utilized to elucidate sublethal toxicity and mechanisms of action of road runoff pollutants among *Oncorhynchus mykiss*, *O. kisutch*, and *O. tshawytscha*, were chosen to represent a range of sensitivities to 6PPD-q and fill current data gaps in the literature. Juvenile fish were exposed to established sublethal concentrations of 6PPD-q and AQ, both separately and in combination, over a five-day period. Non-targeted MS-based metabolomics analysis and RNA-sequencing were conducted on liver samples. Using in-house and open-source spectral libraries, 260 metabolites were confidently annotated in liver tissues. More differentially expressed genes were identified in coho exposed to 6PPD-q compared to rainbow trout and chinook. This study provides mechanistic insights into 6PPD-q and AQ toxicity in salmonids, highlighting oxidative stress, mitochondrial dysfunction, and impaired detoxification as key drivers of sublethal effects. Furthermore, the observed increase in metabolic activity in AQ and co-exposure treatments suggests that AQ may influence the biological response to 6PPD-q, potentially by inducing antioxidant defenses and xenobiotic metabolism. In the co-exposure scenario, this may contribute to an antagonistic interaction, attenuating some of the toxic effects of 6PPD-q, though this should not be interpreted as a beneficial or protective effect of AQ.

4.12.B.T-05 Assessing toxicity of tire wear particle associated chemicals in the estuarine fish, sheepshead minnow (*Cyprinodon variegatus*)

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Tire wear particles (TWPs), a significant source of microplastic pollution, introduce toxic chemical additives such as 6PPD and its transformation product 6PPD-quinone (6PPD-Q) into aquatic environments via urban runoff. While the acute toxicity of 6PPD-Q has been established in freshwater species, its effects on estuarine

organisms remain poorly understood. This study investigates the toxicological impacts of TWP leachate, 6PPD, and 6PPD-Q on *Cyprinodon variegatus* (sheepshead minnow), a model estuarine species. Using a 96-hr exposure assay, 24h post hatch larval minnows were subjected to increasing concentrations of 6PPD (under UV light), 6PPD-Q, and diluted TWP leachate. Results revealed no mortality from 6PPD-Q at concentrations up to 500 µg/L but demonstrated significant mortality with exposure to 6PPD and TWP leachate, indicating that the presence of unidentified degradates of 6PPD and/or toxicants in the TWP mixture are leading to mortality rather than 6PPD-Q alone. To explore this, both targeted and non-targeted chemical analyses were conducted. While targeted methods employing solid-phase extraction (SPE) for sample preparation and LC-triple quadrupole mass spectrometry (LC-MS/MS) with native calibration curves for quantification were used to measure 6PPD-Q concentrations, non-targeted high-resolution mass spectrometry (HRMS) was employed to screen for additional, uncharacterized compounds potentially driving toxicity. This research establishes acute toxicity thresholds for TWP contaminants in estuarine systems and underscores the importance of assessing the chemicals in TWPs in environmental risk evaluations. Our findings offer critical insights into the ecological risks associated with TWPs in coastal ecosystems and inform regulatory frameworks for emerging tire-derived pollutants.

4.12.B.T-06 Effects of tire rubber particles on biotransformation enzymes in three trout species

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Recent work has demonstrated that leachates of tire and road wear particles that enter aquatic environments as a constituent of road runoff can induce severe toxicity in aquatic species. Especially salmonid fish appear to be susceptible to toxicity at low concentrations of toxicants like 6-PPD-quinone (6-PPD-Q), polynuclear aromatic hydrocarbons (PAH) and other components of tire rubber. However, significant differences have been found between various fish species, and even within the salmonids. The objective of this study was to further investigate if differences between biotransformation enzyme induction and activity could explain some of the observed species sensitivity differences. Rainbow trout, brook trout and brown trout were exposed to pulsed doses of tire crumb rubber over 96-h at environmentally relevant concentrations, after which gall bladder, gill, liver and intestinal tissues were collected. Bile analysis demonstrated that PAHs and 6-PPD-Q metabolites were actively absorbed during the exposure. Slot blots using specific antibodies for cytochrome P450-1A (CYP1A), and glutathione transferases (GST) were performed to analyze expression levels of these enzymes in gill and liver. This was correlated with catalytic activity of these enzymes through enzyme assays, using the ethoxyresorufin O-deethylase assay for CYP1a activity, 1-chloro-2,4-dinitrobenzene for GST activity, and 1-naphthol for phenol-type glucuronosyltransferase activity in subcellular fractions of liver and gill. Results showed significant species differences for some of the enzymes, with overall a lower expression in brook trout. This could explain the higher sensitivity of this species to road runoff pollutants compared to the other trout species. Differences in expression and activity were also observed between the tissues analyzed, which will have an effect on the toxicokinetics of different chemicals. The obtained information will be useful for risk assessment of tire wear particles as a subclass of the larger group of microplastics.

4.12.P-Tu From Tread to Watershed: How Tire Wear Particles and Chemicals Affect Aquatic and Terrestrial Ecosystems

4.12.P-Tu-036 Sensitive optical detection of 6PPD in drinking water with absorbance-transmittance excitation emission matrix (a-TEEM) spectroscopy

Adam Gilmore, HORIBA Instruments Incorporated

Tire-wear particles are globally associated with leaching and runoff of the toxic chemical, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine, commonly referred to as 6PPD. This chemical is also known to be converted through oxidative processes in water to a much more toxic quinone form known as 6PPD-Q. Numerous toxicological studies indicate that the 6PPD family of chemicals has been attributed to large scale die-offs of aquatic species, in particular Coho salmon in the United States Pacific Northwest. In June 2024, the

United States Environmental Protection Agency (USEPA) addressed the National Registry with Aquatic Life Acute Screening Values for Freshwater of 8.9 micrograms/L for 6PPD. Recent reports have also shown that 6PPD can be detected using HORIBA's patented Absorbance-Transmittance Excitation Emission Matrix (A-TEEM) spectroscopy, although functional detection and quantification limits were not formally defined. In this study we show that A-TEEM spectroscopy can rapidly scan drinking water matrices with significant background levels of natural dissolved organic matter to resolve and quantify 6PPD. The simple sample preparation requires only filtration (0.45 micrometer) and a complete scan requires less than 8 minutes. The sensitivity limits are significantly below the USEPA screening value with a standard deviation of <0.05 micrograms/L over a range of 0 to 5 micrograms/L and a coefficient of determination (r-squared) of 0.99962. The standard deviation was less than 1.5 micrograms/L for a range of 0 to 100 micrograms/L with an r-squared of 0.99909. We thus conclude that effective screening of drinking water sources for 6PPD can be made using A-TEEM spectroscopy. Importantly, this ability to monitor 6PPD can facilitate evaluation of toxic effects as well as treatment processes aimed at its removal, which can be relevant to pollution remediation.

4.12.P-Tu-037 Recycled-tire playground pavements release rubber particles

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The European sales of tires exceed 300 millions per year (2023 data from the European Tyre and Manufacturer Association). At the end of their life, a large portion of tires go to landfill or incineration. There is therefore a need to find alternative solutions to reuse them safely in other products. In 2018 in Europe, approximately 2 million tons of end-of-life tires were used for material recovery in construction, civil engineering, and automotive applications. In our recent work, we showed that an old and a fresh paver made of recycled tires release particles and chemicals to stormwater due to degradation. In the present work, we will discuss new data on particles released from multiple playgrounds paved with crumb rubber. We showed that an old playground gets damaged and clogged over time therefore releasing rubber particles to the surrounding soil. Our results showed that rubber particles can be efficiently filtered out by soil planted with grass. We evaluated how far from the rubber pavement and how deep in the soil rubber particles from the playground can transfer. These data indicate that while reusing used tires in alternative materials is a seducing idea, their use require a particular attention to the design of their surroundings to mitigate the release of particles and chemicals in receiving environments. Current work is now evaluating the potential of these rubber particles exported from playgrounds to release 6-PPD-quinone and other tire-leachates.

4.12.P-Tu-038 Optimization of extraction methods for assessing rubber additives in soil

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Rubber products like end-of-life tires are difficult to recycle chemically, and often end up physically transformed as crumb rubber, to be used as infill for artificial turf fields. These fields transport rubber-derived chemicals (RDCs), a broad group of additives used during manufacturing, to water and soil surrounding the fields. With recent studies highlighting the ecotoxicological effects of some RDCs such as 6PPD-quinone, new methods to extract these chemicals from complex environmental matrices have become critical for preparing RDCs for analysis. Despite this progress, the absence of a comprehensive extraction method for RDCs in soil hinders the study of the fate and transport of these contaminants. Two popular extraction methods, accelerated solvent extraction (ASE), and quick, easy, cheap, effective, rugged, and safe (QuEChERS), were compared to determine the baseline approach for optimization of RDCs from soil. Variables for both were optimized to maximize recoveries of a standard mixture containing 33 RDCs, including antiozonants such as 6PPD, vulcanizing agents, and plasticizers. ASE parameters tested included extraction times, solvents, in-line clean-up sorbents, and temperature while QuEChERS parameters included the aqueous/organic layer ratio, soil amounts, and dispersive solid phase extraction sorbents. All recovery samples were assessed by quantitative liquid chromatography mass spectrometry using an Altis Triple Quadrupole Mass Spectrometer. Data analysis was conducted using open-source software Skyline and Microsoft Excel. The method with the highest standard

recoveries was chosen for further testing of real-world samples. Soil samples were collected from several sites adjacent to an artificial turf field in Massachusetts to show proof-of-concept of the optimized method.

4.12.P-Tu-039 Investigating environmental tire and road wear particle derived pollutants in Seattle's stormwater system

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Tire and road wear particles (TRWPs) are generated through the physical abrasion of tires on the roadway during regular vehicle use and are subsequently transported via urban stormwater runoff into nearby receiving waters. As a result, TRWPs are ubiquitous in the urban environment and threaten aquatic ecosystems by serving as ongoing and legacy sources of 6PPDQ and other tire-derived contaminants. However, most studies to date have investigated the chemical fate of TRWPs using manually generated or synthetically aged TRWPs, which may or may not accurately represent the chemical characteristics of real-world TRWPs. To address this knowledge gap, we investigated environmentally isolated TRWPs collected from street sweepings, roadside soils and sediments collected from various locations within the stormwater conveyance system in Seattle, WA. TRWPs were separated from environmental samples via density separations and manually isolated after visual identification with optical microscopy. TRWP-containing sediments were then analyzed for 6PPDQ and other tire-derived compounds using both aqueous and solvent extraction techniques. In addition, isolated TRWPs were examined for their potential for 6PPDQ formation and tire-derived contaminant leaching. Findings from this study will help determine the need for roadway and stormwater management strategies to prioritize the physical removal of TRWPs from roadway and stormwater infrastructure relative to removing dissolved phase 6PPDQ from roadway runoff to effectively mitigate contaminant loading in roadway runoff impacted watersheds.

4.12.P-Tu-040 Monitoring at urban salmon streams indicates that multi-hr periods of elevated 6PPD-quinone concentrations may be widespread in urban waterways during surface runoff

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Automobile-source N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q) is flushed from roadways and other reservoirs to streams during wet weather, high stream flow conditions. Therefore, assessing temporally dynamic contaminant transport and hazard to aquatic species can be achieved with time- and flow-resolved stream 6PPD-Q concentration data. We monitored small, urban streams where salmon spawn and rear during dry weather (baseflow) before rainfall and for a duration of 72 hr from the initial rise in stream flow (stormflow) after the start of rainfall. For five streams in the metropolitan area of Vancouver, British Columbia, Canada, we collected water samples with portable autosamplers that were then analysed for 6PPD-Q, and obtained data on rainfall and stream flow (discharge). Stormflow concentrations of 6PPD-Q regularly exceeded published government guidelines (e.g. 10-12 ng/L BC, WA, USA) and toxicity thresholds (e.g. coho salmon LC50s 41-95 ng/L) for multi-hr time periods, but not during baseflow conditions. Accordingly, estimates of 6PPD-Q transport must account for high-flow periods to generate accurate watershed loads. We conclude that high-concentration, multi-hr 6PPD-Q pulses are widespread in the urban salmon streams of metropolitan Vancouver, a phenomenon likely representative of other urban watersheds with high roadway density.

4.12.P-Tu-041 Tire and road wear particle flux into a riverine estuary from a heavily trafficked bridge

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Tire and Road Wear Particles (TWRPs) have been identified as major contributors to marine microplastic pollution, and increasing dependence on personal vehicles suggests their prevalence in the environment will continue to grow. In much of the United States, the lack of stormwater treatment infrastructure results in road

runoff being deposited directly into adjacent retention basins or nearby waterbodies, facilitating the input of TRWPs into marine and aquatic environments. Due to the relatively high density of TRWPs, it is expected that TRWPs accumulate in sediments proximal to their initial points of deposition, or in stormwater retention basins when present. Bridges overlying waterbodies are also a suspected major source of TRWPs to the environment, as their storm drainage systems often allow for an amount of runoff to directly reach the water below. This study aims to investigate TRWP flux from a well-traveled bridge overlying a riverine estuary with several points of road runoff deposition. To accomplish this goal, sediment and water samples will be collected from a stormwater retention basin adjacent to one downspout, an estuarine saltmarsh adjacent to a second downspout, as well as the stormwater effluent from the downspouts themselves. Samples collected before, during, and after a precipitation event will be analyzed for TRWP mass using density separation techniques and pyrolysis-GC/MS. In both sites, TRWP concentrations are expected to peak in the water column during the precipitation event as particles are transported through the downspouts. We expect concentrations to fall at a faster rate in the saltmarsh than the retention basin, as tidal flushing will pull particles out of the marsh into the riverine estuary. Higher concentrations are anticipated in the sediments as particles settle out of suspension, with greater concentrations observed over time in the drainage basin. Overall, the work presented here will provide a unique case study on environmental factors, both built and natural, and their influence on TRWP distribution in coastal estuaries.

4.12.P-Tu-042 Transport and occurrence of tire-related chemicals and their transformation products in sediments of densely populated Newport Bay, CA

Oluniyi Olatunji Fadare¹, Andrew Gray¹, Clare Murphy-Hagan¹, Zhongkun Du² and Jay Gan¹, (1)University of California, Riverside, (2)College of Resources and Environment, Shandong Agricultural University, China Limited knowledge exists on the fate, transport and distribution of tire-and-road wear particle (TRWP)-related chemicals into the aquatic environment. The combined effect of mechanical abrasion, UV radiation, temperature, and precipitation results in volatilization, leaching, transformation, and release of tire-related chemicals into urban watershed systems. Recent studies have shown that TRWP-related chemicals, including organic accelerators, anti-scorching agents, plasticizers, and antioxidants, used in tire vulcanization, protection, and processing, pose an emerging threat to aquatic ecosystems. This study investigated the abundance, distribution, and variability of TRWP-related chemicals in sublittoral sediments of Newport Bay, CA. Subtidal sediments (0-5 cm), collected with grab samplers, were extracted and analyzed using UPLC-MS/MS with ESI (+) source in multiple reaction monitoring mode. We detected 19 target compounds in sediments of two particle size ranges (< 100 µm and 100-250 µm) with a total concentration range of 77.6 to 2957.4 ng g⁻¹ and 70.1 to 2.883.1 ng g⁻¹, respectively. Benzothiazole and its transformation products exhibited the highest maximum concentrations (3.6-1605 ng g⁻¹ and 4.3-1412 ng g⁻¹ for < 100 µm and 100-250 µm particle sizes, respectively). Antioxidant derivatives p-Phenylenediamine (PPDs) and PPDs-derived quinones (PPDQs) were also detected at concentrations of up to 28.6 ng g⁻¹ and 9.7 ng g⁻¹, respectively. Our results show that contaminant concentrations are particle-size dependent, with sediment-bound contaminants preferring particles < 100 µm in size. This study highlights the pivotal role of sediment in TRWP-related chemical transport and storage and discusses the potential impact of these contaminants on water quality and aquatic ecosystems.

4.12.P-Tu-044 Nontargeted chemical identification of micron cryo-milled tire tread (CMTT) and electric vehicle tire leachates: A comparison study

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Tire tread particles (TTPs) are generated through road friction and are known to leach toxic chemicals into aquatic ecosystems. Previous nontargeted chemical analysis has identified more than 50 chemicals from passenger car tires, such as hexa(methoxymethyl)melamine (HMMM), N-cyclohexyl-N'-phenylurea (CPU), and N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). However, there is little information regarding the chemical profiles of electric vehicle (EV) tires, despite their faster tread wear compared to conventional tires

due to their heavier components. This study analyzed the chemical profiles of TTPs leachates in water under dark conditions over a 24 hr period. Composite cryomilled tire tread (CMTT) from passenger and heavyweight vehicles was obtained from the United States Tire Manufacturers Association (USTMA), and EV tire tread was provided by Oregon State University. Chemical profile comparisons of the leachates were conducted by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS). A total of 613 chromatographic features were detected across both sample types, of which 109 were tentatively identified compounds (TICs) based on mass spectral library matching. TICs will be assessed for persistence, bioaccumulation, and toxicity properties in order to prioritize compounds for further analysis and environmental health implications. Due to their increased mechanical demands, it is expected that EV tires will contain unique chemical constituents compared to CMTT or other passenger tires. These findings will provide greater perception into the chemical diversity and complexity of tire leachates, which will improve environmental health assessments regarding tire pollution. **Disclaimer/Disclosure:** The National Science Foundation (CBET Project # 2327008) and California State University COAST State Science Information Needs Program (COAST-SSINP-2020-001) provided support for this study.

4.12.P-Tu-045 Comparing 6PPD/6PPD-Q and tire wear particle concentrations in aquatic sediments across the inland northwest

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6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine), an antioxidant increasingly found in tire rubber formulations, is widely recognized as an emerging environmental contaminant. Since 2021 its highly toxic transformation product, 6PPD-quinone (6PPD-Q), has received significant attention due to its environmental persistence and its determination as the cause of Coho salmon mortality in urban streams. Work is ongoing to determine the potential risk of 6PPD-Q to other ecological systems, including risks to human health. Tire wear particles (TWPs), generated by vehicular tire abrasion, are a significant source of 6PPD/6PPD-Q contamination to aquatic systems primarily due to stormwater runoff, which transports both TWPs and leachate into aquatic systems. This study hypothesizes 6PPD/6PPD-Q concentrations will be higher where TWPs are present in the aquatic sediments because these particles act as a continued contaminant source post emplacement. To test this hypothesis we are collecting and analyzing sediment samples from multiple sites across the inland Northwest and developing analytical methods to extract and quantify these contaminants. Samples collected from the field are homogenized to ensure uniformity and dried to remove all moisture content. The samples are then split for 6PPD/6PPD-Q and tire wear particle extraction. A three-step liquid-solid extraction protocol uses sequential extractions with acetonitrile, methanol, and a final acetonitrile rinse to extract 6PPD/6PPD-Q. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), optimized for high sensitivity and specificity, is utilized to detect and quantify these contaminants. Tire wear particles are extracted using a novel density and oil separation protocol. Particle identification and counts are made using dissecting microscope with subsample material confirmation using micro-FTIR analysis. Results from this study will help inform pollution prevention, monitoring, and cleanup efforts for these emerging contaminants. **Disclaimer/Disclosure:** This work is supported by EPA cooperative agreement number 02J19601.

4.12.P-Tu-047 Portable autosamplers are suitable for monitoring 6PPD-quinone in the field over several days

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In urban streams, contaminants such as N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q) are flushed from impervious surfaces during wet weather. Portable autosamplers can collect time- or flow-paced stream water samples that can help to understand the transient contaminant fluxes and aquatic exposure caused by dynamic stream flow. To support deploying these systems to monitor road runoff contaminants, we

evaluated the suitability of portable autosamplers to collect water samples for tracking 6PPD-Q in streams over time, assessing whether carryover, sorption, interference, or instability of the target compound would bias results. Our laboratory and field tests indicate that samples are generally free of interference from tubing and bottles, and that analyte losses were minor over a multi-day period. Finally, time-matched samples taken directly into storage jars compared well with autosampler results. We conclude that portable autosamplers are fit for collecting water samples for 6PPD-Q analysis when used with appropriate cleaning and field protocols. Automated sampling will aid in estimating time-resolved transport and hazard from concentration data.

4.12.P-Tu-048 Monitoring tire road wear particles in stormwater-dominated streams by LDIR

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Stormwater runoff is a major route of transport for microplastics, including tire road wear particles (TRWPs), to surface waters. Quantifying the environmental loading of TRWPs is a high-priority data gap to inform management. The wide range in size and density of TRWPs generated on roadways presents challenges for collecting representative samples during storm events, but this may be overcome by sampling well-mixed locations in stormwater-dominated channels. To test this strategy, we identified two urban streams in San Francisco with sampling points currently being monitored by USGS water gauges where previous studies found that turbulent conditions created well-mixed conditions for suspended solids. Grab samples (1 liter) were collected at three depths (0.2x, 0.6x, and 0.8x total depth) at 4 time points over a storm hydrograph (during the rise, at the peak, just after the peak, and at the tail) at one location within each of these channels. Sample duplicates and blanks were also collected. The concentration (particle number per L) and size (area dimensions) of TRWPs were determined by Laser Direct Infrared (LDIR) spectroscopy. Additional water samples at each time point were collected for analysis of total suspended solids (TSS) and particle size distribution. Our results will inform on LDIR methods for TRWP enumeration in environmental samples, elucidate the relationship between TSS and TRWPs, and test whether there is significant variation across depth in TRWP concentration or size during the runoff events. This research will characterize stormwater inputs of TRWPs to a vital estuarine system while informing on strategies for watershed-scale monitoring.

4.12.P-Tu-049 A robust extraction method for monitoring 6PPD-Q and other PPD-quinones in fish tissue

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6PPD-quinone (6PPD-Q) is an ozonation by-product of the tire antioxidant 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine), which emerging research shows poses a significant threat to aquatic ecosystems. Tire manufacturers are looking towards alternatives to 6PPD, such as other p-phenylenediamine derivatives. However, these compounds also form their own quinones, and those toxicities are beginning to be studied. As the concern over this class of contaminants grows, developing methods to extract and analyze various matrices is of the utmost importance. A high level of sensitivity is necessary, as it has been discovered that the LC 50 for 6PPD-Q in juvenile coho salmon is as low as 0.094ng/mL. This poster displays a QuEChERS (Quick, Easy, Cheap, Efficient, Robust, and Safe) extraction of a subset of PPD-Qs in salmon, combined with push-thru cartridge clean-up using PSA (Primary-Secondary Amine), C18, and UCT proprietary sorbent Lipifiltr® in series. The extracts are analyzed on a core-shell C18 HPLC column using LC-MS/MS. A matrix-matched calibration, followed by limit of detection and limit of quantitation studies, determines accuracy and precision. 6PPD-Q, the compound of interest, is calculated using extracted isotope dilution, with the remaining PPD-Qs calculated using an extracted internal standard. The final analysis can achieve a 0.073 ng/g detection limit and minimize matrix effect to below 12%. **Disclaimer/Disclosure:** Research performed by UCT, Inc

4.12.P-Tu-052 It's everywhere, but is it toxic to brook trout? A basin-wide survey of 6-PPDq in the Delaware River Basin

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Brook trout (*Salvelinus fontinalis*), the only trout species native to the Delaware River Basin, holds significant economic, recreational, and cultural importance to the region. Their population in the basin is historically low and is threatened by rising water temperatures, which will further shrink their suitable habitat range. Additionally, a recent USGS study found that juvenile brook trout are vulnerable to 6-PPDq at concentrations similar to those affecting coho salmon. Brook trout sensitivity to 6-PPDq could be a factor in their population decline in the Delaware River Basin. To investigate the presence of 6-PPDq in the watershed, a basin-wide survey of trout streams was conducted from April 2024 through April 2025. Triplicate samples were collected quarterly from 14 tributary or main-stem sites of the Delaware River, as well as selected sites during several rain events after at least a two-week dry period. At least one sampling event quantified 6-PPDq at each of the 13 sites. Concentrations ranged from below detection up to 150.9 ± 0.5 ng L⁻¹, with an average of 4.7 ± 19.8 . The highest concentration was recorded during a storm event in July 2024 in an ephemeral runoff drainage from the Pennsylvania Turnpike that discharges into Valley Creek. However, apart from samples associated with Valley Creek, no other concentrations exceeded 10 ng L⁻¹ during the study. The maximum concentration observed was above the 100 ng L⁻¹ 24-hr LC50 for 3-week-old brook trout, though it is unlikely that this concentration persisted for 24 hr. Additionally, during a basin-wide drought in fall 2024, when water flows were significantly reduced, the quarterly November sampling was the sole event in which 6-PPDq was quantified in every collected sample. This indicates that 6-PPDq is consistently present in basin surface waters, even in those with limited influence from paved surfaces. However, concentrations are diluted to below detection limits under typical flow conditions. The widespread presence of 6-PPDq, mostly well below acute thresholds, underscores the need to establish sub-lethal toxicity values for brook trout. Based on this study, it is too soon to determine the role of 6-PPDq in the decline of brook trout in the Delaware River Basin. Still, the pollutant can reach acute toxicity levels for juveniles in one system and is broadly present in brook trout streams throughout the basin.

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4.12.P-Tu-053 Monitoring and modelling exposure to 6-PPD quinone in UK rivers

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6-PPD is a chemical antioxidant used in tyres to prevent degradation and extend the tyre's lifespan. Following release, it reacts with ozone to form 6-PPD quinone (6-PPDq) which can then be transported to surface water. The presence of 6-PPDq in highway runoff in the North-West of the United States has been linked to impacts on Coho salmon. While the UK, is a heavily populated country with areas of high traffic density, the occurrence and risks of 6-PPDq in the country have yet to be established. The aim of this study was to develop and evaluate a high temporal and spatial exposure assessment model for 6-PPDq and other highway-derived pollutants in UK surface waters. The model, which was developed for the Yorkshire region in England, utilized information on traffic densities, 6-PPD release rates, rainfall alongside a hydrological model and a river model to estimate concentrations of 6-PPDq in 16,000 sub-basins across Yorkshire. Alongside the modelling, continuous monitoring of surface water was performed at 19 locations across the region to generate weekly data on the occurrence of 6-PPDq to provide a dataset to evaluate the model. In the monitoring study, 6-PPDq was detected in 27% of the samples obtained and in between 70-80% of samples from the more urbanized monitoring locations. Maximum measured concentrations were seen in the more urbanized areas and were in the low ng/l range. Comparison of model predictions with the monitoring results showed model predictions to be fair and ranging from 1.3 to 6.6 times higher than the observed concentrations. Use of the model for the 16,000 sub-basins showed that more than 95% of sub-basins had concentrations lower than 5 ng/l but there were some situations where levels were high. The sub-basin with the highest exposure concentration had an annual average concentration of 72 ng/l and a peak concentration of 3500 ng/l. Our modelling approach provides a useful approach for characterizing exposure and risks of 6-PPDq and other highway-derived contaminants and for

identifying hotspots of exposure. **Disclaimer/Disclosure:** This project was funded by the UK Natural Environment Research Council

4.12.P-Tu-055 Relevant exposure window for 6PPD-quinone acute toxicity

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Dose and exposure duration are critical determinants of toxic effects. The novel tire-derived toxicant 6PPD-quinone is acutely lethal to various salmonids within days of exposure to concentrations below 2 ppb. Coho salmon are the most sensitive species currently known, with acute mortality occurring within 24 h at most exposure concentrations. Mortality is preceded by changes to swimming behavior, progressively including increased surfacing, continuous surface swimming, and loss of equilibrium. Lethal body burden appears to be acquired hr in advance of development of these symptoms. I will explore critical exposure windows for triggering mortality in coho salmon through review and analysis of prior research exposing coho salmon to 6PPD-quinone or whole roadway runoff. The practical consideration of this analysis is time periods during static laboratory tests over which to average exposures concentrations to best estimate the median lethal concentration (LC50).

4.12.P-Tu-056 Effects of tire wear particles degraded by UV irradiation on marine amphipod

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Amphipods are a typical benthic invertebrate in aquatic environment. The species inhabit marine water areas close to the thousands and are important prey for a lot of organisms as fish. Generally, because they are very sensitive to chemicals, the serious polluted sites possibly threaten their biodiversity. Recently, some research groups showed that a large amount of tire wear particles (TWPs) have been carried into the aquatic environment. Tires are composed of many kinds of chemicals and possibly affect aquatic organisms especially inhabit the benthic environment. Most TWPs settle on the road and stay there, unless it rains. As a result, the TWPs continuously exposed to ultraviolet (UV) from sunlight, they and their materials and components possibly degrade. A certain amount of degraded TWPs move into aquatic environment with the weather events as raining. Toxicities derived from TWPs degraded by UV on aquatic organisms are not well-known and the information is limited. The present study investigated the effects of UV irradiated Cryo-Milled Tire Tread (CMTT), obtained from U.S. Tire Manufacturers Association, on marine amphipod, *Ptilohyale barbicornis*. CMTT was irradiated with UV for 8 weeks in a Weather-Ometer at 80 degree. Additionally, we prepared CMTT without UV irradiation. CMTTs with or without UV irradiation were added to artificial seawater in a glass vessel as concentrations of 12.5, 0.5, 0.1, and 0.02 mg/mL. Separately, CMTTs at same concentrations were stirred in artificial seawater for 48 hr, and then allowed to stand until the particles had completely settled. The supernatant was then collected and transferred to a glass vessel. Individual marine amphipods were loaded into each vessel and exposed to CMTT particles and leachate for 14 days. CMTT particles without UV irradiation showed 0.500, 0.245 and 0.0399 mg/mL of LC50s at exposures for 4, 7, and 14 days, respectively. Those derived from leachates were also 1.23, 1.12, and 0.792 mg/mL, respectively. On the other hand, UV-irradiated CMTT showed 2.50, 1.50, and 0.358 of LC50s for 4, 7, and 14 days, respectively. Their leachates were also 2.50, 1.69, and 0.356 mg/mL, respectively. UV irradiation certainly loaded the degradation of CMTT particles, and their toxicities caused in marine amphipod from exposures the particles and leachates were significantly reduced. The results suggest that sunlight causes the reductions of toxicities in TWPs settled on the road.

4.12.P-Tu-057 Impacts of nanoscale tire particles and zinc on freshwater algal productivity

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Tire particles generated as tires deteriorate from being driven on roads are a major contributor to micro and nanoplastic pollution in the environment. Tire particles can accumulate on roadways and be transported into

aquatic ecosystems during precipitation events. Zinc oxide is a tire additive used for vulcanization that has the potential to leach into aquatic environments from tire particles. This research aims to understand the role of zinc oxide leaching relative to other chemical constituents with respect to algal productivity using the freshwater algae *Raphidocelis subcapitata*. Microplate algal toxicity experiments were conducted on two types of nanoscale tire particles as well as leachate, and three different-sized zinc oxide particles (1 mm, 70 and 50 nm) along with zinc chloride salt, to determine the minimum inhibitory concentrations affecting algal growth. Both tire and zinc oxide particles had significant inhibitory effects on algal growth. Tire particle IC₅₀ values ranged from 0.4 to 0.7 mg tire particles/L, depending on tire type. Zinc oxide toxicity was related to particle size and ranged from 0.3 mg/L for the 1 mm particles to 0.07 mg/L for the 50 nm particles, similar to the 0.04 mg/L value for zinc chloride salts. This suggests that smaller zinc oxide particles are more toxic to freshwater algae, likely due to the increased surface area for dissolution. Given that zinc is typically around 1-2% of tire mass, the leached zinc is not sufficient to explain the algal growth inhibition observed from tire particles, regardless of the form of zinc used during manufacture. Thus, other chemicals involved in tire manufacturing and known to leach into aquatic systems must also be investigated for their impacts on algal productivity. Nonetheless, tire particles entering aquatic environments pose a risk to algal productivity at the bottom of the food web and could have concomitant impacts on higher-level species.

4.13.P-We Greener and Smarter Sample Preparation for Per- and Polyfluoroalkyl Substances (PFAS) and Contaminants of Emerging Concern (CECs): Innovations and Sustainable Approaches

4.13.P-We-101 Aqueous PFAS passive sampling with ceramic cups

Jessica Goin, Erik Naylor, Gillian Reay Williams and Michael Gefell, Anchor QEA LLC

The fate and transport of per- and poly-fluoroalkyl substances (PFAS), such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), are not yet fully understood in sediments and aquatic environments. This lack of understanding, coupled with an increased interest in PFAS, creates a need for more efficient and representative environmental sampling methods. In some settings, it is impractical to collect water samples by pumping, due to low permeability or in surficial sediments where surface water short-circuiting and dilution would be difficult or impossible to avoid. This creates a need for a passive sampler for PFAS. Ceramic cups are a proven tool for passive sampling of aqueous volatile organic compounds and polycyclic aromatic hydrocarbons in porous media. This study was designed to assess the feasibility of porous ceramic cups for diffusion-based, passive sampling of dissolved-phase PFAS. Ceramic cups were filled with water, capped, and deployed in a water bath with a known aqueous concentration of select PFAS. Water inside the ceramic cup samplers was PFAS-free and spiked with an isotopically labeled tracer compound to track equilibration of PFAS and tracer concentrations. Samplers were retrieved after 7, 14, and 31 days, and aliquots of water from inside and outside the samplers were sent for analysis. Adsorption of PFAS onto the ceramic cups was measured after 31 days. For most of the tested PFAS compounds, approximately 100% equilibration was reached between the inside and outside of the ceramic cup samplers after 31 days of equilibration. Some of the longer chain compounds, especially those with adsorption to the ceramic cup material, had more variability in the degree of equilibration achieved. This study showed that modified porous ceramic cup sampling devices are feasible for sampling many PFAS in water. Future testing will assess using ceramic cups for passive sampling of porewater PFAS concentrations in sediment.

4.13.P-We-102 Direct injection LC-MS/MS workflow for ultrashort, short and long chain PFAS in water: Eliminating SPE To improve sustainability without compromising sensitivity

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PFAS continue to face increasing regulatory attention, but current analytical methods often rely on solid-phase extraction (SPE)—a step that adds time, cost, and a lot of solvent and plastic waste. In this work, we evaluated a more sustainable approach: a direct injection LC-MS/MS method that eliminates SPE entirely, while still

meeting regulatory sensitivity for short-chain PFAS. Using the latest purpose-built inert mixed-mode column from Agilent and a guard column setup, we ran samples of surface water and municipal effluent filtered through a 0.2 µm membrane—no other prep required. The column retains C1 to C18 PFAS in a single run, and the inert hardware helps avoid issues like metal adsorption that can suppress C1–C3 analytes. Results show good reproducibility, clean baselines, and detection limits below 1 ng/L for short-chain PFAS. The method also passes EPA Method 1633A's retention time requirement of ± 0.40 min. Compared to SPE workflows, solvent use was reduced drastically, plastic waste was eliminated, and overall sample turnaround time dropped to 10 minutes. This direct injection approach offers labs a practical way to meet trace-level detection needs with fewer consumables and less complexity.

4.13.P-We-103 Using liquid chromatography tandem mass spectrometry (LC-MS/MS) large volume injection to eliminate the need for offline solid phase extraction (SPE) sample preparation

Elizabeth Brown, Sujan Fernando and Thomas Holsen, Clarkson University

LC-MS/MS is a highly sensitive, accurate, and established technology used to quantify water-soluble ionic per- and polyfluoroalkyl substances (PFAS). As legislation works towards an enforceable contaminant level, at or near zero, improvements to current LC-MS/MS methodologies will be necessary to quantify PFAS at environmentally relevant concentrations. Large volume injection (LVI) technologies are a promising approach for increasing the sensitivities of the current LC MS/MS methods. The inline sample preparation of LVI eliminates the need for a separate sample processing step leading to a more sustainable analysis that does not utilize additional and costly consumables. The aim of this research, with the overall goal of quantifying PFAS contaminants in the parts-per-quadrillion (ppq) range, is to understand the effects LVI has on the limits of detection (LOD) and limits of quantification (LOQ) of the already established LC–MS/MS methods and develop new methodologies to overcome issues typically experienced using LVI technologies (i.e., increased signal-to-noise ratio, poor chromatography, longitudinal diffusion).

4.13.P-We-104 Advancing PFAS detection in soil: A greener, faster and streamlined approach to EPA method 1633 with automated online SPE-LC-MS/MS

Helmer Jonathan Korb, iChrom Solutions

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants with significant potential for adverse human health effects. Due to their widespread presence and resilience in the environment, effective detection and analysis are crucial. Traditional methods for analyzing PFAS, such as those defined by EPA 1633 require labor-intensive and solvent-heavy sample preparation processes. This presentation explores the use of online solid-phase extraction (SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) as a more efficient and environmentally friendly approach for analyzing PFAS in soil, specifically within the framework of EPA Method 1633. Existing methods, including ASTM 8421, ASTM 7979, and EPA 8327, focus on non-drinking water samples and typically involve co-solvation with methanol, which can be categorized as "dilute and shoot" or direct injection. In contrast, EPA Method 1633 mandates manual SPE for all matrix types, requiring the entire water sample and its associated sample bottle to be rinsed. By incorporating automated online SPE-LCMSMS (Symbiosis) for co-solvating non-drinking water samples and automating key sample preparation steps for soil samples, this approach improves sample throughput and analytical efficiency. Additionally, it maintains the integrity of EPA Method 1633 while reducing both time and solvent consumption, thus offering a greener, more sustainable solution for PFAS analysis in future laboratories.

4.13.P-We-105 A simplified method for analyzing gaseous fluorotelomer alcohols using SPME-GC-MS/MS in outdoor air

Md. Abdur Razzaq and Natalia Soares Quinete, Florida International University

Fluorotelomer alcohols (FTOHs) are volatile, semi-persistent precursors to perfluoroalkyl acids (PFAAs), formed through environmental and biological degradation. Despite their relevance, FTOHs remain

underrepresented in environmental monitoring compared to ionic, water-soluble PFAS. They are often the dominant PFAS in indoor air and in the ambient air of landfills and wastewater treatment plants, where they primarily exist in the gaseous phase. This study presents a streamlined, solvent-minimized method for measuring gaseous FTOHs using solid-phase microextraction (SPME) coupled with gas chromatography–tandem mass spectrometry (GC-MS/MS). Air samples were collected using a cascade impactor air sampler to assess phase distribution and enable targeted extraction of gaseous compounds. Method development focused on six FTOHs (4:2 to 10:2), with a systematic evaluation of multiple SPME fiber coatings and the optimization of key parameters including temperature, equilibration time, and desorption conditions. Ethyl acetate was used as the working solvent. The developed protocol enables qualitative to semi-quantitative screening of FTOHs without the need for pre-concentration equipment or large sample volumes, making it ideal for lab-scale or resource-limited settings. This work contributes to greener, more sustainable sample preparation practices and supports expanded PFAS monitoring in air, an emerging environmental matrix of concern.

4.14.P-We Human Exposure to Organic Contaminants of Current and Emerging Concern

4.14.P-We-106 Novel application of low-density polyethylene passive samplers to quantify phthalate exposures in artificial turf environments and turf-air exchange among infill types

Kelly O'Malley, Alison Clark, Lane Tidwell and Kim Anderson, Oregon State University

Artificial turf field users may be exposed to hazardous chemicals that emit into the air from some of the components used in artificial turf fields, like crumb rubber. Phthalates are one of the chemical classes that have been detected in crumb rubber in previous studies and are associated with having reproductive and developmental toxicity following exposure. Artificial turf field managers are beginning to install fields with more sustainable alternative infill to reduce chemical exposure. It is unknown how phthalates move between the artificial turf and the overlying air among different infill types. Low-density polyethylene (LDPE) passive samplers have been used extensively to measure a variety of chemical classes but have not been previously used to measure phthalates. The objectives are to demonstrate a novel application of LDPE sampling to quantify phthalates in artificial turf environments using a phthalate performance reference compound to estimate chemical uptake rates, to investigate the fugacity of phthalates between the turf and the air to assess potential sources, and to assess if newer alternative infill practices improve the air quality and exposure concentrations for field users. We hypothesized that fugacity would indicate volatilization and alternative infills would reduce exposure concentrations of phthalates. Paired air and turf pore air LDPE passive samplers were deployed concurrently at indoor and outdoor artificial fields for 28 days during the summer and late winter. LDPE samplers were analyzed for 31 phthalates and alternatives using a gas chromatography mass spectrometer method. Preliminary summer results show seven phthalates were detected among all air and turf pore air samples, including three that are recognized as reproductive toxicants by REACH (bis(2-ethylhexyl) phthalate, di-n-butyl phthalate, and diisobutyl phthalate). Bis(2-ethylhexyl) phthalate was detected in all artificial turf samples and generally had greater concentrations in the air than its paired turf pore air sample. Results showed the oldest field had the overall highest sum phthalate concentrations, and the newest field had the lowest. Results showed diisobutyl phthalate volatilizing at most sites, and bis(2-ethylhexyl) phthalate and bis(2-ethylhexyl) adipate depositing to turf at all sites. This may indicate the presence of other sources of phthalates in turf indoor and outdoor field environments releasing into the air that go on to deposit to the turf.

4.14.P-We-107 Meta-analysis of long-term per- and polyfluoroalkyl substances (PFAS) trends in groundwater from Miami-Dade county, Florida

Maria de Navarro, Natalia Soares Quinete and Richard Zurbaran, Florida International University

PFAS are water soluble and resistant to biodegradation due to the exceptional stability of C-F bonds. The physicochemical properties inherent to their structures have made them attractive to be used in many industrial applications. Their difficulties to be removed by traditional drinking water treatments and the health risks associated with exposure to PFAS have brought the need to better understand its fate and transport in the

environment. Once these compounds are released, they follow different transport mechanisms and might infiltrate groundwater and surface water, which are usually drinking water sources. Miami-Dade County presents a complex setting with several interactive factors that could affect the aquifer systems, including extensive urban development, military facilities, and airports operations. The availability of long-term PFAS monitoring data from the Miami-Dade Water and Sewer Department provided the opportunity to evaluate PFAS contamination patterns. This study presents a meta-analysis of PFAS concentrations in monitoring wells from Miami-Dade County over five-years period, utilizing publicly available records from the county's groundwater monitoring program. The analysis includes data from multiple monitoring wells, including Biscayne and upper Floridian aquifers, located in different land-use categories, with focus in seasonal trends, spatial distribution, and the functionalities of detected PFAS. Concentrations were statistically evaluated to account for detection frequency, regulatory thresholds (US. EPA health advisory levels), and compound specific trends such as chain length (size) and functional group (class). Preliminary results indicate that concentrations of legacy PFAS, as well as short-chain and replacement compounds, remained relatively stable throughout the monitoring period. Geographic patterns revealed localized hotspots of PFAS contamination, near highly urbanized regions and airport facilities, emphasizing the influence of historical and ongoing point sources. The results display the need of continued long-term monitoring for assessing regulatory compliance, evaluating public health risks, and communicating vulnerabilities for water management strategies.

4.14.P-We-108 Identification of volatile organic compound biomarkers of PFAS exposure

Patrica Ivy Agorsor, Husam Kafeenah and Michael Eze, Missouri University of Science and Technology
Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants linked to hepatotoxicity, reproductive toxicity, impaired lung function, lung cancer, and developmental abnormalities. Inhalation of PFAS-contaminated dust and aerosols represents a critical yet understudied exposure route that directly impacts airway epithelial cells, the first barrier of the respiratory system. However, distinct volatile organic compound (VOC) signatures associated with PFAS exposure remain unknown. To understand the metabolic changes associated with PFAS inhalation and lay the groundwork for a non-invasive diagnostic tool, this study investigated VOC profiles in human alveolar basal epithelial cells (A549) exposed to varying concentrations of PFAS at 12, 24, and 48-hr intervals. Principal component analysis showed distinct clustering of individual treatments, indicating concentration- and time-dependent metabolic alterations following PFAS exposure. PFAS exposure induces distinct changes in cellular metabolites. Across all time points, greater metabolic perturbations were observed with increased concentration and longer exposure, with an increasing number of upregulated and downregulated metabolites. Key metabolites that were upregulated include methylthioethane, glycidyl methyl ether, ethyl acetate, and (E)-2-butenal, while iprovalicarb, phenethyl acetate, dicyclohexyl phthalate, and 2-ethylphenol were downregulated. The identification of VOC biomarkers of PFAS exposure in this study will lay the foundation for the development of a noninvasive breath-based method for early diagnosis of PFAS exposure and consequently mitigate the negative impacts of exposure, especially in vulnerable populations such as pregnant women and infants.

4.14.P-We-109 Characterizing urinary metabolites by non-targeted analysis and their associations with endogenous metabolism in children From South Florida

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Understanding exposure to environmental contaminants during the early years of life is essential to assess potential impacts on metabolism and human health at critical stages of development. This study aimed to detect and identify organic compounds of potential health risk concern and assess their relationships with endogenous

metabolites in urine samples collected from children aged between 6 months and 8 years (n = 183 samples from 54 families), residing in Miami-Dade and Broward Counties, Florida. Urine samples were filtered using 0.2 µm Acrodisc syringe filters, followed by enzymatic hydrolysis with β-glucuronidase/arylsulfatase in ammonium acetate buffer, and incubated overnight at 37 °C. The resulting extracts were diluted, fortified with internal standards, and subsequently analyzed using online solid phase extraction (SPE) followed by liquid chromatography coupled to a Q-Exactive Orbitrap mass spectrometer. Data acquisition was performed in full scan mode combined with data-dependent acquisition (dd-MS²), operated in both positive and negative ionization modes. Chromatographic separation of the analytes was performed using a Hypersil GOLD C18 column with mobile phases composed of water, methanol, acetonitrile, and formic acid. Blank and quality control samples were included to ensure analytical robustness and minimize interferences. Data processing and molecular feature extraction were conducted using the Compound Discoverer software, which enabled peak detection, alignment, and grouping, as well as molecular formula generation and preliminary annotation based on spectral databases. To investigate potential associations between the detected contaminants and endogenous urinary metabolites, we conducted a Spearman correlation analysis to allow for the identification of covariation patterns among various compounds. The exploratory correlation analysis revealed co-occurring compounds and patterns. These findings may reflect simultaneous exposures or biologically relevant metabolic interactions, emphasizing the complex nature of the childhood exposome. Additionally, the study demonstrated good applicability of the non-targeted online SPE and HRMS approach in the biomonitoring of children's exposure to organic compounds and provides a preliminary basis for future investigations in vulnerable populations.

4.14.P-We-110 Impact of industrial activity from petrochemical plant on environmental health in Beaver County, PA

Oindrila Ghosh, *Fatemeh Shabani, Carla A. Ng, Helen Farnham and Jeanine Buchanich, University of Pittsburgh*

In November 2022, an ethylene cracker plant was established in Beaver County along the Ohio River. A recent study showed that residents in Beaver County, Pennsylvania were surveyed to explore their perceptions on health in the months leading up to the November 2022 opening of a petrochemical plant and indicated high concern about current and future exposure to pollution in Beaver County. A follow-up survey to determine changes in perceptions since plant operations began coupled with community-engaged sampling campaign collecting blood, silicone wristband samplers, and household dust was carried out between September-December 2024, to estimate the residents' exposure to PFAS and SVOCs. Pitt researchers set up a sampling/information booth at a locally owned office space to meet with residents periodically, collect blood samples, distribute wristband and dust samplers with instructions, and answer relevant questions related to the study. Blood sampling was carried out by Pitt researchers using Eurofins PFAS blood sampling kits and shipped to Eurofins lab for analysis. In house-dust collecting kits were provided to residents to collect dust samples from all horizontal surfaces that are at least 1 meter above the floor level. In addition, a stationary-wristband (SW) kit and a wearable wristband (WW) kit were also provided: the SW to sample indoor air at the home while the WW would sample the individual personal exposure from indoor and outdoor air, for each participant over a period of 7 days. All samples were shipped to the Pitt lab and stored at -20°C until analysis. A total of 60 residents from 10 zip codes were sampled to analyze for a total of 40 PFAS (EPA 1633 method) and 90 SVOC (EPA 8270 method) compounds. Initial observations suggest most blood samples only had PFOS and PFOA. Some samples also had detectable levels of PFOSA. These suggest legacy exposures. Ongoing analysis will allow comparison of the blood levels to PFAS and SVOCs present in indoor environments and picked up by the personal wearable wristband samplers.

4.14.P-We-111 Pollution characteristics and health risks of atmospheric polycyclic aromatic hydrocarbons and their derivatives in an iron and steel industrial city in South Korea

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Polycyclic aromatic hydrocarbons (PAHs) and their derivatives, including nitrated PAHs (NPAHs) and oxygenated PAHs (OPAHs), are primarily emitted from incomplete combustion. Large quantities of PAHs and their derivatives are released from various industrial activities, particularly in the iron and steel industry, which is a significant source of these compounds. Some NPAHs and OPAHs are known to be more toxic than their parent PAHs; however, studies on these compounds in industrial cities remain limited. This study aimed to monitor atmospheric PAHs, NPAHs, and OPAHs in Pohang, a representative iron and steel industrial city in South Korea, to characterize their profiles, determine spatial distribution patterns, and assess associated health risks. Polyurethane foam passive air samplers (PUF-PASs) were deployed during the winter of 2023 across the port, industrial complex, and residential areas of Pohang, which has a port dedicated to iron making and trade, as well as an industrial complex for steelmaking. Collected samples were Soxhlet-extracted, cleaned using silica gel and silica gel/alumina columns, and analyzed for 21 PAHs, 17 NPAHs, and 9 OPAHs using gas chromatography/mass spectrometry (GC/MS). In the port area, the mean concentrations of PAHs, NPAHs, and OPAHs were 54.6 ng/m³, 530 pg/m³, and 4.80 ng/m³, respectively, which were 3.9, 10.6, and 2.5 times higher than those in the industrial and residential areas. These elevated concentrations were primarily attributed to emissions from ironmaking and steelmaking processes, as well as cargo handling activities at the port. Among NPAHs, 1-nitropyrene (1-NPyr), a marker of diesel exhaust, was the most abundant compound in the port area, reflecting emissions from large vessels and heavy-duty vehicles such as container trucks. Risk assessment indicated that the estimated carcinogenic risk in the port area was approximately 20 times higher than in the industrial and residential areas, although all values remained below the United States Environmental Protection Agency (US EPA)'s acceptable risk threshold of 10⁻⁶. While port emissions appeared to have limited health impacts on Pohang's residential areas, continuous monitoring is essential to evaluate unregulated pollutant emissions from ironmaking and steelmaking processes.

4.14.T Human Exposure to Organic Contaminants of Current and Emerging Concern

4.14.T-01 Assessing environmental and dietary exposure to trifluoroacetic acid (TFA) formed from HFO-1234ze(E) emissions in prospective pressurized metered-dose inhalers: A case study in the Rhine Basin

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HFO-1234ze(E) is a next-generation propellant under development for use in pressurized metered-dose inhalers (pMDIs). Upon atmospheric release, it can degrade into trifluoroacetic acid (TFA), an ultra-short-chain per- and polyfluoroalkyl substance (PFAS) that is persistent, highly mobile, and increasingly detected in the environment. TFA's presence in surface waters, precipitation, and biological tissues has raised increasing concerns regarding its long-term environmental fate and potential impacts on human health. This study presents a case assessment of environmental fate and transport modeling and human exposure to TFA resulting from HFO-1234ze(E) emissions from pMDIs, with a focus on the Rhine River basin. Using an EPA-recommended environmental fate and transport modeling framework, we simulated TFA concentrations in surface water, soil, groundwater, and the food chain across sub-basins of the Rhine based on model-predicted atmospheric TFA deposition fluxes. Human exposure was estimated by age and gender across multiple pathways, including inhalation, ingestion of drinking water, soil contact, and dietary intake. The results provide valuable insight into the fate and distribution of TFA in the environment and associated exposure pathways. This work supports informed decision-making for regulatory bodies and contributes to the sustainable development of low-impact medical propellants.

4.14.T-02 A resource to empirically establish drug exposure records directly from untargeted metabolomics data

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Drugs represent one of the most important human chemical exposures. To study the relationships between drug exposure and health outcomes, clinical research relies on medical records or self-reported medication usage.

However, such information is costly to acquire and often inaccurate, and does not reflect drug exposure from non-medication routes. Untargeted metabolomics offers the opportunity to provide objective drug read-out, but it suffers from the limited number of reference spectra of drugs and even lower coverage for drug metabolites. Moreover, interpretation of the annotations requires extensive literature searches to understand the therapeutic indications of the drugs. To overcome these challenges, we developed GNPS Drug Library, a tandem mass spectrometry (MS/MS) based resource that integrates MS/MS references of drugs and their metabolites/analogs with standardized vocabularies on their exposure sources, pharmacologic classes, therapeutic indications, and mechanisms of action. It enables direct analysis of drug exposure and metabolism from untargeted MS/MS data, supporting flexible summarization at multiple ontology levels to align with diverse research goals. We demonstrate its utility by stratifying participants in a human immunodeficiency virus (HIV) cohort based on detected drug exposures and uncovering drug-associated alterations in microbiota-derived N-acyl lipids.

4.14.T-03 Non-targeted analysis of amniotic fluid reveals metabolic and environmental signatures associated with birth defects: A pilot study

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Birth defects remain a significant global public health concern, with increasing evidence pointing to environmental exposures as critical contributors alongside genetic and maternal health factors. Despite this, the full range of exogenous chemicals potentially involved in adverse fetal outcomes remains poorly characterized. Amniotic fluid, which reflects both maternal and fetal exposures, serves as a valuable matrix for profiling metabolic and chemical signatures relevant to fetal development. In this study, we applied liquid chromatography–Quadrupole Time-of-Flight Mass Spectrometer (LC-qTOF)-based non-targeted analysis (NTA) to amniotic fluid samples collected from pregnancies affected by birth defects. Our objectives were to: (1) identify potential case samples without prior classification using unsupervised clustering combined with machine learning; (2) annotate discriminative chemical features through database matching and *in silico* fragmentation; and (3) examine the presence of exogenous compounds and their associations with altered metabolic profiles. Analysis of a pilot cohort (40 samples) revealed distinct exposomic and metabolic patterns in samples associated with birth defects. K-means clustering identified a subgroup of nine samples with characteristic perturbations in tyrosine/phenol metabolism and amino acid-related pathways. Notably, changes in the abundance of fatty acids and their conjugates, as well as bile acids and alcohol derivatives, suggest disruptions in unsaturated fatty acid biosynthesis and primary bile acid metabolism—two processes critical for normal fetal development.

4.14.T-04 Characterizing fragrance substances in air fresheners based on detection, co-occurrence patterns, and inhalation risk assessment

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Fragrance substances are emitted from consumer products and account for approximately 20% of indoor VOCs. Their production and use are increasing, posing potential health risks. However, insufficient information on fragrance compositions remains a challenge. For a total of 58 allergenic fragrance substances regulated in South Korea or Europe, this study aimed to identify structure-based hazards, determine their concentrations in air fresheners from South Korea (n=50) and Europe (n=41) using GC–MS, analyze co-occurring scent combinations via association rule mining (ARM), and assess inhalation risks using a two-tiered approach, applying conservative assumptions in Tier 1 (95th percentile) and more realistic scenarios in Tier 2 (75th percentile). The 58 substances were associated with five major hazards: respiratory irritation, developmental/reproductive toxicity, neurotoxicity, genotoxicity, and endocrine disruption. Three predominant compounds of linalool, limonene, and geraniol in South Korean products were detected at 78% (19–133,773 mg/kg; mean: 14,643 mg/kg), 60% (7–60,181 mg/kg; 6,182 mg/kg), and 58% (13–28,175 mg/kg; 1,834 mg/kg),

respectively. In European products, linalool, limonene, and α -pinene were predominantly detected at 95% (61–185,017 mg/kg; mean: 33,969 mg/kg), 90% (57–132,808 mg/kg; 15,334 mg/kg), and 90% (13–28,175 mg/kg; 1,834 mg/kg), respectively. A comparative analysis showed similar detection frequencies ($p = 0.883$) but lower similarity in concentration distributions ($p = 0.601$). ARM results revealed common floral–floral (e.g., linalool–geraniol) and citrus–related (e.g., limonene) combinations, with musky–floral (lift = 1.36) distinctive in South Korean products, whereas floral–floral had the highest lift in European products (1.31). In Tier 2, hazard quotients for α -hexylcinnamaldehyde and linal were 2.3 and 1.1, exceeding safety thresholds. This study provides comprehensive information on which fragrance substances are used, in what amounts and in what combinations. More importantly, this study highlights that some fragrance substances exceed the safety thresholds under current use conditions, contrary to previous expectations that they are safe. Several measures should be taken to ensure the safe use of fragrance substances, including continuous monitoring of their content in products as well as evaluation of actual exposure levels.

4.14.T-05 Discovery and characterization of chemicals from wildland-urban interface wildfires in the western United States

Kelly O'Malley, Christine Ghetu, Diana Rohlman and Kim Anderson, Oregon State University

Wildfires have become an emerging air quality concern as the number of large wildfires continue to increase and occur more frequently in wildland-urban interface (WUI) areas. This has led for the need to quickly measure human exposure to complex wildfire smoke mixtures, which still have knowledge gaps of vapor-phase (bioavailable) organic contaminants that may be of toxicological concern. The objectives of this study are to discover and quantify novel bioavailable chemicals linked to wildfire smoke, to characterize their potential sources and toxicity, to determine if the concentrations of wildfire chemicals increase at sites with worse air quality, and to explore chemical composition in relation to WUI wildfires. Low-density polyethylene (LDPE) passive samplers were deployed by trained community members in air cages before and during wildfires at twelve locations in the Western United States. Additionally, LDPE was co-deployed in soil air boxes at four locations. All air and soil air wildfire samples were quantitatively analyzed for 64 PAHs and 13 oxygenated PAHs (OPAHs) using tandem gas chromatography mass spectrometry (GC-MS/MS) and GC-MS respectively. A 1530 chemical semi-quantitative GC-MS screening method was also performed. Air quality was assessed with publicly available PM_{2.5} Air Quality Index (AQI) data. 85 bioavailable chemicals are potentially linked to wildfire smoke, including 32 PAHs, 18 alkylated PAHs, 7 OPAHs, and 28 from the 1530 screen. Over 30 of these chemicals have not been previously linked to wildfire smoke to the best of our knowledge. Dibenzofuran, 4-methoxyphenol, and 9-fluorenone, an OPAH, were some of the most frequently detected chemicals among all wildfire samples. The OPAHs present may be degradation products of the PAHs in wildfire smoke. Only a few compounds had higher concentrations at sites with worse air quality during a wildfire, which were diisobutyl phthalate, di-n-butyl phthalate, and benzyl salicylate. Diisobutyl phthalate and di-n-butyl phthalate are chemicals found in housing and structure materials that could have been burned and released into the smoke plumes during WUI wildfires. PM_{2.5} AQI alone does not appear to be a good indicator of exposure during a wildfire, meaning other wildfire characteristics may be needed. Identifying bioavailable components of wildfire smoke can improve human exposure assessment to wildfire events and can facilitate further understanding of the health impacts.

4.14.T-06 Flame retardant and combustion byproduct exposures in wildland vs structural firefighters assessed using silicone wristbands

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Firefighters are routinely exposed to a range of hazardous chemicals generated during fire suppression activities. This study investigated chemical exposures among 29 structural firefighters and 55 wildland firefighters using passive sampling and biomonitoring techniques. All participants wore silicone wristbands

during work shifts to assess personal exposure to a broad spectrum of emerging & legacy contaminants. In addition, wildland firefighters provided a post-shift urine sample, enabling paired analysis of external (wristband) and internal (urinary) chemical biomarkers. Silicone wristbands assessed exposure to 123 different semi-volatile organic chemicals, including polycyclic aromatic hydrocarbons (PAHs), flame retardants, plasticizers and some pesticides. Among wildland firefighters, PAH levels detected on wristbands were significantly correlated with corresponding urinary PAH metabolites, validating wristbands as an effective tool for assessing personal chemical exposure in wildland firefighters. Notably, wildland firefighters exhibited significantly higher levels of PAHs on wristbands and in urine compared to structural firefighters, suggesting elevated exposures due to either closer contact with combustion zones, differences in protective gear, and/or duration on the fireground. Wristbands from wildland firefighters also showed elevated exposure to some flame retardants, including decabromodiphenyl ether (BDE-209) and decabromodiphenyl ethane (DBDPE), compounds associated with adverse health outcomes and commonly used in consumer products and building materials. Statistical analyses revealed that chemical exposure levels among wildland firefighters were significantly influenced by job-specific tasks and gear cleaning frequency. Firefighters who engaged in direct fire line construction or who reported infrequent gear cleaning had higher levels of PAHs and some flame retardants. These findings highlight the importance of monitoring occupational exposures in diverse firefighting contexts and suggest that silicone wristbands, in combination with biomonitoring, offer a practical and sensitive method for assessing chemical exposure. Differences in exposure profiles between structural and wildland firefighters underscore the need for targeted interventions, including improved gear decontamination practices, task-specific exposure mitigation strategies, and continued health surveillance.

4.15.T In Memoriam: Dr. Katherine Peter—Expanding Environmental Horizons to Unknown Limits

4.15.T-01 Kathy Peter's scientific legacy: Expanding environmental horizons to small urban watersheds using innovative mass spectrometry 215s

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Dr. Katherine T. Peter was a profoundly gifted environmental researcher, the best collaborator that you could ever hope for, and an even better human being. Here, we will celebrate her many intellectual and personal contributions to the field of environmental engineering and chemistry by looking through the work and recognizing the many studies and people who have followed her footsteps. I will provide a data driven overview of Kathy's research contributions as a post-doctoral scholar and research scientist while she was at the Center for Urban Waters (Tacoma, WA). Topics addressed in this presentation will be Kathy's work on the Thornton Creek hyporheic treatment system for urban stormwater runoff, her contributions to small stream hydrology and water quality assessment via studies on Miller Creek (Burien and Normandy Park, WA, USA), her foundational studies on the chemical causation of urban runoff mortality syndrome in coho salmon, her use of non-target high resolution mass spectrometry fingerprinting to accomplish source apportionment and fingerprint tracking, and her work with citizen science groups. Throughout, Kathy's research was defined by her innovation and ideas, her emphasis on applied real world problem solving, and her development of environmental mass spectrometry techniques and strategies to investigate contaminant fate and transport. Kathy most certainly left us all too early, but here I intend to show that so many of us will be following her footsteps and exploring her ideas for the remainder of our professional careers.

4.15.T-02 Building a better brita: Simple yet elegant fabrication of high-performing nanomaterials for water treatment

David Cwierthy, University of Iowa

While Kathy would go on to become a highly regarded environmental analytical chemist during her time at NIST and the Center for Urban Waters, her doctoral and undergraduate research were rooted in materials

science and environmental nanotechnology. This talk will detail Kathy's contributions and influence in the areas of material fabrication for applications in water treatment. At the University of Iowa, Kathy crafted her thesis around the development of electrospun nanofiber membranes for point-of-use water treatment. Her work was motivated by the numerous challenges confronting Iowa's private well owners, over 300,000 vulnerable consumers that fall outside of Safe Drinking Water Act protections and often have the need for multi-functional, broad-spectrum purification technologies in a small device footprint. Through Kathy's genius and drive, she produced a series of studies in which she creatively designed materials to exhibit not only high reactivity but also material strength that made them practically applicable under the constraints of point-of-use treatment systems. Collectively, Kathy's thesis research advanced the application of engineered nanomaterials in drinking water treatment. Moreover, her work has gone on to inspire several new lines of investigation, including at Iowa where future members of Kathy's group have gone on to build a prototype filter using the nanofiber membranes inspired by her work.

4.15.T-03 From the road to the field: Decoding chemical transformation in aging tire and artificial turf crumb rubber

Madison McMinn, Yuqiao Tang, Phillip Berger, Katherine Poisson, Andresa Lima, Aron Stubbins, Arzu Tugce Gueler and Zhenyu Tian, Northeastern University

Increasing tire use worldwide raises concerns about the impact of rubber-derived chemicals (RDCs) on human and environmental health. Of particular concern is the aging of RDCs, which may lead to the formation of unknown, potentially harmful transformation products (TPs). Existing studies have not comprehensively assessed different aging mechanisms and their impact on tire from various sources. To address current knowledge gaps, we performed aging of 2 tire and 1 artificial turf crumb rubber samples exposed to natural and accelerated artificial photoaging, in dry and wet conditions. Sample extracts underwent targeted quantitation, suspect and nontarget screening (NTS) via liquid chromatography-mass spectrometry (LC-MS) followed by temporal trend analysis and molecular networking. Comparison between aging conditions indicates that accelerated photoaging and contact with water results in faster transformation/degradation rates than expected. NTS prioritized 572 potential TPs of which ~63 were tentatively identified. Analysis of molecular networking clusters without known RDCs, revealed 180 2,2,4-Trimethyl-1,2-dihydroquinoline-related (TMQ) features based on MS/MS, including 2 bioavailable compounds previously thought to be para phenylbenzene diamines (PPDs). We tentatively identified 14 TMQ-related TPs, including, unexpectedly, reactions between TMQ and other RDCs. Our analysis highlights the complexity of tire and turf crumb rubber aging and the need for further investigation of RDCs and their TPs.

4.15.T-04 Standardizing reporting in non-targeted analysis with the study reporting tool (SRT)

Allison Phillips, Independent Scientist

Non-targeted analysis (NTA) is rapidly becoming a powerful tool used in many research disciplines. The rapid rise in NTA use has led to inconsistent scientific reporting, which creates challenges for peer-review and threatens the reproducibility of key scientific findings. Under the leadership of Dr. Katherine Peter, the Best Practices for Non-Targeted Analysis (BP4NTA) working group developed the NTA Study Reporting Tool (SRT) to address the need for standardized reporting criteria in untargeted exposomics. The SRT is the first easy-to-use tool for rigorous evaluation of NTA reporting practices and is available for download as an Excel or interactive PDF file on the BP4NTA website (www.nontargetedanalysis.org/srt). It is organized by NTA study chronology and contains 13 sub-categories for scoring that cover all aspects of study design; data acquisition, analysis methods, and outputs; and quality assurance/quality control metrics. The SRT uses a color-coded, 4-level scoring system to evaluate research reporting quality and reserves space for reviewers to explain their rationale for assigned scores. Designed with ease-of-use in mind, the SRT contains examples of information that might be reported in each sub-category. Sub-category headings are hyperlinked to reference content on the BP4NTA website, providing detailed background information for users less familiar with certain subject matter. Since its initial publication in 2021, the SRT has been updated several times based on user feedback and to

expand its relevance to multi-omic workflows. The SRT has been viewed over 3,500 times, downloaded 1,400 times, and cited 120 times, demonstrating its value and current application in the NTA community. Efforts are underway to interlink the SRT with BP4NTA's Study Planning Tool (SPT), a companion guide for NTA study design and execution, and to connect with other -omic working groups (i.e., Metabolomics Quality Assurance & Quality Control Consortium [mQACC], Metabolomics Society, International Lipidomics Society [ILS]) around consensus-based reporting standards and tools for untargeted mass spectrometry applications. Dr. Katherine Peter was the driving force behind the SRT's conception and initiated many of the ongoing efforts and collaborations to expand its impact.

4.16.P-Mo Mass Spectral Solutions to Environmental and Toxicological Challenges

4.16.P-Mo-040 Linking (non)targeted analysis and chemical risk assessment in kale from urban and rural farms

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This work evaluates the presence and potential human hazards associated with pesticides and other human-made chemicals detected in kale cultivated in both urban and rural settings. To screen and quantify the pesticides and related compounds in kale samples, we employed a nontargeted data acquisition for targeted analysis (nDATA) method that utilized a pesticide mixture containing over 1,000 compounds. We modeled population level exposures and assessed non-cancer hazards to DEET, piperonyl butoxide, prometon, secbumeton, terbumeton, and spinosyn A using nationally representative estimates of kale consumption across life stages in the US. Our results suggest that individuals, even those in sensitive populations, are unlikely to experience hazards from the selected compounds if they consume kale grown in this study. However, employing a purely non-targeted chemical analytical approach uncovered a total of 1,822 features across all samples, and principal component analysis revealed that the kale chemical composition may have been impacted by agricultural growing practices and environmental factors. Notably, certain compounds were found to be significantly more abundant in urban samples compared to rural ones, including chemicals categorized as 'flavoring and nutrients' and 'surfactants' in the EPA's Chemicals and Products Database. Furthermore, using the US-EPA's Cheminformatics Hazard Module, we identified many of these non-target compounds as having predicted toxicity scores of 'very high' for various human health endpoints. This underscores the importance of our approach, as traditional targeted analysis methods might have overlooked these aspects. However, further investigation is necessary to determine whether the compounds identified through non-targeted analysis pose environmental or human health concerns.

4.16.P-Mo-041 Investigating per-/poly-fluoroalkyl substances in food packaging using atmospheric pressure gas chromatography/ultra-performance liquid chromatography/mass spectrometry: Targeted, non-targeted methods

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Per- and polyfluoroalkyl substances (PFAS) are used in food contact packaging materials (FCMs), firefighting foams, textiles, and other products owing to their hydrophilic and hydrophobic properties. PFAS are bioaccumulative and pose a risk to human health. This study advanced sample extraction and instrumental analysis to investigate PFAS in 19 commercial food packaging varieties. A 100 cm² section was homogenized and extracted using an EDGE system with two 10-mL methanol cycles at 70 °C, then concentrated to one mL. APGC-MS/MS with a ZB-WAX column (30 m × 0.25 mm, 0.25 μm) quantified three FTOHs. UPLC-MS/MS with UPLC BEH C18 (50 × 2.1 mm, 1.7 μm) and Premier BEH C18 AX (50 × 2.1 mm, 1.7 μm) columns analyzed 6:2 PAP/8:2 PAP and remaining 26 PFASs using negative UniSpray ionization. Non-targeted analysis

used UHPLC-Q-TOF MS at ESI- with Premier BEH C18 AX column (100 × 2.1 mm, 1.7 μm), collecting data in DDA and DIA modes and processing with MSDIAL and FluoroMatch software. Perfluoroalkyl carboxylic acids were detected in all 19 samples, with PFHxA reaching 14.1 ng/dm² in a grease trap paper bag. PFBS and PFOS were present in every sample, with PFBS peaking at 25.5 ng/dm² in oilproof paper. 6:2 FTCA showed a highest concentration of 32.7 ng/dm² in a grease trap paper bag. 8:2 diPAP was found in all samples (1.2-2.1 ng/dm²). 6:2 PAP and 8:2 PAP were detected in most samples, exceeding 10 ng/dm² in popcorn bags and egg cartons. 8:2 FTOH and 10:2 FTOH appeared in all samples, reaching 53.7 ng/dm² and 24.7 ng/dm² in a popcorn bag. 3,6-OPFHxA reached 25.1 ng/dm² in one popcorn bag. DIA data processed with MSDIAL showed superior performance compared to DDA using FluoroMatch. This study compared results from 19 FCM samples analyzed with UPLC-MS/MS to PFAS identified in DIA data processed by MSDIAL from UHPLC-Q-TOF MS. UPLC-MS/MS detected PFBA, PFHxA, PFBS, and 6:2 FTCA in oil-proof paper bags, while UHPLC-Q-TOF MS identified PFBA, PFBS, non-targeted (E)-2:2 FTUCA, PFPrA, and FBSE. Both oil-proof and microwave popcorn paper bags contained non-targeted 2-((3,3,3-Trifluoropropyl)thio)acetic acid and PFSM-N-MeFSAA (C₄H₆F₃NO₄S). This study refined sample preparation procedures for APGC-MS/MS and UPLC-MS/MS using UniSpray ionizations to determine 31 PFAS in FCMs. UHPLC-Q-TOF MS with MSDIAL software identified nine targeted and non-targeted PFAS from FCM samples, including PFCAs, PFSAAs, FTUCAs, and degradation products, complementing targeted analysis in PFAS monitoring.

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4.16.P-Mo-042 Streamlining environmental data analysis by artificial intelligence (AI): A deep learning approach

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Per- and polyfluoroalkyl substances (PFAS), a class of emerging persistent organic pollutants (POPs), are present at trace levels not only in the environment (water, soil and air) but also in food. The quantitative analysis of PFAS is typically conducted using liquid or gas chromatography-tandem mass spectrometry (LC-MS/MS, GC-MS/MS). Despite the high sensitivity of these instruments, PFAS analysis remains challenging. The data analysis of PFAS often requires time-consuming manual steps to eliminate false positive or negative quantifier and qualifier peaks of the corresponding compound. These steps include, among others: • Adjusting peaks from early eluting PFAS and PFPAs (e.g. PFBA, PFOPA); • Combining partially or fully separated peaks of linear and branched isomers of some PFAS (e.g. PFOS, PFHxS), while accounting for variations in their ratios; • Removing false positive or negative peaks caused by matrix interferences or contamination. In this work, we tested a complete workflow integrating several DL architectures, tailored for liquid chromatography-tandem mass spectrometry (LC-MS/MS) data in multiple reaction monitoring (MRM) mode for the quantitative analysis of PFAS. The data preprocessing workflow was designed to account for chemically relevant metadata, such as retention time shifts and quantifier-qualifier correlation. Data acquired for the analysis of PFAS in different environmental matrices following the EPA 1633 method, and from different LC-MS/MS instruments with varying sensitivities, are used for the model training and validation. Several convolutional neural network (CNN)-based architectures and a transformer-based model are evaluated and their performance compared. Preliminary results show that both models improve upon existing automatic integration algorithms. When a trained DL model is deployed, data review time can be significantly reduced by eliminating most of the manual data analysis steps, on a compound-by-compound basis.

4.16.P-Mo-043 Science communication and community partnership in suspect and nontarget environmental monitoring study

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Rohlman², Robyn Leigh Tanguay² and Manuel Garcia-Jaramillo², (1)ToxStrategies, (2)Oregon State University, (3)Portland Harbor Community Coalition

Community engaged research provides an opportunity for scientists to co-design and conduct research that aligns with local concerns and interests and can expand scientific literacy within a community. In this study, we partnered with Portland Harbor Community Coalition (PHCC) to conduct environmental monitoring of emerging contaminants in the lower Willamette River, a historically industrialized and urbanized waterway and listed Superfund site. PHCC initiated the study and co-developed the sampling strategy and hypotheses. Throughout the duration of the project, research results were reported back by participating in online and in-person public forums and creating materials to share the project goals, outcomes, and results. An ongoing effort in the science communication strategy was to define the capabilities and constraints of suspect and nontarget screening which were communicated via infographics and videos. The community expressed interest in surface water contaminants, so the methods employed focused on detecting polar and moderately polar compounds. While this approach limited our ability to assess nonpolar Superfund contaminants of concern in the water column, these compounds are regularly monitored by the US Environmental Protection Agency and Oregon Department of Environmental Quality. The suspect and non-target analysis revealed an urbanized river with influences from wastewater effluent and industrial activity. We detected thousands of chemical features, identified over 750 by suspect screening and prioritized 114 detected compounds for future monitoring efforts based on hazard, detection frequency, and compound classes. We assessed the biological effects of a selection of surface water samples using high throughput embryonic zebrafish assays. The results showed few adverse effects, providing evidence that typical interactions with the river system are unlikely to be hazardous for community members. Following the completion of the study, we produced an accessible report of the research findings and held a community forum to share our findings and answer questions. This study provides a process of codeveloping and communicating study aims and results with a community partner with a specific focus on translating suspect and nontarget analysis results to a non-technical audience.

4.16.P-Mo-044 Characterizing deep groundwater wells in a estuarine aquifer in south Florida using various analytical techniques

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In Miami-Dade County, groundwater serves as an important source of potable water, much like other areas around the globe. The Biscayne Bay watershed encompasses a highly interconnected groundwater, canal system, and Bay, meaning the Biscayne Aquifer interacts frequently with these other sections of the watershed. The Bay serves as an important ecological, economic, and environmental resource for the area. Over the past few years, declining water quality such as eutrophication has been linked to the presence of anthropogenically-sourced contaminants in the watershed. Phosphorus acts as the limiting nutrient of Biscayne Bay and is especially relevant to monitor as previous studies have found high levels of phosphorus within the groundwater. Through a variety of analytical techniques encompassing High-Resolution Mass Spectrometry (HRMS) and nutrient analyses for nitrate and nitrite, SRP, TP, and TN, a better understanding of groundwater's chemical space can be developed. Non-Target Analysis (NTA) can be used for the identification of unknown or suspected organic compounds within groundwater samples using online-SPE HESI-HPLC-HRMS and Compound Discoverer (CD) 3.4. Nine sites were sampled across Miami-Dade County in cooperation with the USGS and underwent analyses to gain a better understanding of the chemical space, the workflows included known contaminants and suspect screening. With the exception of one, these wells are considered to be deep and were visited each month for a total of six months. The sites showed temporal and spatial variation in the total phosphorus concentration, as well as in the anthropogenic wastewater tracers sucralose and caffeine. The sucralose concentrations ranged from levels of 0 - 532 ng/L. Out of the nine sampling locations, six sites had TP levels above the Nutrient Numeric Criteria, 12 µg/L. TP concentrations ranged from 6.3 - 117.87 µg/L. The Level 2 compounds detected by NTA came from a range of compound classes. By gaining a better understanding of the chemical makeup of the groundwater, we can begin to develop an idea of which

compounds are particularly relevant for future monitoring efforts and, those that serve as tracers for the magnitude of impact from external pollution sources as well as for potentially tracing the movement of groundwater throughout the interconnected watershed.

4.16.P-Mo-045 Differentiating PFAS isomers: A multi-pass cyclic ion mobility mass spectrometry approach for detection, identification and relative quantitation

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Per- and polyfluoroalkyl substances (PFAS) are industrial compounds of concern due to their negative health effects and widespread presence in the environment. Due to the manufacturing process, several PFAS can exist as mixtures of linear and branched structural isomers. While regulatory PFAS testing sums all isomers, there is interest in the distribution of individual branched isomers for fingerprinting the source of PFAS contamination. In this study, PFAS isomers in technical mixtures of perfluorooctanesulfonic acid (T-PFOS) and perfluorooctanoic acid (T-PFOA) were characterized by combining liquid chromatography with ion mobility separation (IMS) and high-resolution mass spectrometry. These mixtures included documentation of the relative amounts of individual branched isomers, as measured by ¹⁹F NMR. Due to their structural similarity, IMS with high resolving power was needed to differentiate branched isomers. The SELECT SERIES™ Cyclic™ IMS Mass Spectrometer (Waters Corporation) enables high-resolution IMS measurements by directing ions through its unique circular ion mobility path multiple times. For the identification and relative quantitation of PFOS isomers, the branched isomers are separated chromatographically from the linear isomer, but they are not well separated from each other. Adding the extra dimension of IMS helped differentiate individual isomers. To characterize the T-PFOS sample, a 6 pass IMS acquisition was used to measure the distribution of PFOS isomers. In addition to the 11 isomers previously reported, 7 additional branched isomers were detected. However, 3 of the branched isomers needed more separation in the IMS space as they could not be differentiated with 6 passes in the IMS cell. An advanced IMS experiment that utilized ion mobility selection and a "top and tail" acquisition mode with 25 passes in the IMS cell was able to isolate and differentiate those 3 isomers. Relative quantitation of individual isomers was calculated using the peak area from drift-time specific extracted ion chromatograms. The relative amounts of individual isomers in T-PFOS by LC-IMS-MS differed by less than 2% compared to ¹⁹F NMR results. Similar to PFOS, the branched isomers of PFOA were separated chromatographically from the linear isomer. Three passes were needed in the IMS cell to separate the known isomers of PFOA. The relative amounts of individual isomers by LC-IMS-MS differed less than 5% compared to amounts reported by ¹⁹F NMR for T-PFOA. **Disclaimer/Disclosure:** The authors are employees of Waters Corporation.

4.16.P-Mo-046 Identification of known and novel PFAS in aqueous film-forming foam (AFFF) by non-targeted screening with high-resolution mass spectrometry and pattern analysis

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Per- and polyfluoroalkyl substances (PFAS) are a group of industrial compounds that have been identified as a class of environmental contaminants of high concern due to their negative health effects and widespread presence in the environment. Current targeted methods focus on a short list of legacy and emerging PFAS and are therefore incapable of measuring all potential PFAS contamination in environmental samples. Non-targeted analysis (NTA) with high-resolution mass spectrometry (HRMS) is a promising technique for the discovery and identification of new PFAS. Here, HRMS was coupled with liquid chromatography and ion mobility separation (IMS) on the SELECT SERIES™ Cyclic™ IMS mass spectrometer (Waters Corporation) to analyze a sample of aqueous film-forming foams (AFFF) (NIST Reference Material 8690). As PFAS may be present at low levels in this complex sample, data independent acquisition was utilized to yield as much information as

possible from a single injection. Detected peaks were first compared to an internal PFAS mass spectral library for identification. Identifications were based on metrics of accurate mass, retention time error, diagnostic fragments and measured collision cross section (CCS). The reference material does contain several known PFAS and those along with a few additional compounds were identified. For the discovery of new PFAS not in the suspect library, an additional data reduction tool, the Pattern Analysis Application, can be used to discriminate the detected compounds which are most likely to be PFAS. The tool takes advantage of characteristic properties of compound classes such as PFAS, that separate them from naturally occurring compounds. Flagging of detected compounds is based on class-specific product ions and neutral losses, mass defect, $^{12}\text{C}/^{13}\text{C}$ isotope ratio, trendlines on Kendrick plots and the distinctive relationships between both m/z and CCS. IMS adds an additional advantage as PFAS have lower CCS values compared to other compound classes of similar m/z . The application can also search for sequences of compounds with a repeating mass difference. Given that many PFAS are a part of homologous series with repeating CF_2 units, this functionality was used to look for sequences in which subsequent m/z values differ by 49.9968 (CF_2). The sequence detection tool revealed two unexpected series of PFAS that were previously unreported and were tentatively identified in the AFFF sample. **Disclaimer/Disclosure:** The authors are employees for Waters Corporation

4.16.T Mass Spectral Solutions to Environmental and Toxicological Challenges

4.16.T-01 Dual pathways of heavy metal contamination in urban environments: A comparative study of road-deposited sediments and water-soluble fractions

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Toxic heavy metals present in urban situations represent substantial health hazards through both particulate sediments and soluble aqueous forms. This study investigates the concentrations, pollution indices, and origins of heavy metals within road-deposited sediments as well as their water-soluble form from 29 urban locations. The samples underwent analysis utilizing inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectroscopy (AFS) methodologies. Source apportionment conducted through the UNMIX model designated industrial activities as the predominant source of contamination (34.8%), succeeded by agricultural practices, copper mine tailings, atmospheric pollution, and natural origins. Conversely, the soluble metals displayed distinct contamination trends, characterized by elevated lead (Pb) levels and variable concentrations of mercury (Hg). Principal Component Analysis (PCA) of the water-soluble metals delineated three components that accounted for 86.85% of the variance: PC1 denoted industrial contributions, PC2 represented agrochemical influences, and PC3 indicated input from alloy and electroplating sectors. Water-soluble copper (Cu) and cadmium (Cd) posed considerable risks, underscoring the critical necessity for environmental management strategies aimed at safeguarding public health and mitigating the enduring repercussions of metal contamination in both particulate and soluble forms.

4.16.T-02 Start to finish: Discovery, quantification and toxicity assessment of emerging contaminants in tap drinking water using mixed and new approach methods

Chloe Louise Fender, Manuel Garcia-Jaramillo, Miranda Elizabeth Jackson, Lisa Truong, Robyn Leigh Tanguay, Jason Schindler, Kenneth Lee and Peter William Bright, Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis

Securing the quality and safety of drinking water is an escalating global challenge. A key step in characterizing the risk associated with consuming tap drinking water is comprehensively characterizing the chemical composition of these waters. Without detailed and spatially resolved characterization, linking specific health outcomes to exposure is difficult. Despite this, a growing body of evidence indicates that epidemic diseases can be associated with chronic consumption of contaminated drinking waters. Nontarget analysis (NTA) utilizing high-resolution mass spectrometry offers a broad assessment of the chemical composition of tap drinking waters. We present findings from a 2022 sampling campaign where 12 tap water sources across western Oregon

and 1 commercial water source were sampled and analyzed using NTA methods. These water samples were analyzed with multi-modal extraction and chromatographic techniques to target physiochemically polar to non-polar organic contaminants and increase the overall chemical coverage of the water analysis. Over 3,000 unique spectral features were observed, and high confidence chemical annotations were made for over 50 features. Prioritization to select contaminants for further confirmation included rapid toxicity screening achieved through the USEPA's Cheminformatics Hazard Comparison Dashboard, with hazard profiles revealing potential developmental, reproductive, and carcinogenic risks to humans. A total of 13 compounds were confirmed by reference standard comparison including 5 chemicals of emerging concern. Confirmed water contaminants with little or no prior in-vivo toxicity data were evaluated for toxicity using the zebrafish 215, demonstrating common adverse outcomes in behavioral endpoints and some adverse morphological outcomes. Finally, contaminant mixtures representative of tap water samples were tested for toxicity to more holistically assess the risk associated with Oregon tap waters. Water contaminants were identified in both analytical extraction and chromatographic methods, demonstrating the value of methods focused on comprehensive contaminant screening. The data presented here, including detections and complementary toxicity data for lesser-known water contaminants, aim to support advancement for the best practices for water quality monitoring, complement ongoing regulatory efforts, and ultimately enhance the quality and safety of public tap water sources.

4.16.T-03 Sample preparation, nontarget and suspect screening of persistent and mobile organic contaminants in water

Katherine Poisson, Anna Casey, Jessica Eig, Phillip Berger, Madison McMinn and Zhenyu Tian, Northeastern University

Persistent and mobile organic contaminants (PMOCs) are a class of small molecule contaminants and transformation products whose high polarity enables them to persist in aquatic environments. These contaminants enter waterways by way of household, industrial, and agricultural waste, encompassing many use classes such as pharmaceuticals, pesticides, industrial chemicals, and short- and ultra-short-chain PFAS. Due to their hydrophilicity, PMOCs do not sorb well to sediments, soils or biomaterials, allowing them to slip through common water treatment and occur in treated effluent. In addition to this protection gap, high polarity makes these compounds difficult to analyze by conventional means, posing an analytical gap and leaving PMOCs under-monitored, understudied, and undiscovered. To enable PMOC analysis, an enrichment protocol utilizing evaporative concentration was developed and assessed for recovery for 25 model PMOC standards, including known PMOCs metformin, melamine, and trifluoroacetic acid. These methods were optimized for enrichment of PMOCs in drinking water and surface water samples for high recovery versus conventional methods. Targeted zwitterionic hydrophilic interaction liquid chromatography methods were developed for model standards and paired with electrospray ionization high resolution mass spectrometry using an Orbitrap Exploris 240. These methods were then expanded to suspect and nontarget analysis of PMOCs in drinking and surface water, revealing unmonitored organic contaminants. This study reinforces that conventional means of enrichment and chromatographic separation are often not amenable to highly polar analytes and tailored analytical methods are necessary to perform broad screening of this emerging class of contaminants.

4.16.T-04 Assessing environmental degradation of microcystin-LR using targeted and non-targeted mass spectrometry approaches

Kerry Jacobs, Isabella Feldmann, Hailey Brant, Kimberly Halsey and Manuel Garcia-Jaramillo, Oregon State University

Harmful algal blooms (HABs) have become an emerging environmental health problem driven by global agriculture and a warming climate. Cyanobacteria, a major HAB contributor, release microcystins, with microcystin-LR (MC-LR) being the most common and most potent hepatotoxic congener. The blooms impact drinking water sources for humans, pets, livestock, and wildlife, often resulting in their liver failure or death. While government agencies test public bodies of water and drinking water for the toxin, the burden of testing

private lagoons and ponds used for watering livestock is on farmers, many of whom cannot afford to run expensive water quality tests. This same problem applies to untreated drinking water sources in developing countries where there are documented cases of human fatalities from MC-LR. The current rule of thumb for gauging water safety is to avoid water that is actively green from cyanobacteria, although there is no scientific evidence that MC-LR is absent in the water once the bloom is over. UV radiation, chloride, pH, ozone, and biodegradation have all been cited as possible mechanisms for toxin degradation, but there is no complete kinetic or mechanistic data to apply to contaminated water sources in the environment. Our study aimed to identify and assess the toxicity of degradation products under different environmental conditions. We performed laboratory incubation studies for each of the five known degradation mechanisms using multiple reaction monitoring (MRM) liquid chromatography tandem mass spectrometry (LC-MS) to track changes in MC-LR concentration over time and to calculate half-life. Additionally, non-targeted screening (NTS) was used to identify transformation products unique to each of these degradation mechanisms. By using the kinetics data and known transformation products, we can analyze environmental samples using LC-MS and look for these same transformation products to determine which mechanisms have contributed the most to environmental degradation of MC-LR. Our study aims to provide data to create guidelines for predicting microcystin degradation using easily monitored environmental characteristics such as UV-index and pH. Preliminary data found 11 UV-degradation products previously described in literature in incubation, and of these, 4 were found in environmental samples.

4.16.T-05 Optimizing wastewater-based epidemiology for illicit drug monitoring using mass spectrometry

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The substance use epidemic in the United States has intensified in recent years. This complex and evolving landscape of substance use underscores the urgent need for comprehensive surveillance tools to inform public health responses. Wastewater-based epidemiology (WBE) has emerged as a cost-effective and population-wide approach to monitor community drug use, but its implementation presents several challenges, including sample variability, analyte stability, and reproducibility. In this study, we present a practical and standardized analytical workflow for multi-class drug detection and quantification in wastewater, leveraging liquid chromatography-tandem mass spectrometry (LC-MS/MS) and optimized sample preparation strategies. 24-hr composite wastewater samples were collected weekly from more than 20 treatment facilities across four major U.S. metropolitan areas over a period of one year. We evaluated two solid-phase extraction (SPE) sorbents, Oasis HLB and Oasis MCX, to compare analyte recovery, precision, and consistency. The MCX cartridge demonstrated superior performance across most compounds, offering improved reproducibility and reduced variability despite isolated outliers. All analytes showed calibration curve coefficients >0.99 , with limits of detection and quantification within the three lowest calibrants, confirming high instrument sensitivity. Recoveries ranged from 65–120% with $<20\%$ RSD for most analytes. A broad spectrum of substances was quantified, including opioids, stimulants, cocaine-related metabolites, hallucinogens, and other emerging compounds, capturing both parent drugs and their major metabolites. Drug consumption rates were normalized to population size and adjusted for known metabolic transformations of parent compounds. The resulting data revealed distinct regional and temporal trends across the sampled metropolitan areas, highlighting differences in drug usage patterns that may align with various factors that deserve further investigation. This work highlights the successful application of WBE to assess drug consumption trends at a population level and establishes a validated and standardized protocol for the simultaneous detection of multiple drug classes in complex wastewater matrices. These findings underscore the value of advanced mass spectrometry and WBE as high-throughput, non-invasive tools that complement public health surveillance and support timely, evidence-based responses to the substance use crisis. **Disclaimer/Disclosure:** This project was supported by a supplement from

the National Institutes of Health, NIDA, to the parent award U01DA051126, the National Drug Early Warning System (NDEWS).

4.16.T-06 3D Molecular cartography of the International Space Station

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The transition from terrestrial to extraterrestrial living poses a significant and anticipated challenge for humanity. Despite growing awareness of the substantial health impacts of indoor chemical exposure, there is a remarkable lack of knowledge regarding the chemical composition of extraterrestrial living systems. The International Space Station (ISS) stands as a distinctive environment, isolated from Earth, and has hosted human inhabitants, their microbiomes, and self-contained experimental organisms for over two decades. With long-term space travel on the horizon, it becomes crucial to unravel the molecular and microbial composition of the surfaces within the ISS. In this presentation, we unveil the first 3D environmental metabolome survey of the ISS. We collected 803 environmental surface samples aboard the ISS using a specially designed dual-headed cotton swab device. Upon transportation back to Earth, the samples underwent untargeted metabolomics profiling employing reverse-phase liquid chromatography and tandem high-resolution mass spectrometry, utilizing both positive and negative electrospray ionization. The untargeted metabolomics data were analyzed through MS/MS library matching, molecular networking, reference-based metabolomics for chemical source tracking, and 3D Molecular Cartography. We detected diverse array of molecules on the ISS surfaces, including industrial materials (e.g., perfluoroalkyl and polyfluoroalkyl substances [PFAS], rubber-derived chemicals), surfactants and disinfection products (e.g., polyethylene glycol derivatives, amidopropyl betaines, quaternary ammonium disinfectants), plant and food-derived compounds (e.g., polyphenols, flavonoids, alkaloids, and derivatives), human or animal metabolites (e.g., urate, carnitines, long-chain fatty acids), and microbial metabolites (e.g., phenylacetyl-glutamine, hippurate acid, indoxyl sulfate, asperphenamate). Unique chemical markers were observed at different functional modules of ISS, suggesting that ISS surface chemistry is strongly driven by human activities.

4.17.A.T Mercury Biogeochemistry, Exposure Pathways and Risk to Wildlife

4.17.A.T-01 Isotopic fractionation of gaseous mercury in a large industrial city: Spatio-temporal variations and source apportionment

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The source-receptor relationship of atmospheric mercury is a critical environmental concern, particularly in industrialized coastal regions where complex meteorological patterns influence pollutant dispersion. Comprehensive evaluations of mercury pollution based on spatially resolved and time-averaged data have not yet been conducted in Korea. In this study, the spatio-temporal variations of total gaseous mercury (TGM) and mercury isotopes were examined using passive air samplers at 30 sites in Ulsan, the largest industrial city in Korea, over one year. TGM concentrations ranged from 2.26 ng/m³ to 68.5 ng/m³ with a mean of 6.89 ng/m³, which was significantly higher than the national average of 1.98–2.09 ng/m³. Mean TGM concentrations by season were highest in summer (9.28 ng/m³), followed by spring (7.31 ng/m³), winter (6.57 ng/m³), and fall (4.41 ng/m³). Industrial sites exhibited significantly elevated concentrations compared to suburban and urban areas. The highest concentration occurred in a non-ferrous industrial complex (21.9 ng/m³), which was attributed to zinc smelters processing over 1.2 million tonnes of non-ferrous metals annually. Seasonal winds significantly influenced gaseous mercury dispersion to surrounding areas. Southeasterly monsoon winds in summer enhanced widespread transport of industrial emissions to urban and suburban regions, while northwesterly winds in winter minimized this dispersion. Mercury isotope analysis of 13 samples each from

summer and winter indicated distinct isotopic signatures, with $\delta^{202}\text{Hg}$ values of -0.14 ± 0.34 ‰ and $\Delta^{199}\text{Hg}$ values of -0.06 ± 0.05 ‰. Through a ternary mixing model with distinct isotopic end-members, the mean contributions of anthropogenic emissions, surface evasion, and background effects to TGM levels were 73%, 2%, and 25% during summer and 49%, 12%, and 39% during winter, respectively. The anthropogenic contribution decreased with distance from industrial sources, demonstrating a clear source-receptor relationship. The results indicate that anthropogenic emissions are the major source of gaseous mercury in the coastal city of Ulsan, particularly during summer when southeasterly winds are prevalent. This study is the first to present spatial and seasonal distribution maps of source contributions to TGM concentrations using mercury isotope analysis and a ternary mixing model.

4.17.A.T-02 Mercury in small ponds: The role of mixing regime in lower food web bioaccumulation

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Mercury (Hg) contamination across landscapes continues to pose a risk to human and wildlife health. Research on mercury in aquatic systems has spanned across lakes, tributaries, reservoirs, and marine environments, among others. Small ponds, while making up a considerable portion of water bodies globally, are often left under-studied, although it is known Hg can be methylated and mobilized into food webs across diverse ecosystems and environments. To fill this gap, we investigated the role of mixing regime on Hg trophic transfer in small ponds across four bioregions in New York State. We hypothesized that ponds with less mixing (more stratification) would promote higher methylmercury concentrations in zooplankton due to hypoxic conditions known to promote Hg methylation by anaerobic microbes. Sediments and size fractionated (53 and 253 μm) zooplankton were collected from 16 small ponds in summer of 2024. We found a wide range of sediment and zooplankton Hg concentrations across the four bioregions. A strong significant relationship ($R^2=0.49-0.62$, $p < 0.0001$) between sediment total Hg concentrations and zooplankton MeHg concentrations in both size-groups suggests a tight coupling between biotic and abiotic compartments in ponds. Results from this study will place small ponds in our global understanding of how Hg cycles across diverse aquatic ecosystems. Additionally, understanding the propensity of Hg methylation in small ponds will provide context on the risk small ponds pose as a contaminated lower food web energy source to adjacent food webs.

4.17.A.T-03 The influence of native versus non-native wetland vegetation communities on methylmercury production

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Wetlands are often associated with production and elevated concentrations of methylmercury (MeHg) because the conditions (stagnant/low oxygen water and higher nutrient and organic carbon concentrations) are favorable to methylating microbial communities, compared to other aquatic systems (i.e., rivers, lakes). Wetlands can be dominated by native vegetation species or exotic/invasive species. A common invasive wetland species is reed canarygrass (*Phalaris arundinacea*), which is distributed globally and can out-compete native vegetation. Reed canarygrass is a quick growing, high-density plant species that can produce large quantities of organic material though high root densities and seasonal senescence and re-growth. The objective of this study was to determine if different wetland plant species have a significant influence on MeHg production and if the removal of invasive reed canarygrass and replacement with native species can result in a decrease in MeHg production. To address these questions, controlled experiments were performed in replicate mesocosms containing different wetland vegetation communities (reed canarygrass, common/soft rush, sitka willow, daggerleaf rush, and wooly sedge). The plant species were selected to identify if variables such carbon storage and nitrogen fixation have the potential to influence sediment redox conditions and microbial methylation. In addition, field measurements from wetlands that are dominated by reed canarygrass were compared to areas that have been restored to native vegetation. Measurements of sediment and porewater were analyzed for total-Hg (THg) and MeHg

concentrations as well as common ancillary parameters associated with methylation (i.e., major anions, sulfide, reduced iron, oxidation-reduction potential, dissolved oxygen). Preliminary results show that wetland plant community composition can have a significant impact on MeHg production, and that wetlands that have had reed canarygrass removed and restored to lower density native species have significantly lower THg and MeHg in the porewater as well as lower dissolved organic carbon and higher a higher oxidation-reduction potential. Overall, these results highlight the potential to utilize wetland vegetation management as a strategy to decrease MeHg production.

4.17.A.T-04 Quantifying hydrologic controls on mercury speciation and fluxes in a beaver-dominated vs. unimpounded stream reach

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Atmospheric deposition of mercury (Hg) is increasing in some regions of the Western U.S. When transported to anoxic environments, inorganic Hg can be transformed into methylmercury (MeHg), a bioaccumulating neurotoxin. Simultaneously, American beaver (*Castor canadensis*) populations are increasing, and their modifications of ecosystems (e.g., construction of dams, lodges, and food caches) expand anoxic conditions. Previous research has documented increased surface water MeHg concentrations downstream of new beaver dams, but to date has focused only on individual ponds. However, the cumulative effects of multiple beaver ponds likely exert a stronger influence on Hg methylation processes, highlighting a need to understand how beaver activity alters MeHg production, storage, and transport at the reach scale. To address this knowledge gap, we ask: How does Hg concentration, speciation, and dissolved versus particulate fractions change in response to hydrologic variability at the outlet compared to the inlet of a beaver-dominated and an unimpounded stream reach (~1.5 km)? We hypothesize that, compared to the unimpounded reach, the beaver-dominated reach will 1) exhibit higher dissolved MeHg transport due to enhanced particulate settling and microbial methylation in anoxic pond sediments and 2) have the greatest MeHg enrichment during midsummer, when prolonged water residence time, warm temperatures, and anoxia stimulate high rates of microbial reduction. We collected surface, ground, and porewater in paired beaver-dominated and unimpounded reaches in Crested Butte, Colorado, throughout the snow-free 2025 season (late May - late October). We collected samples at the inlet, outlet, and on the floodplain in both reaches. Surface water samples were paired with stream discharge measurements to quantify the mass flux of Hg. We analyzed the water samples for dissolved and particulate total Hg/MeHg along with sulfate, dissolved organic carbon, and physical water quality parameters. Our research contributes to our quantification of how beaver activity alters the storage of mercury throughout the snow-free season and develops a novel reach-scale Hg mass balance. The outcomes of this work will provide valuable information to land and water managers and restoration practitioners who must consider the expanding presence of beaver activity and its impacts on water quality and the bioavailability of MeHg in the river corridor. **Disclaimer/Disclosure:** The research is supported by the Critical Zone Dynamic Water Cluster: EAR-2012669

4.17.A.T-05 Do temporal shifts in DOM composition affect Hg bioaccumulation across the subtropical wet season in a hypereutrophic lake?

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The composition and quantity of Dissolved Organic Matter (DOM) in freshwater ecosystems can affect how much mercury (Hg) is available for uptake and bioaccumulation in food webs. While these relationships have been recently explored spatially in northern ecosystems, few studies have focused on temporal effects and within subtropical ecosystems. Florida, one of the most freshwater dense locations in the U.S.A., experiences

distinct wet and dry seasons, and this climatic variability is likely to affect the source – and therefore the composition and quantity – of DOM within lakes seasonally, with implications for Hg cycling. The goals of this study are to examine whether DOM composition and quantity varies temporally within a Florida lake, and if this affects Hg concentrations of pelagic and littoral invertebrates and fish. We intensively sampled Lochloosa Lake, a hypereutrophic lake representative of lakes in central Florida, during the wet season of 2025. Specifically, we collected monthly water samples for common chemistry parameters (i.e., nutrients, sulfate, DOM quantity), as well as optical-based DOM composition measurements, from April through October. We concurrently collected zooplankton, macroinvertebrates (e.g. midge larvae, grass shrimp), and forage fish (shad species, mosquito fish) samples. While field and lab work are on-going, methylmercury concentrations and total Hg concentrations of biota will be evaluated to determine temporal variation, and their relationship to DOM composition and quantity. We predict that inputs of Hg will increase during the wet season due to 1) increased wet Hg deposition and 2) increased imports of terrestrial DOM and bound Hg caused by frequent rewetting of riparian areas. As a result, the Hg concentration in lower trophic level organisms (i.e. grass shrimp, midges, and zooplankton) may increase in the wet season and this increase may be reflected in forage fish during the dry season. Ultimately, this work will provide new knowledge on what limnological parameters drive biotic Hg levels in understudied subtropical lakes, and how they vary seasonally. Further, this study will assist in the development of future monitoring plans for Hg risk, and conservation and remediation efforts of Floridian freshwater resources.

4.17.A.T-06 Methods of understanding the impact of increasing global temperatures on microbial mercury methylation

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Due to increased greenhouse gas emissions, temperatures across the globe are expected to rise throughout the twenty-first century, which will affect the rates of biogeochemical processes in sediments. As part of the mercury cycle, microorganisms in sediments transform mercury from inorganic mercury (Hg(II)) to toxic methylmercury (MeHg). MeHg is a pollutant of great public health and environmental concern due to its neurotoxic effects and ability to bioaccumulate. The role of temperature in the mercury cycle is not well understood, and therefore it is not known how rising global temperatures will affect the rate of toxic MeHg formation. To test the effects of temperature on Hg(II) methylation, laboratory incubations were performed on intact sediment cores from five locations representing four ecological regions in North America, including Arctic tundra (Alaska), Mediterranean California, subtropical wetlands (Florida), and the Great Plains (Michigan). At each location, fifteen replicate cores and pore water were collected and pore water was collected for laboratory experiments. Additionally, porewater was characterized for inorganic anions (Cl⁻, NO₃⁻, SO₄²⁻), particulate metals (Fe, Mn), dissolved organic carbon (DOC), filter-passing total mercury (THg), filter-passing methylmercury (MeHg), small organic acids (SOA), methane (CH₄), and dissolved inorganic carbon (DIC). Incubation temperatures (n=4 for each location, in experimental replicate) simulated air temperature increases projected in each location from the present day to the year 2100, constrained using several climate models. Experiments used enriched stable isotopes of Hg(II) to quantify how temperature influenced rates of Hg(II) conversion to MeHg. Adenine triphosphate (ATP) quantification and genetic sequencing were used to assess the response of microorganisms. Microbial activity, and therefore the magnitude of microbial methylation, were expected to increase with temperature. The results of this study will aid the understanding of both the role of temperature in the mercury biogeochemical cycle, as well as the impact of climate change on the severity of MeHg pollution.

4.17.B.T Mercury Biogeochemistry, Exposure Pathways and Risk to Wildlife

4.17.B.T-01 Exploring mercury biodynamics for a freshwater biosentinel in the literature, laboratory and field

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Mercury exposure risk can be regulated by complex biogeochemical drivers at multiple scales that determine mercury loading, bioavailability, and trophic transfer. Tissue data from biosentinel taxa can be useful to estimate mercury risk and protect wildlife and human health. Freshwater predatory invertebrates like dragonfly larvae are increasingly being used as biosentinels to monitor mercury regimes. Because the intrinsic and extrinsic drivers that regulate their mercury assimilation are not fully understood, we analyzed trends in the literature, laboratory, and field. We conducted a meta-analysis and estimated that freshwater predatory invertebrates biomagnify mercury by factors of 2.1 ± 0.2 to 4.3 ± 0.3 , with 95% credible intervals ranging from 1.6 to 5.3. Methylmercury trophic magnification factors for communities including vertebrates and primary producers were 18.6 ± 6.2 to $54.1 \pm 7.7\%$ higher than invertebrate sub-communities alone. Biomagnification was influenced by exposure concentration and invertebrate taxonomic and functional diversity. We also conducted a series of laboratory experiments to measure mercury trophic transfer from dosed prey to dragonfly larvae over time across exposure levels. Preliminary data suggested that dragonfly mercury concentrations responded to dietary exposure in as little as 7 days and reached equilibrium in as little as 40 days. Dragonflies biomagnified methylmercury by preliminary factors of 1.0 ± 0.1 to 3.4 ± 0.2 , influenced by exposure concentration and intrinsic drivers like development and taxonomy. We also explored the influence extrinsic drivers like surrounding land cover on mercury levels by sampling dragonfly larvae from 104 diverse sites throughout the complex Willamette River Basin in Oregon, USA. Preliminary dragonfly mercury concentrations varied by $88 \pm 14.7\%$ within tributary catchments, negatively correlated with finer-scale surrounding urban cover and positively correlated with broader-scale surrounding forest cover. Agriculture had habitat-dependent influences, associated with higher dragonfly mercury in wetland sites and lower dragonfly mercury in lentic sites. This research helps expand scientific understanding of the intrinsic and extrinsic determinants of mercury cycling in dragonfly larvae and other freshwater predatory invertebrates.

4.17.B.T-03 Do cursorial wolf spiders accumulate mercury from aquatic or terrestrial prey?

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Substantial research effort has focused on better understanding the consequences of aquatic pollution for aquatic organisms such as fish and piscivorous predators. More recently it has become clear that aquatic contaminants can also enter terrestrial food webs with dire consequences for the predators there. For example, the cross-ecosystem transport of mercury from polluted rivers can lead to deleterious effects on terrestrial songbirds. Spiders exist atop long invertebrate food chains that can biomagnify contaminants and pass them on to higher trophic levels, including birds. Previous studies have documented that songbirds in a mercury-contaminated floodplain obtain most of their mercury from wolf spiders. There are two trophic pathways by which the mercury could enter wolf spiders. One hypothesis is that terrestrial wolf spiders may consume large numbers of emergent aquatic insects that have accumulated contaminants. The other hypothesis is that historical flood events deposited contaminants on floodplain soils, which now circulate independent of aquatic mercury, in the terrestrial food web from plants to herbivores and eventually to spiders and birds. Our objective was to determine whether cursorial wolf spiders living in the floodplain of a mercury-contaminated river obtain a large portion of their diet from aquatic prey, using DNA metabarcoding of gut contents from 550 spiders collected on the floodplain from April - September, 2024. We found that the vast majority of wolf spiders ate prey species of terrestrial origin; very few ate those with an aquatic life stage. The ecotoxicological implications of this study go beyond this one river floodplain or contaminant as terrestrial spiders are widely recognized vectors for cross ecosystem subsidies of contaminants from aquatic to terrestrial systems. If, as demonstrated here, terrestrial spiders generally obtain contaminants by eating terrestrial invertebrates that have become contaminated through biomagnification of food webs that start in terrestrial soils, then successful remediation of injury to terrestrial wildlife must include wide swaths of floodplain, and remediation of river channels alone will not effectively reduce contaminant exposure of terrestrial wildlife such as songbirds.

4.17.B.T-04 Implications of glacial retreat for mercury export and bioaccumulation in nearshore food webs and key subsistence species of Alaska

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High latitudes are warming rapidly due to polar amplification, which accelerates the melting of the cryosphere, thus increasing glacial retreat. Glaciers have long acted as repositories for contaminants that undergo long-range atmospheric transport – particularly mercury (Hg). The heavily glaciated Gulf of Alaska (GoA) region is losing glacial mass faster than other glaciated parts of the world, and concerning, Hg yields in glacial streams entering the GoA are some of the highest ever reported. Subsequent microbial transformation processes that occur in aquatic systems can convert Hg to methylmercury (MeHg), the highly neurotoxic and bioaccumulative form of Hg present in fish muscle. MeHg also biomagnifies and can reach potentially toxic levels in the muscle of high trophic level and/or long-lived fish species. Thus, the purpose of this study was to investigate temporal and spatial trends in Hg accumulation in biota from glacially influenced waters in Kachemak Bay, Alaska. Black rockfish (*Sebastes melanops*) and Pacific halibut (*Hippoglossus stenolepis*) were selected as high trophic level fish species of interest because they are long-lived, occupy different habitats within the study area (e.g., pelagic versus benthic-dwelling), and are regularly consumed by humans. Total Hg (tHg) was measured in skeletal muscle and MeHg was measured in concentric eye lens layers of both species to examine potential temporal trends in Hg accumulation in large-bodied fish. We also analyzed tHg in crescent gunnel (*Pholis laeta*; a low trophic level prey fish) and several invertebrate species from three Kachemak Bay watersheds spanning a gradient of glacial coverage (0% to 62%), in order to examine spatial trends driven by glacial export of Hg in meltwater. Glacial coverage, watershed characteristics, foraging styles, and feeding ecology were among the factors that were found to be significant drivers of Hg accumulation in biota. These findings highlight the important role of glaciers in the global Hg cycle, including the influence of glacial meltwater on Hg uptake into coastal food webs.

4.17.B.T-05 The modifying effects of maternal diet (fish vs. rice) on the associations between prenatal methylmercury exposure and children's neurodevelopment: An investigation utilizing stable mercury isotopes

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Methylmercury (MeHg) is a potent neurotoxicant. Depending on dietary practices, fish or rice consumption can be the main dietary exposure pathways of MeHg in humans. We hypothesize that neurodevelopmental impacts due to MeHg may vary depending on the maternal dietary source of MeHg, due to beneficial nutrients found in fish, not rice. To investigate this hypothesis, we leveraged our birth cohort study in rural China where rice is an important dietary staple (n=261 mother/offspring pairs), using stable mercury (Hg) isotopes. We previously reported adverse associations between maternal hair Hg concentrations, a biomarker for prenatal MeHg exposure, and children's neurodevelopment assessed at 12 months of age using the Bayley Scales of Infant Development, 2nd Edition. For the same cohort, we reported that mass independent fractionation (MIF) (represented by maternal hair D199Hg) can distinguish MeHg intake primarily from rice versus fish. In the present study, we investigated the modifying effects of measures of maternal diet on child's 12-month Bayley scores using 1) maternal hair D199Hg, 2) maternal self-report fish consumption frequencies, and 3) three indices for peripartum serum long-chain polyunsaturated fatty acids (PUFAs), a biomarker positively correlated with fish intake. Results: In adjusted models, hair D199Hg modified the adverse associations between maternal hair Hg and child's Bayley scores. Mothers who obtained a majority of MeHg from rice, not fish, were primarily in the lowest tertile of hair D199Hg. For those mothers, a doubling in maternal hair Hg was associated with

statistically significant lower (i.e., more adverse) Mental Developmental Index (MDI) scores [Beta: -4.19, 95% Confidence Interval (CI): -7.51, -0.878]. A similar trend was observed for Psychomotor Developmental Index scores (Beta: -4.13, 95% CI: -7.90, -0.363). The other indices, including maternal fish consumption frequencies and serum PUFAs, did not modify the associations between maternal hair Hg and child's Bayley scores. Using hair MIF, our results indicated the dietary source of MeHg (fish versus rice) modified the adverse associations between a biomarker of prenatal MeHg exposure and children's neurodevelopmental scores. Relevance: Our results demonstrated that MeHg-associated decrements in Bayley scores were observed for mothers whose MeHg dose was primarily from rice, not fish. **Disclaimer/Disclosure:** This study was funded in part by a grant from the U.S. National Institute of Environmental Health Sciences at the U.S. National Institute of Health (NIH/NIEHS) to Sarah E. Rothenberg (grant #R21 ES032600). This work was also supported by the University of Rochester Environmental Health Sciences Center, an NIH/ NIEHS-funded program (P30 ES001247).

4.17.B.T-06 Tracing mercury cycling from gold mines to crops

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Mercury (Hg) is a toxic global pollutant with major public health impacts. The largest anthropogenic source of Hg is artisanal and small-scale gold mining (ASGM), which contributes to more than one-third of global Hg emissions. ASGM activity often surrounds agricultural fields and local gardens, but our understanding of how ASGM Hg use impacts terrestrial food systems is limited. We first conducted a global review of mercury (Hg) cycling in agroecosystems by analyzing how Hg concentrations in the edible biomass of crops vary by crop type (i.e., bulb, grain, leafy vegetable, legume, root vegetable, and tree crop), Hg source, proximity to source, and pathways of entry. We then compared these concentrations to values reported in reviews of plant biomass from natural systems, including forests, wetlands, and grasslands. We then quantified the Hg concentration in crops from farms and gardens in two different ASGM communities in Ghana, which has one of the largest ASGM sectors globally. Crop samples (root, leaves, fruit/vegetable), soil samples, and air samples were collected from farms and gardens along transects extending from locations where Hg-gold amalgams are burned to the surrounding gardens and farms. We hypothesize that crops within gardens close to ASGM Hg burning sites and elevated air Hg concentrations will have predictably higher total Hg concentrations than those on farms located further away with lower air Hg concentrations. We also hypothesize that root vegetables and below-ground plant biomass will accumulate higher concentrations of total Hg than other crops (i.e., leafy vegetables and tree crops) and above-ground biomass. In this presentation, we will discuss global mercury (Hg) cycling trends in agroecosystems, along with results and inferred implications from our sampling efforts conducted during the summer of 2024–2025.

4.17.P-Tu Mercury Biogeochemistry, Exposure Pathways and Risk to Wildlife

4.17.P-Tu-058 Elevated mercury concentrations in riparian predators unaccounted for by trophic magnification

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Metamorphosis is central to the flow of nutrients and aquatic contaminants to riparian consumers and is thus important both ecologically and in terms of exposure risk. Physiological changes during metamorphosis can result in markedly different chemical compositions of adults compared to juveniles, which may propagate to consumers of these different life stages. Chemical changes include large variances in contaminant concentrations depending on their loss or retention during metamorphosis, along with non-trophic enrichment of nitrogen stable isotopes ($\delta^{15}\text{N}$) potential confounding our ability to use these types of tracers in food web exposure models. Riparian and aquatic consumers within the same food web would therefore be expected to reflect these compositional differences; however, scant data are available to assess the effects of metamorphosis

on consumer chemical composition across aquatic-terrestrial boundaries. Here, we measured total mercury in terrestrial and aquatic consumers in the headwater streams of southern Appalachia to compare biomagnification in both systems. We found that biomagnification rates (i.e., change in Hg concentration per unit increase in $\delta^{15}\text{N}$) were similar, but that mercury concentrations in riparian organisms were approximately 6 times higher than neighboring aquatic organisms. We propose possible ecological mechanisms, occurring separately or simultaneously, that might explain these surprising observations: 1) aquatic insect metamorphosis bioamplifies mercury, 2) insect metamorphosis enriches $\delta^{15}\text{N}$ values, and/or 3) the fractionation of $\delta^{15}\text{N}$ that occurs during predation is lower for riparian predators than for aquatic predators. Further study is needed to elucidate the exact mechanisms driving elevated mercury concentrations in riparian predators.

4.17.P-Tu-059 Assessing mercury export and cycling in abiotic media across a gradient of glacial influence in the Gulf of Alaska

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Coastal ecosystems in high latitude regions are especially vulnerable to the adverse impacts of climate change as a result of disproportionately high rates of warming driven by polar amplification. This is particularly evident in Alaska (AK), where increasing temperatures are causing rapid retreat of coastal glaciers, a process known to release large quantities of mercury (Hg) to downstream environments. Recent studies conducted in the heavily glaciated regions of North America have found some of the highest measured concentrations of Hg ever reported in coastal meltwater streams, suggesting that the melting cryosphere (e.g., glaciers, permafrost) may be an increasingly important source of Hg in coastal watersheds. Glacially derived inorganic mercury (Hg(II)) exported to downgradient aquatic systems can bind to organic matter (OM) and/or undergo microbial transformation processes, resulting in the production of an organic form of Hg, methylmercury (MeHg). While the bioavailability of Hg(II) is relatively low, particularly when adsorbed onto OM, MeHg readily accumulates in aquatic biota and biomagnifies in aquatic food webs, reaching potentially harmful levels in high trophic level and/or long-lived species. MeHg is a potent neurotoxicant that adversely affects the health of wildlife and humans and poses a much greater ecological and human health risk relative to the inorganic or elemental forms. We quantified, speciated, and identified sources of Hg exported from glacially influenced watersheds in Kachemak Bay, AK, in an effort to understand the implications of glacial retreat for coastal ecosystem health. Water and sediment samples were collected from intertidal zones and glacial meltwater streams in three watersheds spanning a gradient of glacial influence (0%–62%). All samples were analyzed for total Hg, MeHg, and OM content. Water samples were also used for Hg isotope analysis to identify the relative importance of Hg source contributions within the watersheds of interest. Preliminary results reveal spatial variability in Hg concentrations across watersheds, shaped by environmental and geochemical conditions. These findings underscore the importance of climate-driven glacial retreat on Hg dynamics at the local and global scales, including implications for bioavailability and uptake in coastal food webs.

4.17.P-Tu-061 High-resolution porewater profiling with equilibrium passive sampler enables novel treatment options for mercury contaminated sediments

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High resolution depth profiling of porewater methylmercury (MeHg) in surface sediments is critical for the assessment of benthic organism exposure, flux into the water column, and performance evaluation of engineered remedies. Traditional porewater sampling methods are challenging due to the redox-sensitive nature of MeHg and difficulty extracting porewater at the fine-scale. Here, we demonstrate the use of an equilibrium passive sampler (PS) made of a 1-mm thick agarose gel polymer sheet embedded with activated carbon (AC). When inserted in the sediment, MeHg in the porewater diffuses through the agarose gel and adsorbs to the

embedded AC, eventually reaching equilibrium enabling estimation of the porewater MeHg based on measured concentration in the sampler sheet and an equilibrium partitioning calculation. The activated carbon in the PS stabilizes the sampled MeHg so that it is resistant to transformation upon retrieval. Mercury contaminated sediment from field sites were used to construct laboratory mesocosms with different treatments: 1) untreated control, 2) 2-cm sand cap, 3) powdered activated carbon placed as a layer on sediment (5% by weight) 4) activated carbon mixed into the sediment, and 5) 2-cm sand cap over a layer of AC on sediments. After allowing treated sediments to stabilize for 8 weeks, the PS was vertically inserted to traverse the depth of the sediment, allowing at least 1 cm exposed to the surface water and allowed to equilibrate for 4 weeks. The PS were then removed from sediment and sectioned (0.5-cm) alongside sediment cores (1-cm sections) and surface water grab samples. All four treatments resulted in reduced surface water MeHg. The highest surface water reductions were observed where AC was added as a cap either alone or beneath the 2-cm sand cap. The passive sampler was able to generate 0.5 cm increments of estimated porewater concentrations and showed that the most effective treatment (based on porewater MeHg reductions) was the placement of a layer of AC under a 2 cm sand cap followed by AC cap, mixed AC, and a sand cap. Fully mixing AC into the sediment resulted in higher sediment MeHg compared to the unamended control although porewater MeHg remained low. The high resolution depth profiling of MeHg porewater shows that strategic placement of the AC treatment layer under a thin sand cap provides the greatest protection from benthic and aquatic exposure to MeHg produced in sediments. **Disclaimer/Disclosure:** This research has been supported by funding from the DoD SERDP program, The NIEHS Superfund Research Program, and the Dow Chemical Company.

4.17.P-Tu-062 Temporal trends of mercury and condition factors in arctic char from paired lakes influenced by increased turbidity and other climate-related drivers

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Climate-driven changes including rising air temperatures, enhanced permafrost degradation, and altered precipitation patterns are having profound effects on biogeochemical cycling of mercury (Hg) in the High Arctic. Two physically similar lakes, East Lake and West Lake (74°50'N, 109°30'W) on uninhabited Melville Island, Nunavut, are being affected by climate change differently. The West Lake catchment has experienced significant permafrost degradation and had multiple underwater sediment slumping events between 2007 and 2011, leading to a sustained 50-fold increase in turbidity. Neighboring East Lake has had only minor changes in water chemistry. West Lake is now recovering from the impacts of sediment loading; turbidity has declined by about 50% from a maximum 161 NTU in 2013 to 78 NTU in 2024 in West Lake. Meanwhile turbidity has remained low in East Lake (~10 NTU in 2024). These contrasting changes have provided a unique opportunity to study the potential impacts of permafrost degradation, turbidity, and other climate-related effects on concentrations of Hg in landlocked Arctic char (*Salvelinus alpinus*), the only species of fish in the lakes. Over the period 2008-2019, there was a significant decrease in length-adjusted Hg concentrations in East Lake char averaging 6.5 %/yr. In contrast there was a significant increase in char Hg in West Lake, averaging 7.9 %/yr for length-adjusted means over the period 2009 to 2017, correlated with turbidity. However, length-adjusted Hg declined in West Lake from 2017 to 2024 (7.7%/yr) while increasing in East Lake (8.8%/yr), although neither trend was statistically significant due to limited sampling years. The high turbidity impacted char diets and growth rates. From 2013-2018, the char condition factor (CF) was significantly lower in West Lake (averaging 0.55 g/cm³) than East Lake (0.66 g/cm³), but fish condition has improved with the lower turbidity and CFs are now the same in both lakes (0.65 g/cm³). Another sign of recovery in West Lake is that the char growth rates have increased (age-at-size has declined from 18 yrs to 14 yrs from 2017 to 2024) and thus decreasing Hg concentrations may be partly explained by somatic growth dilution. Nevertheless, carbon (δ¹³C) stable isotope

ratios continued to differ between the two lakes averaging -28.3‰ and -22.2‰, in East and West Lake, respectively in 2024 implying continued greater contributions of benthic and terrestrial carbon in West Lake char due to the past inputs.

4.17.P-Tu-063 Selenium:mercury molar ratios in biota from a Superfund site in Lavaca Bay, Texas

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Located in Lavaca Bay on the mid-Texas coast, the Alcoa (Point Comfort) Superfund site is contaminated with mercury (Hg) due to industrial activities in the 1960s and 70s. The sediment and biota at the site have elevated concentrations of Hg and the area has been closed to the retention of fishes and blue crab (*Callinectes sapidus*) since 1988. While Hg concentrations are monitored in red drum (*Sciaenops ocellatus*) and juvenile blue crab, little is known about Hg concentrations in other species, and nothing is known about the selenium (Se) concentrations and Se:Hg molar ratios. Selenium has an antagonistic relationship with Hg and a Se:Hg molar ratio >1:1 suggests that Se may have a protective effect against Hg toxicity. Using a direct mercury analyzer and ICP-MS, this study investigated the Hg and Se concentrations and calculated the Se:Hg molar ratios in two mollusk species, five crustacean species, 11 teleost species, and three elasmobranch species collected from the Alcoa Superfund site. Mean Hg concentrations (µg/g dry weight) were greatest in black drum (*Pogonias cromis*; 2.85), scalloped hammerhead shark (*Sphyrna lewini*; 2.74), and blue crab (2.66), and lowest in white shrimp (*Litopenaeus setiferus*; 0.120), grass shrimp (*Palaemonetes pugio*; 0.112), and mud crab (*Panopeus obesus*; 0.108). Mean Se concentrations were greatest in hooked mussel (*Ischadium recurvum*, 6.75), Atlantic croaker (*Micropogonias undulatus*; 6.54), and black drum (5.53), and lowest in mud crab (1.00), and gafftopsail catfish (*Bagre marinus*; 1.00). The mean Se:Hg molar ratio was >1:1 in all species, ranging from 2.0 in gafftopsail catfish to 89.8 in white shrimp, suggesting that Se may have a protective effect against Hg toxicity. However, 10% of hardhead catfish (*Ariopsis felis*) and 28.6% of gafftopsail catfish had a Se:Hg molar ratio <1:1, indicating that Se may not have a protective effect in all individuals. If a more conservative 5:1 Se:Hg molar ratio is applied, 16 of the 21 species examined had a mean Se:Hg molar ratio >5:1, and five species [hardhead catfish, gafftopsail catfish, red drum, scalloped hammerhead shark, and bull shark (*Carcharhinus leucas*)] had a mean Se:Hg molar ratio between 1:1 and 5:1. Overall, Se concentrations may be great enough to protect against Hg toxicity; however, the Se:Hg molar ratio that is truly protective and whether the ratio varies among taxa needs to be determined before Se:Hg molar ratios can be extensively used in risk assessment.

4.17.P-Tu-064 texas needs an updated mercury advisory for recreationally caught offshore fishes

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Mercury (Hg) is a nonessential trace element that bioaccumulates in fishes and biomagnifies in marine food webs. Humans are predominantly exposed to Hg through seafood consumption. Frequent consumption of fish high in Hg can result in deleterious neurotoxic and cardiovascular effects and Hg can be maternally transferred via the placenta and breast milk. The Texas Department of State Health Services (TDSHS) can issue a Hg advisory for recreationally caught fish when the mean muscle Hg concentration exceeds the 0.7 µg/g wet weight human health-based standard. The current advisory, based on mean Hg concentrations, has not been updated since 2013. Furthermore, several high Hg species were not examined, and body length was only included in the advisory for king mackerel (*Scomberomorus cavalla*). Using a Direct Mercury Analyzer, this study measured Hg concentrations and investigated the relationship between Hg concentration and body length in muscle tissue from 12 fish species commonly caught in Texas recreational fisheries [blacktip shark (*Carcharhinus limbatus*; n = 51), blue marlin (*Makaira nigricans*; n = 16), crevalle jack (*Caranx hippos*; n = 26), cobia (*Rachycentron canadum*; n = 61), golden tilefish (*Lopholatilus chamaeleonticeps*; n = 45), great barracuda (*Sphyrna barracuda*; n = 66), greater amberjack (*Seriola dumerili*; n = 69), red snapper (*Lutjanus campechanus*; n = 208), sailfish (*Istiophorus platypterus*; n = 14), scamp grouper (*Mycteroperca phenax*; n = 11), Spanish mackerel (*Scomberomorus maculatus*; n = 38), and swordfish (*Xiphias gladius*; n = 12)]. Mercury concentrations (mean ±

standard deviation; $\mu\text{g/g}$ wet weight) were greatest in blue marlin (9.29 ± 4.24), swordfish (1.43 ± 0.445), and blacktip shark (1.27 ± 0.853), and lowest in scamp grouper (0.466 ± 0.225), Spanish mackerel (0.258 ± 0.0888), and red snapper (0.184 ± 0.163). A positive relationship was observed between Hg concentration and body length for nine species, whereas three species (blue marlin, sailfish, and scamp grouper) had no relationship. Nine species had >50% of individuals exceeding the Hg advisory level. Blue marlin and swordfish had 100% of individuals exceeding the Hg advisory level, while red snapper and Spanish mackerel had <5% of individuals exceeding. It is recommended that the TDSHS updates their Hg advisory for Texas coastal waters; in particular, species previously not examined need to be included and consumption recommendations should be issued based on body length.

4.18.A.T Occurrence Fate and Effects of Contaminants in Domestic, Agricultural, Landfill and Industrial Waste

4.18.A.T-01 Evaluating the distribution of wastewater-derived contaminants in the Strait of Georgia using caffeine as a tracer

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Wastewater is known to contain a complex mixture of contaminants and may pose risks when discharged into aquatic environments. However, evaluating the impact of wastewater-derived contaminants can be difficult in coastal marine environments because dilution and transport can result in concentrations that are difficult to quantify despite being ecotoxicologically relevant. For example, the predicted-no-effect-concentration of ethinyl estradiol, a synthetic estrogen and endocrine disrupting compound, is 0.1 ng/L, lower than practical method detection limits in seawater. Compounds present at higher concentrations in wastewater, such as caffeine, can be used as tracers to elucidate the spatial distribution of wastewater-derived contaminants. In this study, we used caffeine as a tracer to evaluate the impact of wastewater discharge in the Strait of Georgia (SoG). As an inland sea that receives wastewater discharges from several municipalities in British Columbia, the SoG has relatively well-characterized inputs and transports processes and presents an ideal location to study the distribution of wastewater-derived contaminants in coastal environments. Vancouver, BC, the largest municipality that discharges into the SoG, produced roughly 451000 million liters of treated wastewater in 2021, of which approximately 224000 liters received only primary treatment. These estimates do not account for untreated sewage released episodically from combined sewer overflows, which occur frequently in Metro Vancouver. For this study, we collected over 230 samples at 46 locations broadly spanning the length and width of the SoG, and at multiple depths, in order to characterize the extent of the impacts of wastewater inputs. Each sample was spiked with a deuterated caffeine internal standard, prepared via solid phase extraction, and analyzed using liquid chromatography-tandem mass spectrometry. This presentation will focus on the results of our extensive sampling campaign. Our findings to date indicate that concentrations of caffeine range from <1.33 ng/L to 156 ng/L. Caffeine concentrations are highest near Vancouver, but we detected caffeine throughout the SoG, including at sites far from known wastewater discharge locations. Overall, our results indicate that wastewater-derived contaminants may be transported throughout the SoG, potentially leading to impacts even at sites relatively far from urban areas.

4.18.A.T-02 Estimating flows of per- and polyfluoroalkyl substances (PFAS) through the carpet life cycle in Japan, 2000–2040

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PFAS are widely used in consumer products, such as carpets, for their water- and oil-repellent properties. Because they pose risks to human health, are resistant to degradation in the environment and the body, and can accumulate once released, understanding their lifecycle stocks and flows is crucial. As a first step toward this

goal, this study estimates the PFAS flows associated with carpets in Japan from 2000 to 2040. The analysis has two main features. First, to reflect different disposal routes in Japan, carpets were categorized into two types: tile carpets and other tufted carpets (e.g., rugs). Tile carpets were assumed to enter direct landfill, be converted to refuse paper and plastic fuel (RPF), or undergo material recycling, whereas all tufted carpets were assumed to be incinerated. To reflect product lifespans, the disposal amount was modeled using a Weibull distribution. Future carpet production and imports were scaled to official population projections. Second, concentration scenarios were established based on reported PFAS concentrations in carpets from the literature. To reflect varying national regulations, producing countries were categorized as Japan, China, the United States, or "other countries." The analysis targeted four specific PFAS: PFHxA, PFOA, 6:2FTOH, and 8:2FTOH. The PFAS content in in-use carpets was assumed to decrease by 1% annually to account for volatilization and degradation. Our estimation revealed that annual PFAS inflow to the Japanese carpet market declined from 40 kg/year in 2000 to 6.8 kg/year in 2023, reflecting regulations in Japan, the US and China. In-use PFAS stock is projected to decline from approximately 400 kg in 2020 to 92 kg by 2040. A scenario assuming stalled regulations in other producing countries showed a slower decline and a subsequent stock plateau. Fluorotelomers (FTOHs) dominate at all time, accounting for approximately 90% of PFAS mass. Although disposal also decreases, nearly half of PFAS discarded in 2040 originates from carpets manufactured before regulation. Therefore, high-concentration legacy carpets will likely continue to enter the waste stream. Furthermore, since some tile carpets in Japan are recycled into RPF and other materials, PFAS emissions from these recycling facilities also warrant close monitoring. **Disclaimer/Disclosure:** This research was supported by the Environment Research and Technology Development Fund(JPMEERF20243003) of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan.

4.18.A.T-03 Updated PCB mass balance of an old urban wastewater treatment plant and its implications

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Long after the ban in 1979, industrialized cities such as Baltimore, MD are still contaminated with PCBs that might be transported through their sewer system to the wastewater treatment plants. Post-treatment, PCBs may still pose an environmental risk through effluent release or biosolid application. With the recent advances of passive sampling to monitor freely dissolved PCBs in water or air, it is possible to accurately measure PCBs in various matrices and provide a better understanding of PCB fate and transport in and out of a wastewater treatment plant. In this study, PCBs were monitored in influent, effluent and air using passive sampling through 3 seasons. PCB concentrations in biosolids and pellets were also measured. PCB mass balance from this study was compared to that performed 4 years earlier. PCB levels above US EPA threshold for a cancer risk of 10 in a million were found in both influent and effluent of the wastewater treatment plant. Higher freely dissolved concentrations were found in the effluent compared to the influent, likely caused by solid digestion. Compared to other tributary inputs, the effluent from the treatment plant was the dominant contributor of PCB loading to Back River. PCB levels in biosolids and pellets were at 200 ng/g, which is less than the cleanup level threshold of 1 mg/kg for bulk PCB remediation waste in high occupancy areas, but above the threshold effect level of 34.1 ng/g. Overall 70% PCB decrease was observed compared to 2015 study, but effluent and solids release still exhibited PCB concentrations above PCB TMDL endpoints and still pose an environmental risk.

4.18.A.T-04 Survey and national emissions estimate of per- and polyfluoroalkyl substances (PFAS) released from landfill gas across USA

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Fluorotelomer alcohols (FTOHs) are per- and polyfluoroalkyl substances (PFAS) found in ambient air collected nearby landfills and in landfill gas (LFG). While 6:2 and 8:2 FTOHs are the dominant PFAS in LFG (Titaley, et al., 2023, Goukeh et al., 2023, Lin, et al., 2024), an estimate of PFAS emissions from U.S. landfills across different geographical locations is needed. The knowledge of FTOH emissions from landfills is crucial because

FTOHs are known to undergo atmospheric degradation to perfluoroalkyl carboxylates and, subsequently, long-range atmospheric transport to remote regions. Samples of LFG were collected using thermal desorption (TD) tubes from 30 landfills in 17 states across three categories of annual precipitation levels (e.g., arid, moderate, and wet). The TD tubes were then heated and desorbed, and the resulting volatiles were analyzed using gas chromatography mass spectrometry. The levels of PFAS in LFG were inputted to a model that estimated the U.S. national emissions of PFAS from LFG. The model used data from the Environmental Protection Agency's Greenhouse Gas Inventory, Greenhouse Gas Reporting Program, and Landfill Methane Outreach Program. The PFAS levels in landfills in arid, moderate, and wet categories were in the range of 1.6×10^3 – 2.1×10^5 , 6.2×10^2 – 2.3×10^5 , and 4.8×10^1 – 1.9×10^5 ng/m³, respectively. Levels of PFAS in LFG collected from landfills in arid and moderate categories were not significantly different, but were significantly higher than the PFAS in LFG collected from landfills in the wet category. The median level of PFAS across the sampled landfills was 1.9×10^4 ng/m³, but more than 25% of PFAS in LFG were in the range of below limit of quantitation to 1×10^4 ng/m³. The modeled U.S. national emissions of total PFAS from LFG was 685 kg/year, which was comparable to a prior estimate of PFAS emissions from leachate (600 kg/year, Lang, et al., 2017). Prior works indicated that the emissions of PFAS from LFG will likely continue for another 100 – 1000 years (Washington, et al., 2015, Li, et al., 2017). Combined with the results of this study, continuing emissions of 6:2 and 8:2 FTOHs will continue well into the future—even if both compounds are banned. However, a recent global survey indicated that worldwide atmospheric FTOHs levels are declining or stable (Saini, et al., 2023). Therefore, although landfills emit FTOHs to the atmosphere, this process may not be impacting the global atmospheric levels of FTOHs.

4.18.A.T-05 Land application of biosolids: Bioaccumulation of emerging contaminants in vegetables and estimated human dietary intake

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As pressures on freshwater resources intensify, recycled water and Class A biosolids are gaining recognition as valuable inputs for sustainable agriculture, offering agronomic benefits such as improved soil quality, enhanced plant growth, and nutrient recycling. However, both recycled water and biosolids can contain trace concentrations of contaminants of emerging concern (CECs), including pharmaceuticals, personal care products, plasticizers, flame retardants, and per- and polyfluoroalkyl substances. Despite growing interest in repurposing these materials, field-based evidence detailing the environmental fate, plant uptake, and potential human exposure risks associated with CECs in food production systems remains limited. To address these knowledge gaps, a large-scale field study was conducted to evaluate the accumulation and bioavailability of 24 priority CECs in potato, cucumber, and sweet corn grown with recycled water and biosolids at agronomically realistic rates. Edible tissues, roots, soil, and porewater were sampled and analyzed to quantify compound concentrations and assess plant uptake. Analytical results, generated using validated UPLC-ESI-MS techniques, revealed that 20 CECs were detected in edible plant tissues at concentrations ranging from 0.93 to 455 ng/g dry weight. Dietary intake estimates for these CECs, based on U.S. EPA standardized consumption rates, suggested an adult annual intake of 2,218 µg, equivalent to 0.087 µg/kg body weight per day for a 70 kg person. In comparison to available acute toxicity data, the estimated dietary exposures are several orders of magnitude below levels known to cause adverse effects. These findings provide essential, field-based insights into the environmental behavior and food-chain transfer potential of CECs in agricultural systems, advancing our understanding of how recycled resources can be safely integrated into food production within a circular economy framework. Future work will extend these investigations to fruit trees, examining CEC accumulation in edible fruit tissues following biosolid application. Collectively, these studies will inform comprehensive human health and ecological risk assessments and support the development of best management practices for the responsible, sustainable reuse of treated waste-derived resources in agriculture.

4.18.B.T Occurrence Fate and Effects of Contaminants in Domestic, Agricultural, Landfill and Industrial Waste

4.18.B.T-01 Integrated characterization of PFAS in landfill leachates, septage, biosolids and municipal wastewater treatment influent

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Over one-third of the US population and >95% of Wisconsin communities rely on groundwater as their primary source of drinking water. Despite the growth of relevant literature, relatively few studies have addressed the potential impact of disperse sources of PFAS including landfill leachates, septage, and land-applied biosolids on groundwater. Using an integrated targeted and non-targeted analytical approach, sample extracts (weak anion exchange solid phase extraction) of 15 landfill leachate, eight hauled septage, 17 municipal wastewater biosolid, and six additional municipal raw influent samples from different facilities across Wisconsin were analyzed. Quantification of 36 targeted PFAS compounds was performed using isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS), and results showed summed concentrations ranging from 1.9-40 ng/mL in landfill leachates, 0.2-0.6 ng/mL in septage, 0.03-0.3 ng/mL in municipal influents, and 32-1400 ng/g in municipal treatment plant biosolids. Perfluorocarboxylic acids (PFCAs) dominated the PFAS profiles in landfill leachates and perfluorosulfonic acids (PFSAs) in biosolids. Sulfonamide precursors were a prevalent PFAS class in septage. Only one biosolid sample exceeded current Wisconsin state guidelines for land application (>150 ng/g combined perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS)). Extractable organic fluorine (EOF) levels measured by combustion ion chromatography (CIC) varied substantially among sample types. The fraction of EOF attributable to targeted PFAS quantified was largest in landfill leachates (8-100%). Conversely, the targeted PFAS contributed less to the EOF in biosolids (1-50%), septage (1-10%), and influent (0.8-7%), indicating substantial contributions of unidentified PFAS compounds in these disperse sources. These differences in targeted PFAS concentrations and EOF were used to inform additional suspect screening analyses using high-resolution mass spectrometry. The combination of targeted PFAS, EOF, and suspect screening analyses provides a novel characterization of disperse sources and advances our understanding of their potential impact on groundwater.

4.18.B.T-02 Transfer of pharmaceuticals from aquatic food webs to riparian spiders along river reaches receiving municipal wastewater effluents

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Complex mixtures of pharmaceuticals are continuously released to freshwater ecosystems via wastewater treatment plant (WWTP) effluents, and multiple drug classes are routinely detected in downstream surface waters. However, it is unclear whether these chemicals accumulate in larval aquatic insects, are retained through metamorphosis in emergent adults, and then transferred to their predators, riparian spiders. This study quantified pharmaceuticals and select major metabolites in larval and adult insects, and predatory spiders, to understand their fate in ecosystems impacted by WWTP effluents and transfer to nearby terrestrial food webs. Caddisflies (Hydropsychidae), mayflies (Heptageniidae), and damselflies (Zygoptera) at both life stages and riparian spiders were collected upstream and downstream of eight WWTPs on the Grand River in Ontario, Canada. Sampling occurred in June, July, and August of 2022 and 2023 during periods of peak emergence. Chemical analysis for antidepressants, anti-inflammatories, antibiotics, antimicrobials, and an antiepileptic were done using a QuEChERS extraction followed by LC-MS analysis. Fluoxetine, Carbamazepine, Ibuprofen, and Diclofenac were detected in larval caddisflies across all sites, and total pharmaceuticals in larvae reached 230 ng/g wet weight (ww). Pharmaceuticals decreased ten-fold in emergent adults with total concentrations as low as 28 ng/g ww detected, but increased in riparian spiders with total pharmaceutical concentrations reaching 100 ng/g ww. Pharmaceuticals detected in riparian spiders more closely resembled those found in aquatic larvae. Across all insects, larval caddisflies contained the highest diversity and concentration of pharmaceuticals. Fluoxetine, Carbamazepine, and Venlafaxine were detected across most sites and taxa, and metabolites Norfluoxetine and Desmethylvenlafaxine were detected in larval caddisflies in 2023 samples when select

metabolites were added to the analytical method. Results show that larval insects accumulate diverse pharmaceuticals, retain these contaminants through metamorphosis, and then transfer them to riparian spiders, with higher concentrations detected in samples downstream of WWTPs. As such, insectivorous predators in riparian environments are at risk of exposure to pharmaceuticals found in WWTP effluents from their aquatic prey.

4.18.B.T-03 Fate of wastewater effluent- and biosolids- borne mixtures of select PFAS, pharmaceuticals, chlorinated paraffins and PBDEs in agricultural systems

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Several priority organic contaminants with diverse physico-bio-geo-chemical properties are frequently introduced into contemporary agricultural environments, largely due to application of contaminated biosolids and wastewater effluents. However, studies investigating the fate and interactions of such complex contaminant mixtures are limited. Herein, we conducted a greenhouse study to assess environmental fate of varying concentrations of mixtures of select organic contaminants that were introduced either via biosolids or wastewater effluent applications. The contaminant classes examined included per- and polyfluoroalkyl substances (PFAS), pharmaceuticals, short and medium chain chlorinated paraffins, and polybrominated diphenyl ethers (PBDEs). Three plant species (lettuce, radish, and peas) and earthworms (*E. fetida*) were exposed up to maturity (plants) and 60 days (earthworms). The contaminants were extracted from the biosolids, effluent, soil, plant, earthworm, and leachate via QuEChERS method and analyzed on LC-MS/MS and GC-Orbitrap. The biosolids and effluent samples contained wide array of the target compounds. Data suggest that the majority of each target contaminant (> 70%) remained in the soil, regardless of whether it was applied via wastewater effluent irrigation or biosolids amendment. Carbamazepine and sulfamethoxazole showed nominal degradation based on increased detection of their major metabolites whereas degradation of the other contaminants was negligible. Leaching of ciprofloxacin and BDE209 was minimal (<1%) whereas ~6-10% (each) of target PFAS, sulfamethoxazole, carbamazepine, and BDE 47 and 99 leached over two months. While most contaminants showed similar leaching potentials irrespective of their source (biosolids or effluent), ~1-2% chlorinated paraffins leached in case of biosolids treatments whereas >7% leached in case of effluent treatments. Plants did not accumulate significant amounts of any of the target contaminants, but the data suggest significant accumulation in earthworms, with bioaccumulation factors estimated to be greater than 3 for most compounds. The leaching and bioaccumulation of the contaminants highlight the need to better understand implications for downstream aquatic systems and broader ecological impacts.

4.18.B.T-04 Treating short-chain PFAS in landfill leachate with a novel coagulation and flocculation with sorption approach

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Landfill leachate contains high concentrations of per- and polyfluoroalkyl substances (PFAS), including short-chain compounds which are challenging to remove. Due to their unique physico-chemical properties, PFAS are difficult to eliminate with a single treatment method. Granular activated carbon (GAC) is commonly used for PFAS removal from landfill leachate, but its effectiveness and cost-efficiency are often reduced by competition from other leachate constituents, such as organic matter. Biochar has emerged as a more sustainable and potentially cost-effective alternative to GAC, offering a lower environmental footprint. This study evaluates a two-step treatment method combining coagulation and flocculation in step 1 with sorption to biochar in step 2 to address these limitations. Real world leachate samples from Arctic and Southern Norwegian landfills are used. The hypothesis being that residual flocculant, after coagulation and sedimentation, interacts with short-chain PFAS in the solution to form complexes, which are more readily adsorbed onto the mesoporous biochar. Coagulation and flocculation (step 1) were shown to reduce organic matter content in leachate, mitigating

interference with subsequent sorption. Preliminary results from step 1 indicate a removal efficiency for the ultrashort-chain PFAS TFA ranging from -39.2 to 49.8 %. For the short-chain PFAS compounds, removal efficiencies were -2.1 to 83.1 % for PFPeA and 0 to 98.4 % for PFBS. The long-chain PFAS PFOA and PFOS showed removal efficiencies of -0.8 to 97.6 % and 33.9 to 99.7 %, respectively. Additional sorption to biochar (step 2) resulted in a total removal efficiency of -38.1 to 52.0 % for TFA, 45.1 to 98.6 % for PFPeA, 96.3 to 99.7 % for PFBS, 99.1 to 99.7 % for PFOA, and 99.3 to 99.7 % for PFOS, indicating a chain length dependency. There was a distinct difference in removal efficiency between the spiked water and real landfill leachates, suggesting that complex interactions occur during leachate treatment, which enhance removal effectiveness compared to the spiked water. Preliminary results also indicate some attenuation by organic material content for the spiked water. Using this approach addresses critical gaps in PFAS remediation, particularly as it provides an improved method efficiency for short-chain PFAS removal. The outcome of this study will pave the way for better treatment of the short-chain PFAS without having to use very expensive and specialist methods.

4.18.B.T-05 Ion-pair enhanced coprecipitation for the treatment and removal of PFAS from water

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PFAS are a ubiquitous environmental contaminant that have been detected in a variety of matrices and in nearly all corners of the world. Often referred to as “forever chemicals,” this class of compounds are highly persistent in both the environment and in biological systems due to the strength of their carbon-fluorine bond. Treatment of contaminated waters has been performed through a variety of techniques, including granulated activated carbon adsorption, ion-exchange resins, supercritical water oxidation, and foam fractionation. Despite the number of options available for treatment, none have demonstrated a robust ability to treat the full range of PFAS. This is because the classification of “PFAS” is an extremely broad category with a high degree of variation in physical properties due to their varying functional head groups and carbon chain tail lengths. This work seeks to develop a treatment system which utilizes the high surface area of a colloidal structure $\text{Fe}(\text{OH})_3$, as well as a cationic surfactant to form ion pairs with a variety of PFAS, thus reducing the solubility of both the surfactant and PFAS to promote adsorption onto the colloidal material. A variety of cationic surfactants varying by both carbon chain length and polar head group were investigated, as well as varying concentrations of surfactant, PFAS, and iron hydroxide. Our work has demonstrated that longer chained cationic surfactants (C16) improve PFAS adsorption than shorter chained surfactants of the same functional head group when tested at the same concentration. Additionally, employing a more polar head group as the cationic surfactant seemed to dramatically improve adsorption of PFAS with respect to sediment-water partitioning coefficient. This seems to indicate that the ion-pair interaction is not occurring as a 1:1 interaction of cationic surfactant to PFAS, but instead relies on a proposed aggregate formation of cationic surfactant on the surface of our colloidal material, iron hydroxide. This is supported by further experiments showing a decrease in removal efficiency when a critical cationic surfactant concentration is surpassed, as well as a plateauing of performance when iron hydroxide concentration surpasses a different critical concentration. Further work will explore the use of other colloidal materials that are more pH-tolerant than iron hydroxide, as well as the effect of increasing concentrations of short chained cationic surfactant.

4.18.P-Th Occurrence Fate and Effects of Contaminants in Domestic, Agricultural, Landfill and Industrial Waste

4.18.P-Th-085 What are the sources of PFAS in San Francisco Bay area municipal wastewater? A conceptual model

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The class of per- and polyfluoroalkyl substances (PFAS) are of high concern in San Francisco Bay due in part

to fish tissue concentrations that exceed some human health risk thresholds. In the highly urbanized San Francisco Bay region, which has relatively little history of PFAS-related manufacturing and limited PFAS contamination in drinking water, municipal wastewater and urban stormwater runoff are likely the primary pathways for PFAS to reach the Bay. Municipal wastewater effluent is considered a significant pathway for PFAS to reach aquatic environments, as conventional treatment technologies are generally ineffective in removing PFAS from wastewater. The sources of PFAS to municipal wastewater remain relatively poorly characterized. A previous study of San Francisco Bay Area wastewater identified domestic/residential discharges as major contributors to PFAS in wastewater. The preponderance of PFAS in residential discharges suggests consumer products (directly and indirectly via human waste) are likely leading to the bulk of PFAS entering municipal wastewater. We developed a comprehensive conceptual model to establish all relevant PFAS product use patterns and other PFAS sources with potential for transport to San Francisco Bay via municipal wastewater. This conceptual model will inform future product testing for PFAS and the design of future Bay Area municipal wastewater monitoring and management actions.

4.18.P-Th-086 What are the sources of PFAS in San Francisco Bay area urban stormwater runoff? A conceptual model

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The class of per- and polyfluoroalkyl substances (PFAS) are of high concern in San Francisco Bay due in part to fish tissue concentrations that exceed some human health risk thresholds. In the highly urbanized San Francisco Bay region, which has relatively little history of PFAS-related manufacturing, municipal wastewater and urban stormwater runoff are likely the primary pathways for PFAS to reach the Bay. (In California, like most of the USA, urban stormwater runoff usually flows untreated directly to surface waters, through separate storm drain systems designed to prevent flooding and avoid sanitary sewer overflows.) Monitoring PFAS in these two pathways has shown urban stormwater runoff concentrations to be commensurate with those from wastewater effluent. Because the region's annual stormwater runoff volumes exceed annual total wastewater discharges, this suggests that urban runoff contributes significantly to the quantity of PFAS in the Bay ecosystem. The sources of PFAS to stormwater runoff remain relatively poorly characterized. Stormwater monitoring data, some of which involves sampling upstream of areas potentially affected by historic contamination (e.g., use of fire-fighting foams) suggest PFAS-containing products as likely sources for detected PFAS. We developed a comprehensive conceptual model to establish all relevant PFAS product use patterns and other PFAS sources with potential for stormwater transport. This conceptual model will inform future product testing for PFAS and the design of future Bay Area urban stormwater runoff monitoring and management actions.

4.18.P-Th-087 Characterization of transboundary pollution and emerging environmental contaminants in the Tijuana River Valley

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The Tijuana River has been a major source of pollution in the United States-Mexico border region for over 90 years. Rapid population growth and industrial expansion have overwhelmed local wastewater infrastructure, leading to the discharge of polluted runoff into the Tijuana River, where harmful chemicals and pathogens are carried through coastal communities, posing public health risks. While fecal indicator bacteria and human viruses have been detected in past research and are routinely monitored in the Tijuana River Valley, the full scope of chemical pollutants and their sources is less understood. Nontargeted chemical analysis can be used to identify a wide array of chemicals present in environmental samples, including previously unknown and emerging compounds of concern. The current study conducted nontargeted analysis of water, sediment, and air samples taken from the Tijuana River Valley, with the goal of generating a comprehensive list of chemical

contaminants that can be used to investigate sources of pollution. Coupled water and sediment samples were taken from various locations in the Tijuana River and nearby beaches during the dry season of 2024, and compared to a control site in La Jolla, California. Air samples were taken using silicone wristbands as passive samplers at each location. Nontargeted chemical analysis was conducted using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass-spectrometry (GC×GC/TOF-MS), then collected mass spectra were searched for similar mass spectra in the National Institute of Standards and Technology mass spectral library for compound identification. Tentatively identified compounds will be prioritized based on occurrence, abundance, and hazard ranking. Hazard ranking will be based on bioaccumulation, persistence, and toxicity data obtained from the United States Environmental Protection Agency CompTox dashboard. In preliminary data analysis, we found numerous chemicals associated with industrial and municipal waste, like pharmaceuticals, pesticides, solvents, and human metabolites. By comparing chemicals detected in water, sediment, and air samples, this study provides insight into the fate and transport of Tijuana River pollutants across environmental compartments and informs human health risk analysis for the border community.

4.18.P-Th-088 Effect of pH and water temperature on PFAS removal in landfill leachate by foam fractionation using protein skimmer

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Per- and polyfluoroalkyl substances (PFASs) are frequently detected in landfill leachate due to their widespread use and subsequent disposal in landfill. Therefore, effective treatment technologies for PFASs in leachate are essential to decrease their environmental impact. Activated carbon adsorption, ion exchange, reverse osmosis (RO) membrane separation, electrochemical oxidation treatment, and foam fractionation treatment are considered as the effective PFAS removal technology. However, the specific characteristics of PFAS removal from landfill leachate by foam fractionation remain unclear. The aim of this study is to estimate the effect of pH and water temperature on PFAS removal from landfill leachate by foam fractionation. Untreated leachate from an industrial waste landfill in Japan served as the feed water for foam fractionation. Treatments were conducted in a 10 L container filled by 5.2 L untreated leachate using a protein skimmer (Genesis, DC500) over a 30-minute period, with samples taken at 0, 5, 10, 15, 20, and 30 min. Experiments were carried out at pH 4, 7, and 10 at 20 °C (n=3); additionally, at pH 7 and water temperatures of 10 °C and 30 °C (n=1). No auxiliary agent for foam fractionation such as surfactants were added. The separated foam which returned to liquid was collected after 30 minutes. Thirty-four PFAS congeners in both water and foam condensate were quantified by LC–MS/MS. Most PFAS congeners exhibited pseudo-first-order removal kinetics during foam fractionation. The combined mass of PFAS in the treated water and the collected foam accounted for over 80% of the initial PFAS mass, indicating that foam transfer was the primary removal mechanism. For perfluoroalkyl sulfonic acids (PFSA), PFPeS, PFHxS, PFHpS, and PFOS achieved > 80% removal after 30 minutes. Similarly, for perfluoroalkyl carboxylic acids (PFCA), PFHxA, PFHpA, PFOA, PFNA, and PFDA each exhibited > 80% removal. Thus, the results suggested that longer carbon chain PFSA (C5<) and PFCA (C6<) were more easily removed by foam fractionation treatment. No significant effect of pH or temperature on final removal efficiencies was observed. However, rate constants (k) derived from 0–10 minutes showed a positive correlation with both increasing pH and temperature. Therefore, for foam fractionation treatment, control of pH and water temperature may be necessary to shorten the treatment time. **Disclaimer/Disclosure:** This research was supported by the Environment Research and Technology Development Fund [3-2303:JPMEERF20233003] of the Environmental Restoration and Conservation Agency (ERCA) of Japan.

4.18.P-Th-089 Identifying and prioritizing the unknown chemicals in onshore produced water

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A key barrier to risk assessment and beneficial re-use of produced water is the unknown array of chemicals that

may be potentially present. These chemicals can be broadly grouped into a limited set of natural, geogenic contaminants that co-occur in water where oil and gas are produced and a large list of possible chemicals that are used in the extraction process. For geogenic contaminants such as chloride, hydrocarbons, ammonia, hydrogen sulfide, hydrogen cyanide, and metals, toxicity data and well-established analytical methods are available to support environmental risk assessment. However, for process chemicals there is considerable uncertainty in the identity, ecotoxicity and associated analytical methods. The potential impacts of unknowns can be evaluated several ways, including the use of whole effluent toxicity and other chemical characterization techniques. While this serves as a logical strategy, there remains interest in identifying and prioritizing the specific chemicals that may be present in PW. In the present work we evaluated existing compilations of chemicals found in PW to understand the scope of the chemical space and then compiled or estimated (eco)toxicity thresholds for those constituents, either individually or as categories (e.g., hydrocarbon substances). This was done to support prioritization, and eventual risk assessments of constituents in raw and treated PW. This scope of this activity included both geogenic constituents, and oil field additives used in the operations. To gain further insights an analysis of FracFocus was performed between 2023 to 2024 for company operations in TX and NM. This query resulted in 64,356 records across 1,561 wells resulting in a set of 79 unique CAS Nos. In ~10% of the records, a CAS No was not provided and either an unambiguous chemical name or ambiguous/proprietary ingredient was specified. To estimate exposure concentrations, the amount of chemical reported in the fracturing fluid across records was evaluated. To estimate hazard thresholds, acute HC5s were derived using toxicity models or empirical data building on the initial chemical space mapping activity. Thus, for each process chemical the relative risk was determined by comparing estimated exposure in the actual fluid and hazard concentrations. The results of this analysis document the range of likely constituents in the raw and treated PW and can help prioritize which chemicals for further evaluation.

4.18.P-Th-090 Comprehensive characterization of PFAS in compost samples by both target and non-target analysis

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Per- and polyfluoroalkyl substances (PFAS) are fluorinated carbon chain compounds often referred to as 'forever chemicals' due to their extreme persistence in the environment. Once released into the environment, PFAS can undergo minimal degradation and transformation, usually into terminal perfluoroalkyl carboxylic acids (PFCAs). Their ubiquitous occurrence in the environment poses potential human health risks due to uptake by flora and fauna, accumulation and transport in surface and groundwater, and adsorption to organic matter. As a result of their use in consumer products (e.g., waterproof textiles, electronics, plastic packaging), they are commonly found in solid waste streams and municipal wastewater. In this study, a comprehensive characterization of the occurrence and concentrations of PFAS in compost samples was conducted using both targeted and non-targeted analysis. The compost samples were derived from biosolids from wastewater plants collected at different stages of compost preparation (i.e., before screening, final product, wood chips before and after final composting). A methodology for assessment of PFAS in compost was developed and validated using an automated solvent extraction protocol followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Non-targeted analysis (NTA) was performed on an Orbitrap Astral Mass Spectrometer in full scan (m/z : 100-800) and data-dependent acquisition (m/z : 70-800, normalized collision energy of 30) for screening of PFAS that are not traditionally monitored through targeted methods. The NTA workflow incorporated data processing with Compound Discoverer, enabling more accurate and specific characterization of the PFAS chemical composition in complex matrices. A Total Oxidizable Precursor (TOP) assay was also used to enhance detection of PFAS and identify precursors that are undetectable through targeted methods.

4.18.P-Th-091 Optimizing complementary fluorine-based workflows to characterize known and "total potential PFAS" in compost

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Expanding composting programs support waste recycling goals and divert organics from landfills, producing a valuable soil amendment. However, the presence of persistent contaminants like per- and polyfluoroalkyl substances (PFAS) compromises the intended land-application of compost as a more sustainable fertilizer and poses a human health concern. We know that certain wastes (biosolids, compostable products) may contribute disproportionately to PFAS burden in composts and that concentrations of known terminal PFAS (candidates for regulation) can increase as compost ages, suggesting transformation of unknown precursor PFAS. For compost producers, users, and regulators, there is a need for a more comprehensive understanding of the greatest contributors of PFAS in composts and of the total potential PFAS land-applied with compost not captured by current targeted mass spectrometric workflows. Crowd-sourcing was employed to collect ~300 compost samples from active facilities across the US, including a diversity of feedstock materials, ages, and recycling methods. Samples were analyzed for both 66 known PFAS via ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) and for total fluorine (TF) content via particle-induced gamma ray emission (PIGE) spectroscopy. TF data complements targeted mass spectrometry by estimating an upper boundary of "total potential PFAS" present in composts, including currently unknown precursor PFAS. The PIGE method for analyzing TF is optimized here for soil-like matrices as a rapid screening approach. Comparison of known PFAS and TF in compost samples varying from fresh to mature ages will also advance our understanding of the PFAS mass balance throughout the composting process and help guide subsequent nontargeted analyses via high resolution mass spectrometry to expand the targeted PFAS method for composts. Feedstocks with high concentrations of PFAS may require a different management strategy that does not apply these persistent pollutants to crops that may serve as a route of exposure for humans.

4.18.P-Th-092 Oxytetracycline removal using a novel flow-through coupled adsorption and electrochemical destruction system for shrimp farm wastewater

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The antibiotic oxytetracycline is widely recognized for its effectiveness in treating diseases in shrimp aquaculture and is commonly used by farmers across Indonesia. However, its routine application is often not accompanied by proper wastewater management, resulting in the release of antibiotic residues into coastal waters. Currently, many shrimp farmers employ a one-time filtration method using basic fabrics, which is ineffective in removing dissolved antibiotic residues. Other treatment methods have also shown to fail to destroy these residues, allowing contaminants to persist in the environment. There is therefore an urgent need for a novel, destructive wastewater treatment technology that can effectively remove oxytetracycline and other antibiotics used from these wastewaters. Such a system must meet stringent environmental regulations, minimizing waste generation and reducing operational/logistical costs without producing toxic by-products. To address this issue, a laboratory based single pass flow-through system, which combines adsorption with electrochemical regeneration, has been developed to replicate the traditional water treatment approach used by farmers while enabling more effective removal of dissolved antibiotics before they enter the surrounding ecosystems. However, it remains unclear to what extent this set up can be used to effectively remove antibiotics. In addition, considering that effluent conditions (such as pH, salinity and temperature) in these wastewater systems vary there is a need to assess the impact of these altering conditions on the effectiveness of the system as well. Preliminary analyses indicate that this system can successfully reduce the amounts of oxytetracycline, without the generation of any harmful by products, over a long period of time (nearly 800L treated) without the need to replace the adsorbent material. However, the removal efficiency is directly linked to the loading rate, indicating that it drops significantly at higher antibiotic loadings. Effluent conditions can have a significant impact on the removal efficiency and particularly increasing salinity enhances the removal

efficiency. Combined this indicates that this novel flow through system may be particularly well-suited to treat shrimp farm wastewaters and could prohibit to discharge antibiotics in the environment.

4.18.P-Th-093 Sustainable destruction of phenol and azo dyes using a novel continuous flow-through hybrid coupled adsorption and electrochemical destruction system

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Access to clean water remains one of the most critical environmental challenges, with over 2.2 billion people lacking safe drinking water globally. Industrial activities continue to discharge hazardous organic contaminants such as phenol and azo dyes into aquatic environments, posing significant risks to both human and ecological health. Conventional wastewater treatment technologies are often energy-intensive, costly, and fail to destroy these compounds, allowing contaminants to persist in the environment. This underscores the urgent need for novel, sustainable wastewater treatment systems that meet strict environmental regulatory requirements and are capable of efficiently removing and degrading contaminants without generating toxic by-products, while minimizing waste, energy use, operational and logistical costs. A recently developed process that combines adsorption with electrochemical regeneration (oxidation) could provide solution and has already shown effective removal of various organic pollutants including antibiotics and pesticides without producing harmful by-products. However, its performance under continuous flow conditions, which better simulate real-world applications, remains underexplored. Most prior assessments have focused on batch-style systems. This study addresses this gap by assessing a novel laboratory-based continuous flow-through system to evaluate its removal efficiency for two model organic contaminants: methyl orange (an azo dye) and phenol. The system was operated for over 1,000 hr under alternating adsorption-only and electrochemical regeneration phases without requiring replacement of the adsorbent media. Under optimal operating parameters, approximately 75% removal of methyl orange and complete removal of phenol (below detection limits) were achieved at loading rates of ~15 mg/h and ~3 mg/h, respectively. Notably, no toxic by-products were observed. Removal efficiency declined when contaminant loadings exceeded these thresholds, indicating a limit to the system's capacity. Additionally, increasing energy input beyond baseline levels yielded minimal gains in performance, emphasizing the importance of process optimization over energy intensification. These findings demonstrate the long-term potential of hybrid adsorption–electrochemical systems for continuous degradation of hazardous organic contaminants, offering a scalable and energy-efficient solution to industrial and municipal wastewater challenges.

4.18.P-Th-094 Estimation of 6:2 fluorotelomer alcohol substance flow contained in greaseproof paper and its emissions from waste treatment processes in Japan

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Greaseproof paper, commonly used in food contact materials and other paper-based packaging, is known to contain PFAS due to its oil-resistant properties. In Japan, this paper is typically disposed of as municipal solid waste (MSW) or mistakenly mixed into recyclable paper waste, potentially entering MSW incinerators and refuse-derived paper and plastic fuel (RPF) production facilities. However, few studies have quantified PFAS emissions from these waste treatment facilities. This study aims to estimate the substance flow of 6:2 fluorotelomer alcohol (6:2 FTOH) from greaseproof paper in Japan and to assess associated emissions from waste treatment facilities. First, we estimated the proportion of greaseproof paper within household paper packaging waste using a composition survey. A total of 300 kg of household waste was collected, and approximately 56 kg of paper-based packaging was separated. Greaseproof paper was identified using an oil-resistance test. Based on this, a national material flow model was constructed. In the model, greaseproof paper disposed of as municipal solid waste was assumed to flow into various waste treatment facilities according to national waste treatment ratios. Greaseproof paper mistakenly mixed into recyclable paper waste was assumed

to enter RPF production, as it is unsuitable for standard paper recycling. We applied a representative 6:2 FTOH concentration from previous literature (13,000 ng/g, maximum case) to estimate the mass entering waste treatment. Emissions were estimated for both the original 6:2 FTOH in the paper and additional FTOH formed via degradation of side-chain fluorinated polymers (SFPs). In 2023, approximately 21,000-31,000 tons (95% confidence interval) of greaseproof paper were estimated to have been discarded in Japan. Over 90% was incinerated as MSW, although a portion was processed in RPF facilities. Despite smaller inflows, RPF-related emissions could be comparable to those from MSW incinerators due to higher assumed emission factors. Furthermore, accounting for degradation of SFPs significantly increased the estimated 6:2 FTOH emissions, especially from RPF production and landfills - by up to an order of magnitude compared to models that exclude polymer degradation. These findings highlight the importance of including polymer-derived contributions in PFAS emission inventories and call for further data collection on polymer degradation behavior and emission factors. **Disclaimer/Disclosure:** This research was supported by the Environment Research and Technology Development Fund (JPMEERF20243003) of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan.

4.18.P-Th-095 High throughput automation modifications to OECD 302B biodegradation assay using robotic interface and blue-tooth data handling

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The OECD 302B (Inherent Biodegradability: Zahn-Wellens/ EVPA Test) is a screening test for inherently biodegradable materials that uses activated sludge as an inoculum source and measures the biodegradation through analysis of dissolved organic carbon (DOC). This assay is a powerful tool for analyzing the fate and biodegradation potential of both small molecules and polymers used in various applications, including pharmaceutical, personal care, and industrial products that are released into domestic or industrial wastewater treatment facilities. In this assay, samples must be taken from the bottle at the sampling timepoint, filtered, and then analyzed with a total carbon analyzer (TOC) or CHNS machine. This process can be labor intensive and time consuming and therefore may be a limiting factor in the number of test materials that can be analyzed during a given trial. To mitigate this issue and make this assay higher throughput, a robot was designed in collaboration with Synchron, Etten-Leur, Netherlands. The setup for this system is five trays of 12 test bottles, arranged in a row along a lab bench. There is an overhead track on which the robotic sample arm moves down the row. The automated series for sampling is as follows: (1) The robot uses first a pH probe to record the pH into its internal computer; (2) the robot picks up a sample from the test bottle and then a filter (3) the filtrated sample is deposited into a test tube; (4) the filter is released into a disposal area (5) the needle is rinsed and cleaned; and (6) the bottle is refilled with DI water to its original amount. The study operated will then take the prepared test tubes samples and be able to place them directly to the TOC for analysis of the carbon content by TOC measurements. The OECD 302B analysis method is now fully operational by a robotic interface enhancing degradation measurements on a daily basis taking a sample aliquot every day while at the same time yielding comprehensive repeatable and reproducible results with lower labor cost and improved high throughput capabilities.

4.18.P-Th-096 Sorption behavior of perfluoroalkyl acids in Japanese andosol removed of organic matter or non-crystalline iron and aluminum hydroxides

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Soil is a major site of per- and polyfluoroalkyl substance (PFAS) contamination. PFAS migrate through the soil profile by repeated sorption and desorption, particularly onto soil components such as organic matter, iron/aluminum hydroxides, and clay minerals. Thus, understanding the sorption/desorption behavior of PFAS in soil is essential for understanding the environmental fate of PFAS. To understand more about how the individual soil components contribute to the sorption of PFAS, we performed a sorption experiment using

Japanese Andosol and four environmentally relevant perfluoroalkyl acids (PFAAs; PFOS, PFOA, PFHxS, and PFNA), which are a subgroup of PFAS. The Andosol contains organic matter, iron/aluminum hydroxides and clay minerals. Organic matter was removed from the soil by using sodium hypochlorite solution. Non-crystalline solids including iron/aluminum hydroxides and clay minerals were removed by using oxalate buffer solution (pH 3.0). A batch sorption test was performed using the untreated and treated soils and each PFAA solution of 5–100 ng/mL, and the mixtures were shaken for 48 h. For PFOS, the percentage contribution of organic matter to sorption of the compound was 59–68%, while that of non-crystalline solids was 27–33%. These results were determined by the difference in sorption percentages between the treated soils and the untreated soil. Among the other PFAAs, the percentage contribution of organic matter was also higher than that of non-crystalline solids. However, the percentage contributions differed among PFAAs. The longer the fluorocarbon chain length, the more readily the PFAA was sorbed onto organic matter, while the PFAA possessing sulfonic groups tended to be more readily sorbed onto non-crystalline solids compared with those possessing carboxyl groups. Furthermore, the difference in the length of the fluorocarbon chain, more than the difference in the hydrophilic functional group, markedly changed the percentage contributions of PFAA sorption onto the soil components. This study indicates that PFAA are more readily sorbed onto organic matter than onto non-crystalline solids. However, the sorption ability depends on the specific chemical properties of the individual PFAA, with shorter chain lengths leading to greater sorption onto non-crystalline solids and other soil components.

4.19.P-Mo Use of Chemical Hazard Assessments to Drive Informed Substitution in Alternative Assessments

4.19.P-Mo-049 Is personal care product safety skin deep? Using EWG's publicly available hazard assessments to streamline informed substitution

Kaley Beins, Hong Lin and Alexa Friedman, Environmental Working Group

As the personal care product (PCP) industry continues to innovate, the need for harmonized approaches to alternatives assessment has increased. However, time constraints and conflicting chemical hazard assessments (CHAs) have led to piecemeal attempts at ingredient replacement and formulation improvements. Skin Deep, Environmental Working Group's free, publicly available cosmetics database, provides the foundation for rapid, fit-for-purpose PCP alternatives assessment. While Skin Deep is a consumer education tool, its chemical hazard assessment scoring and product formulation information can be leveraged to support harmonized alternatives assessment. Skin Deep rates over 125,000 personal care products on a 1-10 score range based on hazard scores for 14,500 ingredients. Ingredient hazard assessments are based on data from authoritative bodies including ECHA, CIR, and EPA's CompTox as well as open literature reviews. These data are categorized into 11 endpoints: allergies/immunotoxicity, cancer, cellular/biochemical changes, ecotoxicity, endocrine disruption potential, irritation, neurotoxicity, occupational hazards, organ system toxicity, persistence/bioaccumulation, and reproductive/developmental toxicity. Data on absorption, additive exposure, and ingredient impurities can be factored into the score when available. Base ingredient hazard scores ranging from 0 to 100 are assigned according to endpoint severity and weight of evidence. The highest base score for each endpoint is used to calculate the ingredient raw hazard scores, which are then combined based on endpoint groupings to create the overall product hazard scores. To inform sustainable formulation, the Skin Deep ingredient hazard scores can be combined with functional use class and Skin Deep product-level formulation data to create preliminary alternative ingredient shortlists. In addition to the ingredient hazard scores, the CHA summaries on the Skin Deep ingredient pages allow for quick comparisons between endpoints of concern across multiple ingredients. We used this approach as a proof-of-concept to lay the groundwork for identifying suitable replacements for three personal care product ingredients: an antistatic conditioning agent, a hair-dye, and a denaturant. We found that while using Skin Deep CHAs as the basis for alternatives assessment works best when an ingredient serves only one function in a formulation, Skin Deep can be an effective starting place for driving informed substitution.

4.19.P-Mo-050 Hazard profiling and characterization supporting safe and sustainable by design workflows applied to chemicals and materials

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We describe the methodology and results we have obtained in case studies for the hazard profiling and characterisation step of the Safe and Sustainable by Design (SSbD) framework recently proposed by the European Joint Research Centre. This framework integrates hazard and risk identification at the earliest stages of product and process development, combining scientific approaches with innovative technologies to drive the design of inherently safer and more sustainable solutions. Our case study application includes applying SSbD to a) cosmetic formulations including micro and nano polymeric forms, b) alternative PFAS-free thinner coatings for textiles, c) consideration of biopolymers as alternatives to current polymers used in products such as automobiles, d) use of bio-sourced chemicals as alternatives to carbon in fibres and composites, and e) graphene materials. Valuable assistance in the hazard identification and characterisation work is obtained from data and scientific techniques to perform more accurate, ethical, and comprehensive assessments, embedded into SSbD decision-making workflows. To achieve this goal, full use of existing data, models and knowledge is leveraged into providing relevant information supporting assessment and decision goals between alternatives in early stage innovation. Such data is integrated with evidence-weighting and scoring schemes, including uncertainty, to reach initial decisions on alternatives and to plan for subsequent refinement phases. The findings include recommendations for generating the most meaningful and useful data to address gaps and uncertainty. We will demonstrate the methodology followed and questions arising from current case study work applying SSbD to chemicals, polymers and advanced materials including: Collection, extraction and curation of knowledge from literature, toxicology databases and safety data sheets; Demonstration and access to knowledge collection tools including resources we are sharing openly with the environmental science community; Hazard profiling of product formulations and ingredients based on existing knowledge; Knowledge infrastructure for characterization of materials including AI-assisted labelled image data analysis of experimental microscopy results; Use of biological data from New Approach Methods for enriching hazard characterization; SSbD Workflows documenting case study results. **Disclaimer/Disclosure:** This presentation includes work related to projects which have received funding from the European Union's Horizon Europe research and innovation programme under grant agreements for the following projects: SSbD4Chem (no. 101138475), ACCORDs (no. 101092796, and Biophenom (no. 101135107). UK participants in the projects receive funding from UK Research and Innovation (UKRI). Swiss participants in the projects receive funding from the Swiss State Secretariat for Education, Research and Innovation (SERI).

4.19.P-Mo-051 Evaluating hazard profiles for quantum chip fabrication chemicals

Sahar Shata and Julie Schoenung, Texas A&M University

Quantum computing is poised to revolutionize information processing; however, its practical realization presents substantial green engineering challenges due to the intrinsic toxicity, material complexity, and energy-intensive demands of fabrication and operation. From an alternative assessment perspective, these multiple attributes should be considered. As part of a comprehensive alternatives assessment study currently ongoing, this presentation will highlight the CHA component. The GreenScreen® for Safer Chemicals method is applied to evaluate hazard profiles of functional materials in quantum chips. As the foundational and experimentally variable element of a quantum processor, twelve quantum chips from superconducting, semiconductor quantum dot, and topological platforms are investigated. The superconducting structures (Al/AlOx/Al on silicon, Ta/Al/AlOx/Al on sapphire, Nb/Al/AlOx/Al/Nb on sapphire, and NbN/AlN/NbN on silicon) contain BM1-rated Al and include Nb and Ta LT-UNK metals, with poorly defined hazards, alongside BM2-rated oxides like AlOx and sapphire. For the quantum dot platform, the GaAs/AlGaAs heterostructure on GaAs relies on LT1-rated

GaAs and AlGaAs, highly toxic due to As content. InSb nanowires on Si similarly utilize LT1-rated Sb compounds. In contrast, Ge/SiGe heterostructures on Si represent a significant improvement: both Ge and Si-based alloys are not classified as high-hazard and exhibit lower toxicity. A greener configuration, Si quantum dots on CMOS, avoids BM1-rated functional materials. Topological quantum hardware is chemically burdened by its dependence on heavy-metal chalcogenides and intermetallic compounds required for spin-orbit coupling. The $\text{FeSe}_{0.4}\text{Te}_{0.6}$ on CaF_2 structure includes LT1-rated Te and FeSe, with a fluoride substrate of debated hazard. The Bi_2Te_3 thin films on NbSe_2 structure compounds this with BM1-rated Bi_2Te_3 and LTP1-rated NbSe_2 . InSb/Al nanowires on Si incorporate BM1-rated Al and LT1-rated Sb compounds, while Cr-doped $(\text{Bi,Sb})_2\text{Te}_3/\text{Nb}$ on SrTiO_3 exhibits one of the highest hazard profiles. Among the structures evaluated, the quantum dot platform presents the clearest case for CHA-driven material selection, with two structures offering a compelling pathway toward sustainable scalability, combining favorable hazard profiles with fabrication compatibility. When applied to functional materials, CHA guides informed substitution and directing quantum hardware development toward reduced toxicological hazard.

4.19.P-Mo-052 Fit for purpose: Design principles for pesticide alternatives assessment

Timothy Malloy, University of California, Los Angeles, School of Law

Informed substitution—the considered transition from chemicals of higher concern to safer alternatives—is now widely recognized as a central principle of modern chemicals policy. Just as risk assessment facilitates conventional risk management regimes, alternatives assessment (AA) enables informed substitution approaches. The sophistication and adoption of AA methods and tools rapidly accelerated in the public and private sectors over the last two decades, yet in the United States that momentum was largely lacking in the pesticide context. Even in the European Union, where comparative assessment of pesticides and their alternatives is required in various settings, AA implementation has notable shortcomings. This research has three aims. First, it maps the state of pesticide AA in the regulatory context and the state, federal, and international level. Second, using a mixed method approach, it uses a case study of California's state and county pesticide regulatory programs to develop a set of design principles for pesticide AA. Third, drawing upon those principles, it generates specific recommendations for AA frameworks fit for state and local pesticide regulatory programs, highlighting benefits, limitations, and resource needs for each.

4.19.P-Mo-053 Helping public agencies document mandates and evaluate risks associated with pest management

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Public agencies such as counties, parks districts, school districts, universities, and municipalities are increasingly tasked with developing and implementing integrated pest management (IPM) policies and pest-specific IPM plans to document their pest management strategies and specific tactics used on public properties. Purposes of such policies and plans, especially when made available as public documents, may be to inform constituents of the key pest systems managed by the agency, the decision-making processes associated with management and, commonly, pesticide products and application methods that may be used. Policies and plans may also be used to train agency staff, contractors, and volunteers on system-specific strategies and tactics. One approach used in Contra Costa County, a populous county in the San Francisco Bay Area with urban, suburban, agricultural, and natural ecosystems, has been to produce 'IPM decision documents' to document pest-specific or site-specific activities. Importantly, these documents list all public mandates associated with the IPM system, including those associated with public health and safety. Beginning in 2022, a new risk assessment tool was incorporated into these documents. Adapted from a risk assessment visualization device designed for agricultural pesticide use, this tool generates a "risk footprint" that includes various components of risk associated with pesticide use in urban areas: acute human hazard, chronic human hazard, known carcinogenicity / reproductive hazard, worker safety, bystander safety, environmental hazard to aquatic ecosystems, environmental hazard to terrestrial ecosystems, hazard to pollinator organisms, efficacy, direct cost, indirect

costs due to special training or equipment, and indirect costs due to litigation, bad public perception, or bad press. Considering these risks helps the agency to select and justify appropriate IPM tactics. Considering the public mandates for pest prevention and control as well as the complexity of IPM decision-making helps constituents to evaluate and understand the agency's IPM program and selected tactics.

4.19.P-Mo-054 Environmental safety assessment framework for consumer product ingredient portfolios

Shuo Yu, Kyle Fetters, Tina Cardona and Phyllis Fuchsman, Ramboll Americas Engineering Solutions, Inc.

Consumer products are a primary source of chemical exposures in the environment. Transparent and efficient identification of chemicals of high concern for ecological risk among thousands of ingredients used in product portfolios is a critical challenge. We have developed an environmental safety assessment framework for screening individual constituents used in raw materials and finished formulations of consumer products which either are disposed down the drain into municipal sewage treatment plants or directly enter the ambient environment. This framework is built upon established environmental safety assessment technical guidance of consumer products from various authorities in US and EU, and focuses on persistence, bioaccumulation, and ecotoxicity endpoints, as well as endocrine disruption and mobility hazard classes recently adopted by European Chemicals Agency (ECHA) under the Classification, Labelling and Packaging of Substances and Mixtures (CLP) Regulation. The framework includes 1) initial screening against regulatory chemical lists to quickly flag constituents with high hazard profiles; 2) hazard-level classification of each environmental fate or effect endpoint for the remaining constituents according to accepted scientific criteria; and 3) assignment of an overall environmental safety score for each constituent. Depending on the hazard assessment results, a higher tier environmental risk assessment to further characterize ecological exposures and risks after consumer product use can be recommended on a case-by-case basis. Application of the environmental safety assessment framework will be demonstrated for example product ingredients. The outcomes of the environmental safety assessment will facilitate future applicability for alternatives assessment and green labeling, with the ultimate goal to help move the consumer product industry toward the use of safer chemicals.

4.19.T Use of Chemical Hazard Assessments to Drive Informed Substitution in Alternative Assessments

4.19.T-01 From animal tests to algorithms: Transforming safety assessment with new approach methodologies

Amelie Ott, Dave Allen and Ryan Heisler, International Collaboration on Cosmetics Safety

Chemical hazard assessments (CHAs) serve as a foundational component of environmental risk assessment (ERA), providing essential information for understanding the inherent properties of chemicals and their potential environmental impacts. As ERA increasingly informs regulatory decisions, product development, and sustainability initiatives, the demand for robust, transparent, and comprehensive CHAs continues to grow. Traditionally reliant on animal testing and limited datasets, CHAs are now expanding by adopting New Approach Methodologies (NAMs). These innovative tools include, for example, silico modeling, in vitro assays, and read-across techniques. NAMs enable more inclusive and efficient assessments, thereby enhancing confidence in hazard characterization across a broader array of substances, including those in difficult-to-assess classes. This presentation will highlight how the integration of NAMs is advancing in the field of CHA and strengthening its role in environmental risk assessment. We will explore current best practices and the opportunities in applying NAMs across different scenarios. Together, these advancements indicate a shift towards more predictive, science-based environmental risk assessments, where NAM-enabled CHAs act as a vital tool for protecting ecosystems and promoting safer innovation.

4.19.T-03 Using hazard information to guide preservative selection in cosmetic product development

Sara Farahmand and Junyeol Kim, The Honest Company, Inc.

Preservative selection in cosmetic product development requires a balance between ensuring microbial integrity and minimizing potential risks to human health and the environment. This presentation outlines a practical

framework for incorporating hazard information into preservative decision-making during early formulation stages. Key hazard endpoints—including skin sensitization, systemic toxicity, endocrine disruption, and ecotoxicity—are evaluated using data from regulatory databases, peer-reviewed literature, and predictive toxicology tools. Emphasis is placed on ecotoxicological concerns such as aquatic toxicity and environmental persistence, which are increasingly relevant under evolving global regulatory expectations. Case examples illustrate how hazard-informed selection supports safer product design, facilitates regulatory compliance, and aligns with sustainability objectives. Integrating hazard data into formulation strategy enables more transparent, science-based decisions while mitigating downstream risks in product development.

4.19.T-04 Bridging gaps in hazard assessment: Exploring new approaches within California's safer consumer products program

Kimberly Hazard, Jen Jackson, Thomas A Bruton and Xiaoying Zhou, California Department of Toxic Substances Control

Chemical hazard assessments (CHAs) are a core component of Alternatives Analyses (AAs), a foundational requirement of California's Safer Consumer Products (SCP) Program. Under the SCP regulations, manufacturers of Priority Products must evaluate alternatives to harmful chemicals and submit AAs that compare hazard, performance, and economic viability. However, SCP has noted variability in the approaches and resources available for manufacturers to conduct CHAs, especially during the review of AAs for 6PPD in tires. Many smaller manufacturers lack in-house expertise in hazard assessment and face substantial barriers to conducting robust AAs. To address these challenges, SCP is exploring two new initiatives designed to improve CHA quality, reduce resource disparities across industry, and facilitate informed substitution. The first initiative involves exploring a collaborative AA in which SCP would lead elements such as the CHA, but meaningfully engage with and receive targeted information from industry for a given regulated product. This model could help level the playing field for smaller manufacturers while improving consistency and scientific rigor. SCP is evaluating the feasibility of this approach and may pilot it in the near future. The second initiative is the PEARL (Promoting transparency in Emerging Alternatives Recognition List), a non-regulatory program aimed at increasing chemical transparency for emerging alternatives. PEARL would recognize manufacturers that voluntarily submit hazard and performance data on chemical alternatives early in the regulatory process. While PEARL does not endorse safety or recommend substitutes, it aims to fill critical data gaps and increase visibility of potential viable alternatives, enabling manufacturers to more effectively evaluate alternatives during the AA process. SCP is developing this effort in consultation with interested parties, with plans to pilot in 2025. Both of these initiatives seek to support scientifically sound chemical substitution decisions and enhance transparency. Piloting of these approaches will help test their feasibility and further the successful implementation of informed substitutions in the state-level regulatory context.

4.19.T-05 A Washington State program to assist hair salons with transitioning to safer products

Shari Franjevic¹, Elinor Fanning², Holly Davies², Everetta Rasyid², Iris Deng¹ and Rebecca Bohannon¹, (1)Washington State Department of Ecology, (2)Washington State Department of Health

The Washington State Departments of Ecology and Health are supporting salons in their transition to using safer products, and manufacturers in formulating and certifying safer products, as part of implementing the 2023 Toxics-Free Cosmetics Act. The law also bans the use of some chemicals/chemical classes in cosmetics. This state law defines “cosmetics” the same way as the Food and Drug Administration (FDA) does, which includes beauty and personal care products such as shampoo, lotion, deodorant, and make-up. Our Safer Salons Partnership is a financial incentive program for small businesses in Washington to receive reimbursement for the purchase and use of safer products. The goals of this program include reducing salons' costs associated with switching to safer cosmetic products and reducing people's exposures to toxic chemicals. The Safer Salons Partnership is also compatible with a related technical assistance program from Ecology to help small manufacturers get certifications for their products. Our strategy is to define criteria for tiers of increasingly safer products, then identify external product recognition programs that meet our criteria for each tier, and finally to

create a list of eligible safer products. Challenges include lack of ingredient disclosure in professional products, lack of identified safer alternative products for some product categories such as hair straightening/smoothing products, and limited certified cosmetic products for many third-party certifications. This presentation will focus on the chemical criteria we developed to evaluate product recognition programs. The criteria are based on chemical hazard assessments. While the first tier focuses on a restricted substances list, the second tier is being developed with more in-depth assessment on hazard endpoints. The programmatic criteria identify programs that are transparent and minimize bias in evaluating products. We identified existing product recognition programs that meet the criteria, and we hope to engage more programs in the future. We are launching a pilot program in 2025 that will reimburse salons up to \$5,000 for buying these safer products. We did not identify any product recognition programs for professional products, so we are starting with products that are available to both consumers and professionals.

4.19.T-06 Use of a centralized repository of chemical hazard assessments for alternative assessments

Gabrielle Rigutto, Heather McKenney and Chris Bartlett, ChemFORWARD

ChemFORWARD is a not for profit organization (NGO) that houses a centralized online repository (i.e. the “Data Trust”) of chemical hazard assessments (CHAs) designed to support users in selecting safer chemical ingredient alternatives for use in products in a variety of industry sectors including cosmetics, electronics, food packaging, and plastic additives. CHAs in ChemFORWARD are completed by qualified toxicology firms (“Assessors”), peer-reviewed by independent toxicologists (“Verifiers”), and subject to ongoing quality assurance practices. New Approach Methods (NAMs), including read-across and grouping methods, are increasingly being used to address data gaps by Assessors when classifying the human health and environmental endpoints in ChemFORWARD CHAs. The cosmetics sector has been at the forefront of this effort, as the California Cruelty-Free Cosmetics Act, the European Cosmetics Directive, and other regulations are driving demand for toxicological assessments based on read-across and other NAMs due to prohibitions on animal testing of cosmetic products. CHAs are the foundation of alternative assessments (AA) and proactive design strategies and provide actionable information so that individuals can compare chemicals with similar functions and choose the ones with the least inherent hazard for their application. The use of read-across approaches and other NAMs in CHAs helps drive cost efficiencies when conducting CHAs by avoiding unnecessary testing to characterize hazard endpoints and leads to more complete characterization of chemicals, helping to limit regrettable substitutions. We will highlight the importance of CHAs as a foundation for alternative assessments, discuss overcoming challenges to filling data gaps, illustrate how CHAs can be utilized to make informed substitution decisions and proactively and routinely monitor ingredient portfolio risks using data analytics, and lastly show how further work on hazard classification could lead to predictive CHAs.

4.20.P-We General: Chemistry and Exposure Assessment

4.20.P-We-043 Characterization of fire ash from wildland urban interface (WUI) fire and multi-route exposure assessment

Cynthia Choo¹, David Kalafut¹, Marilyn Black¹, Timothy Nurkiewicz², Anand Ranpara², William Goldsmith², Thomas Batchelor² and Mark Wilson¹, (1)Chemical Insights Research Institute, (2)West Virginia University

Wildland urban interface (WUI) fire ash is the remaining residue after a fire has burned vegetation and synthetic built environment fuel. It may contain volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs) like flame retardants, polycyclic aromatic hydrocarbons (PAHs) and pesticides. These compounds can be emitted in the gas phase during combustion and may also be subsequently partitioned into household dust or onto surfaces. There are significant knowledge gaps associated with the chemical profiles of WUI fire ash and the corresponding human exposure risk through inhalation, ingestion and dermal exposure. My research aims to (i) characterize and quantify VOCs and SVOCs compounds in the fire ash and (ii) estimate human exposure risks through multi-route exposure modeling based on detected concentration. We utilized a novel laboratory-scale combustion system that simulates WUI fires using custom-formulated pellets. These pellets included four

regionally relevant wildland fuel species and synthetic building materials like polystyrene insulation, oriented strand board (OSB), and vinyl flooring. Collected ash samples were weighed into a stainless-steel desorption tube and analyzed by thermal desorption-gas chromatography-mass spectrometer (TD-GC-MS). Quantification was performed using multi-point calibration curves prepared from high purity standards of known concentrations. Exposure modeling was calculated using concentrations from the direct TD-GC-MS analysis, other parameters like ingestion rate, body weight and oral reference dose (RfD) were based on United States Environmental Protection Agency's (USEPA) recommendation. Preliminary results show styrene and plasticizer (phthalate and phthalate related compounds) detected in the ash from combustion synthetic building material. Styrene is classified probably carcinogenic to humans (group 2A) by International Agency for Research on Cancer (IARC). Children's modeled total ingestion and inhalation exposure is as high as 0.45 $\mu\text{g/kg-day}$. Bis(2ethylhexyl)-terephthalate is one of the detected plasticizers. When the modeled ingestion and inhalation exposure for children was calculated, the total exposure rate is 5.43 $\mu\text{g/kg-day}$. Detailed multi-route exposure models will be presented at the conference. Understanding the contaminants in the WUI fire ash would help mitigate health risks to the general public, while also providing guidance for safe clean-up practices.

4.20.P-We-045 Effects of sewage sludge-derived biochar amendment on pesticide residue adsorption and soil microbial activity

Jiwon Yang¹, Jinju Lee², Minhui Kim² and Goontaek Lee², (1)Seoul National University, South Korea, (2)Department of Agricultural Biotechnology, Seoul National University, South Korea

The efficient management of sewage sludge remains a critical environmental challenge. In this context, pyrolyzing sewage sludge to produce biochar (SB) for use as a soil amendment has emerged as a promising and sustainable alternative to conventional disposal methods. In addition, soil contamination by residual pesticides threatens the sustainability of agricultural systems. Among them, tricyclazole (TCZ) is a widely used fungicide characterized by high environmental persistence and the potential to disrupt soil microbial functions. This study aimed to evaluate the potential of SB to mitigate the microbial toxicity caused by pesticide contamination and enhance soil health. The adsorption characteristics of TCZ on SB produced under different pyrolysis conditions were analyzed, and the effects of SB on soil microbial communities and enzyme activities were examined through incubation experiments. SB was produced at 500°C or 700°C for 1 or 2 hr, and batch adsorption experiments were conducted with TCZ. Among the tested pyrolysis conditions, the SB produced at 700°C for 1 hr (SB700 1H) exhibited the highest TCZ adsorption capacity ($Q_m = 9.8 \text{ mg g}^{-1}$) and the lowest desorption rate (11.5%). Application of the Langmuir isotherm and the pseudo-second-order kinetic model adequately described the adsorption characteristics. Through FT-IR, XPS, and BET analyses revealed a chemical adsorption mechanism based on physical adsorption. In the incubation experiment using SB700 1H, the microbial community disrupted by high TCZ concentrations showed signs of partial recovery. The relative abundance of nitrogen-cycling genera *Nitrosospora*, *Nitrospira*, and *Rhizobium* increased following SB application. This suggests that SB may support microbial functionality by providing a niche, modifying soil pH and C/N ratios, and alleviating TCZ toxicity. In contrast, activities of certain soil enzymes, such as alkaline phosphatase and N-acetyl- β -D-glucosaminidase, were suppressed under SB-only treatment, possibly due to heavy metals or adsorption of enzymes or substrates onto SB surfaces. These findings demonstrate the multifunctional potential of SB not only for immobilizing residual pesticides through adsorption but also for stabilizing the microbial community structure and partially restoring ecological functions. This dual role highlights SB as a sustainable soil amendment that integrates pollutant mitigation with biological resilience in contaminated agroecosystems. **Disclaimer/Disclosure:** This work was supported by the National Research Foundation (NRF) grant funded by the Korea government (MSIT)(RS-2024-00405420). This work was also supported by Knowledge-based environmental service Program funded by Ministry of Environment.

4.20.P-We-046 Biosensor for detection of mycobacterium tuberculosis antigen peptide

Sam Li, NUS, Singapore

In this study, we designed and investigated the feasibility of a capacitive biosensor based on molecularly

imprinted polymers (MIP) for detection of mycobacterium tuberculosis antigen peptide, CFP-10. This is accomplished by altering a polysiloxane matrix to function as a tuberculosis (TB) detection tool. The MIP sensor's performance was evaluated using capacitive touch-sensing technology, which revealed a respectable linear response and a limit of detection (LOD) of 60 ppb. After conducting selectivity assays to ascertain the MIP's affinity for CFP-10, an imprinting factor of 2.6 ± 1.3 was successfully obtained. The MIP-based biosensor for CFP-10 is characterized by its portability, low cost, and quick response time, all of which meet the needs of a useful point-of-care medical device. Thus, the study is a step toward enabling more rapid and effective TB tests, opening the door for additional developments in the field.

4.20.P-We-047 Anionic and cationic polar pesticides analysis using a novel mixed mode LC column

Stephanie Marin, Phenomenex

Polar pesticides play a significant role in modern agriculture due to their affordability and high efficiency. However, their widespread use raises concerns as these pesticides are associated to the food chain through various pathways. Glyphosate, one of the most extensively discussed polar pesticides, has been found in numerous studies to be present in soil, crop products, animals that consume these products, humans, and even in freshwater sources. The emergence of concerns regarding their toxicity and potential carcinogenic effects highlight the importance of developing accurate and sensitive analytical methods to detect and quantify these compounds. Furthermore, it is particularly challenging to analyze polar pesticides due to their unique physicochemical properties, which encompass both anionic and cationic groups, making them an extremely difficult group of molecules study. Traditionally, two different separation modes are employed to analyze the positive and negative groups, necessitating the use of multiple columns. In this study, we present two integrated fast and robust methods for separating a suite of anionic and cationic pesticides using a single column. The Luna polar pesticides column is employed for analyzing anionic/negative polar pesticides in a reversed phase mode; while it is utilized for analyzing cationic/positive polar pesticides in a hydrophilic interaction liquid chromatography (HILIC) mode. Additionally, we demonstrate the equilibration time for each method and the impact of switching between the negative and positive modes on the precision and reproducibility of the analysis. By simplifying the analytical process and minimizing the need for multiple columns, our method offers a practical solution for accurately detecting and quantifying anionic and cationic pesticides.

4.20.P-We-048 Using the air liquid interface in vitro system to investigate the respiratory hazard of irritant volatile organic compounds

Olivia Lampe, Weihsueh Chiu and Natalie Johnson, Texas A&M University

Utilizing new mobile-monitoring air quality data, the Johnson lab identified common volatile organic compounds (VOCs) emitted in the air across multiple environmental disasters. A limitation of current human hazard assessment is insufficient respiratory toxicity data for these and numerous other VOCs emitted following disasters, at an individual and mixture level to inform hazard assessment of emissions at disaster sites. We investigated adverse effects of individual VOCs to perform future concentration-addition risk assessment of recreated mixtures detected at these environmental disaster sites. Human bronchial epithelial cells (16HBEs) were cultured at the air liquid interface (ALI) and exposed to the VOCs acrolein, formic acid or filtered air in a CelTox Sampler exposure system for two hr to replicate an acute inhalation exposure. The cells were then moved to an incubator and allowed to recover for 24 hr before cell lysate and culture media were collected for analysis. LDH activity, indicative of cytotoxicity, significantly increased at the highest doses tested for both VOCs. An increase in barrier permeability was observed in cells exposed to 1000 and 3000 ppb acrolein and increased in a dose-dependent manner in cells exposed to formic acid. Acrolein and formic acid induced pro-inflammatory signaling and upregulation of HMOX-1, a gene indicative of oxidative stress. Benchmark concentration (BMC) modeling demonstrated that in vitro acrolein data produced a BMCL (benchmark concentration lower confidence limit) value that supported OSHA's 8-hr permissible exposure limit (PEL) as conservative. Additionally, BMC modeling highlighted a critical gap in regulation for acute in vitro formic acid data. Collectively, findings demonstrate that the ALI cell culture model is a valid in vitro tool to investigate

VOC-induced adverse effects on the human lung and support regulatory decision-making. These data can inform future studies investigating the hazard assessment of these VOCs as a mixture to better replicate and understand human respiratory hazard following environmental disasters. **Disclaimer/Disclosure:** This work was funded by NIEHS grant P30 ES029067 with additional support from P42 ES027704 and R01 ES028866, with trainee support from T32 ES026568.

4.20.P-We-049 Simulation of chemical transfer in the human airway following aerosol deposition using a fugacity model incorporating CFD

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The pharmacokinetics of inhaled chemicals within the human airway are crucial for the safety assessment of aerosol products. We previously developed a model to simulate the deposition of inhaled aerosol particles and their clearance through mucociliary transport on the realistic detailed human airway mesh reconstructed from computed tomography images of a 35-year-old Asian male. In this study, we aimed to construct a new model to describe the transfer of chemicals deposited on the airways from mucus, through epithelial tissue, to blood. Our model is based on OpenFOAM, an open-source software widely used for various computational simulations. In this study, we utilized its functionality for Computational Fluid and Particle Dynamics (CFPD) to simulate fluid-particle interactions. The behavior of airflow and aerosol particles in the airway was predicted using the standard solvers pisoFOAM and icoUncoupledKinematicParcelFOAM, while the clearance process was modeled using pisoFOAM, which was customized with our own implementation of integrated with the advection-diffusion equations for chemical substances. In addition to this clearance model of pisoFOAM, we incorporated equations based on the the Fugacity model (level IV), which is used for non-equilibrium and unsteady state system to represent the chemical transfer between three compartments (mucus, cells, and blood) in the airway. The mass transfer coefficients for each compartment required by the Fugacity model were determined by applying a chemical substance to the in vitro 3D cell model MucilAir™ and measuring the concentration in each compartment over time. Finally, we integrated the transfer processes derived from the fugacity model with the deposition and clearance processes simulated by CFPD to describe the systemic transfer of chemicals in aerosols, from deposition in the human airway to systemic circulation. This model is expected to enhance our understanding of the behavior of inhaled chemicals and contribute to improving safety assessment methodologies.

4.20.P-We-050 Regional trend analysis of copper in California watersheds

Randi Barton and Bushra Khan, University of California, Davis

Trace heavy metals are essential nutrients for most plants and animals but can be toxic at high concentrations. With increasing anthropogenic use of metals, elevated concentrations are expected to be deposited in sediments. Copper is one of the most widely studied metal contaminants and is highly toxic to several aquatic species. Copper is used in numerous industrial, residential, and agricultural applications. Under the Surface Water Ambient Monitoring Program (SWAMP), the Stream Pollution Trends Monitoring Program (SPoT) was initiated in 2008, with goals of determining long-term statewide trends of stream contaminant concentrations and subsequent environmental effects. Copper is one of several targeted analytes monitored statewide across 90 sites annually. Though these data have previously been used to identify stable statewide trends, California varies widely across regions in land use, climate, and topography. Regional trend analyses can identify trends that are missed at a statewide scale but are critical to environmental management. Here we present regional and site-specific sediment copper trends in California between 2008-2024, identified using Mann-Kendall analyses, with an emphasis on land use relationships and regional monitoring priorities. Increasing trends in sediment copper have been identified at several urban sites over the years. In addition, some sites dominated by agricultural influences also show temporal trends requiring sustained monitoring. Though none of the sediment

copper concentrations measured above established LC50 values for aquatic indicator species relevant to the program, these data play a critical role in informing monitoring strategies about effectiveness of changing regulations (e.g., The Brake Pad Law (SB346)), site prioritization, and implications of land use changes on contaminant use and transport. Spatiotemporal analyses at different scales, such as presented in this work, facilitate improved understanding of contaminant sources, fate, and equip monitoring programs to better address management needs.

4.20.P-We-051 Spatial trends and land use relationships of pyrethroid-driven toxicity in California watersheds

Katherine Molinari and Bushra Khan, University of California, Davis

Pyrethroid insecticides are widely used across California and are offered as commercial pest control options. Widespread use of pyrethroids, particularly bifenthrin, in urban landscapes has contributed to surface water pollution in freshwater habitats. Pyrethroids have been quantified in California waterbodies at concentrations known to be toxic to aquatic organisms. Elevated pyrethroid burdens in freshwater systems can adversely impact ecosystem health and community structure leading to degradation of water quality. Under the Stream Pollution Trends Monitoring Program (SPoT) of the California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP), pyrethroids have been monitored annually statewide since 2008. One of the goals of the program is to identify contaminant and toxicity trends and their relationships to land use across California. The analyses presented here serve the program by creating visualizations relevant to management and information needs. Sediment pyrethroid monitoring data and amphipod toxicity results collected for 40 sites between 2010 and 2022 were spatialized using ArcGIS Pro in the context of land use data obtained from USGS National Land Cover Database. The sites used for these analyses represent seven out of nine regional water boards of California. Sites were delineated at 5 kilometers based on site-specific drainage areas using digital elevation models and National Hydrologic Dataset Catchment data, and sums of eight pyrethroids were calculated and carbon corrected to obtain toxic units (OC TUs) for each site. Urban areas including San Diego, Los Angeles and San Francisco Bay show elevated median pyrethroid concentrations, amphipod toxicity as well as increasing trends of pyrethroid sums and OC TUs. These analyses inform site prioritization that supports management and mitigation efforts. Spatial analyses, such as presented here, are a valuable tool that informs monitoring programs about areas of concern and integrates relevant information that contextualizes monitoring data for managers and the public. Continued monitoring with inclusion of mapping in ArcGIS Pro also equips the program to examine the effectiveness of current legislation surrounding pesticide usage.

4.20.P-We-052 A single particle ICP-MS method for elemental characterization of airborne environmental particulates

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Environmental particulates invisible to the human eye pose risks to air quality and the overall public's health. Traditional chemical analyses can provide important information on contaminant identification and concentration, but some methods fail to offer comprehensive data on the characteristics of individual particles, such as their size and elemental composition. While inductively coupled plasma mass spectrometry (ICP-MS) is a valuable tool for bulk elemental analysis, it does not provide the high resolution required for detecting and analyzing single particles that most often fall into nanometer size range. Additionally, ICP-MS is not regularly utilized as a tool for airborne particulate analysis of single particles. This study aims to develop a single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) method to provide individual elemental characterization of airborne environmental particulates. An established validation of SP-ICP-MS using dissolved gold and gold nanoparticle standards with known concentrations and sizes acts as the foundation for building a validated air sampling technique in this study. This active-sampling method specifically tailored to nanoparticles will provide more comprehensive and detailed data for environmental monitoring and risk

assessment due to its ability to detect the smallest trace of harmful environmental particles containing heavy metals like lead, arsenic, and vanadium. The development of this SP-ICP-MS method has the potential to change the way we monitor air pollutants, particulate matter, and other environmental contaminants by allowing for more precise, particle-level analysis. **Disclaimer/Disclosure:** This research was funded by UL Research Institutes, and the authors declare no conflicts of interest.

4.20.P-We-053 Evaluating iron nanogeochemistry within the Nooksack River using single particle inductively coupled plasma-mass spectrometry

Brianna Benner and Manuel David Montaña, Western Washington University

In the past two decades, single particle inductively coupled plasma-mass spectrometry (spICP-MS) has largely been used to the detection and quantitation of engineered nanomaterials. Considerably less attention has been given to naturally occurring nanoparticles (NPs), despite being ~1,000,000x more abundant. The aim of this research is to utilize spICP-MS in the study of naturally occurring nanomaterials within the Nooksack River in Whatcom County, WA. Iron oxide NPs have been shown to play a large role in stimulating photosynthesis in estuaries and oceans after settling out of rivers. The influence that these materials can have on primary production is dependent on their bioavailability, which is in turn controlled by particle size and mineralogy. Furthermore, NPs can serve as vectors for contaminants due to their high reactivity. The floodplain of the Nooksack encompasses agricultural, commercial, and residential land, creating the potential for long-range transport of associated pollutants during high-flow events. In this work, samples were taken along the Nooksack River, from glacial and snowmelt sources, and throughout the mainstem towards the mouth of Bellingham Bay. A combination of spICP-MS, X-ray diffraction, and scanning electron microscopy were used to evaluate how iron oxide NPs are transported and transformed throughout the course of riverine transport, as chemical parameters such as pH, dissolved oxygen, and ionic strength change. As the glacial and snowmelt feedstock of these NPs is expected to decline with rising temperatures, this work will help establish a baseline of the mineralogical NP populations and their behavior so that future work may be done to understand how NP formation and transformation changes in response to a warming climate.

4.20.P-We-054 In-line thermal extraction of silicone wristbands for 47 volatile organic compounds using purge-and-trap gas chromatography-mass spectrometry

Ricky Scott, Kaley Adams and Kim Anderson, Oregon State University

Silicone wristbands have been widely adopted as passive sampling devices since their introduction in 2014, particularly for assessing real-world chemical exposures in populations underserved by traditional methods. However, a frequently cited limitation is the lack of standardized protocols for extraction and analysis across users and laboratories. In this study, we present a robust and streamlined method for extracting and analyzing volatile organic compounds (VOCs) from silicone wristbands using purge-and-trap thermal desorption. The method quantifies 47 VOCs using a Teledyne Tekmar Atomx XYZ autosampler coupled with an Agilent 8890 gas chromatograph and 5977 mass spectrometer, following a modified U.S. EPA Method 8260. Three sets of wristbands were used for validation. The average relative standard deviation (RSD) across sets analyzed on multiple days was 23%, with individual RSDs of 17%, 23%, and 28%. Median percent recoveries were within 87% of known values. Quality control checks on approximately 400 field samples confirmed consistent analytical performance, underscoring the method's reliability in real-world applications. These findings demonstrate that silicone wristbands can be effectively integrated with established analytical techniques. The in-line purge-and-trap method reduces organic solvent use and minimizes labor-intensive sample preparation, offering a cost-effective and environmentally sustainable approach to personal exposure monitoring.

4.20.P-We-055 Assessment of particle and chemical exposure hazards from 3D printing

Qian Zhang and Mark Wilson, UL Research Institutes

3D printing is widely used in industrial, residential and educational settings, given its low cost, flexibility, and ease of operation. However, it can be a source of micro/nano-plastic particles and volatile organic compounds

(VOCs), especially for material extrusion 3D printing that uses thermoplastics. Exposure to the mixture of these emissions could be hazardous for the users and occupants. This study characterized particle and chemical emissions from 3D printing using specialized exposure chamber setups. Airborne particles were measured online for concentration and size distribution. VOCs and low-molecular-weight carbonyl compounds were collected onto sorbent media and analyzed using analytical methods. Metal composition of emitted particles was analyzed using inductively coupled plasma-mass spectrometry. Emission factors of each component were calculated following the chamber testing, which were applied to an indoor exposure model to estimate exposure potential. This study established a database on emission profiles of nine commonly used thermoplastic material types. Particle and VOC emissions are associated with the heating process of the thermoplastic materials during printing, which were affected by printing conditions like printer design, material type and brand, and print temperature. In general, acrylonitrile butadiene styrene, high impact polystyrene and nylon materials tended to emit more particles, especially ultrafine particles. VOC emissions contain a mixture of aromatics, aldehydes, alcohols, ketones, esters and siloxanes. The most abundantly emitted VOCs were typically associated with bulk materials. However, we detected emissions that were not associated with the specified material ingredients, which indicated unknown additives or chemical reactions may also contribute to the emissions. Heavy metals such as chromium, cadmium, copper, and arsenic, were detected in the emitted particles. Model estimation showed that exposure to fine particulate matter may exceed the standard for some printing scenarios like metal composite materials. Personal exposure to VOCs with health concern, such as ethylbenzene, styrene, formaldehyde, benzaldehyde, acetaldehyde, and benzene, may exceed the recommended indoor levels. Thermoplastic 3D printing is a source of ultrafine particles, heavy metals, and hazardous VOCs, which pose inhalation risks for users and occupants indoors, especially in poorly ventilated places and for vulnerable populations.

4.20.P-We-056 Expanding the chemical space of commonly used solid phase extraction methods for contaminants of emerging concern

Michael Shields¹ and **Yina Liu²**, (1)*Geochemical and Environmental Research Group, Texas A&M University*, (2)*Texas A&M University*

Solid phase extraction methods concentrate organic compounds from the dissolved phase while removing interference for chemical analyses. For environmental contaminants, they are meticulously developed to target specific classes or categories of chemicals for monitoring. This targeted approach is essential for reliable quantitative analysis at low detection limits. However, this process limits the chemical space that may be explored for broader exposomics and chemodiversity. Can we apply these extraction methods to a wider chemical space? Here, we assess four standard solid-phase extraction methods for recovering multiple contaminant classes to expand each technique's chemical space. The targeted compounds include per- and polyfluoroalkyl substances (PFAS), pharmaceuticals and personal care products (PPCPs), artificial sweeteners, and the tire-derived contaminant 6PPD-quinone. These SPE methods comprise weak anion exchange (WAX) for the EPA 533.1 and 1633 methods developed for PFAS, hydrophilic-lipophilic balanced (HLB) for the EPA 1694 method created for pharmaceuticals and personal care products (PPCPs), and styrene-divinylbenzene (SDVB) for the EPA 537.1 method targeting PFAS, which is also used for characterizing dissolved organic matter. We assess this in laboratory-grade water (i.e., MilliQ) and estuarine water (salinity = 17.61 and a dissolved organic carbon concentration of 4.02 mg/L). Targeted quantification was performed on an Ultrahigh Performance Liquid Chromatograph (UHPLC) coupled to a Triple Quadrupole Mass Spectrometer. Additionally, non-targeted analysis was carried out on a UHPLC combined with a time-of-flight mass spectrometer.

4.20.P-We-057 Microplastics in commercial beverages from Cape Town, South Africa: Occurrence in sugary drinks, energy drinks, alcoholic beverages and bottled water

Adetunji Ajibola Awe¹, **Gideon Idowu²** and **Conrad Sparks¹**, (1)*Cape Peninsula University of Technology, South Africa*, (2)*Federal University of Technology, Akure, Nigeria*

The increased production and use of plastics have contributed significantly to the growing problem of plastic pollution in the environment. Plastics pose numerous threats to ecosystems, including the entanglement and smothering of aquatic organisms, habitat loss, the transport of toxic chemicals, and the loss of biodiversity. This issue is further exacerbated by the occurrence of microplastics (MPs), which are plastic particles smaller than 5 mm. MPs are pervasive and persistent in nature, and are now widely distributed across the environment and even found within human systems. Although significant efforts have been made to reduce plastic pollution through the prohibition or reduction of single-use plastics and the improvement of waste management systems, MPs continue to present serious environmental and health challenges. This study investigated commercially available consumable drinks as possible pathways of human exposure to MPs. A total of 30 different drinks, including sugary and energy drinks, alcoholic beverages, and bottled water, were sourced and sampled. For each drink, 300 mL was processed in five replicates using a Buchner filtration system fitted with a 10 µm nylon mesh for MP extraction. The particles retained on the mesh were examined microscopically and classified by type, colour, and size. Polymer composition was determined using a Spectrum Two Universal Attenuated Total Reflectance-Infrared (UATR-IR) spectrometer. Findings from this study show that fibers accounted for 78% of the extracted MPs, with most ranging between 0.5 mm and 1 mm in size (46%) and appearing predominantly dark in colour (blue to black, 69%). Most of the analyzed particles were composed of cellulose-based fibers. Other detected polymers included polyethylene, polyethylene terephthalate, acrylonitrile-based copolymers, polystyrene, polypropylene and alkyd resin. These findings provide a foundation for assessing the potentially toxic effects of MPs on humans through the consumption of commercially available drinks in Cape Town, South Africa. Keywords: Microplastics, Consumable Drinks, Polymer Identification, Human Exposure, South Africa

4.20.P-We-058 Comprehensive mapping of emerging chemical threats in the Tampa Bay (FL) region

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The Tampa Bay region is known to be a heavily polluted area because of its high density of sources of pollution, such as wastewater treatment facilities (WWTFs), airports, and military bases. These sources of pollution pose great threats to the bay, which is vital for both the environmental health of the ecosystem, but also for the citizens who rely on the bay for food, agriculture, recreation, and tourism. To date, there is limited knowledge of the presence and concentration of emergent chemicals of concern (e.g., drugs of abuse, pharmaceuticals, antibiotics, and pesticides) in the Tampa Bay region. Thus, the primary objective of this study was to focus on the detection and quantification of these emerging contaminants in surface water, to create a baseline of knowledge of the current state of pollution in the bay. To represent an accurate baseline, we chose to sample across the entire region with over 400 surface water samples collected, where each was investigated for over 50 chemicals of emerging concern. In this study, we employed crowdsourcing efforts (via trained citizen scientists collecting the water) to simultaneously educate the public on these raising concerns, while also achieving a large geographical spread of data. The surface water samples were filtered with glass fiber filters and then extracted using solid phase extraction. Samples were then analyzed with a targeted method using ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). This study provides more detailed information on the overall Tampa Bay contamination landscape, while also allowing for the comparison between classifications (e.g., opioids, depressants, benzodiazepines, stimulants, pesticides, and new emerging environmental contaminants). Furthermore, this data allowed for the comparison of diverse surface water profiles geographically within the sampling region. With the coordinates of each sample, heat maps of concentrations of compounds/classes were created. Heat maps helped to visually identify hotspots across the entire bay, pointing to possible point sources of specific contaminants. We hope that this comprehensive analysis will provide more attention toward solutions, overall community awareness, and ideally, statewide regulations aimed at mitigating these harmful chemicals in our surface water.

4.20.P-We-059 Wildland-urban interface (WUI) fires and the spatial distribution of soil heavy metals in the aftermath of the Lahaina fire

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Wildland-Urban Interface (WUI) is the line, area, or zone where structures and other human development meet or intermingle with undeveloped wildland or vegetative fuels. WUI fires lead to the release of uncharacterized and complex mixtures of chemicals, particles, and heavy metals, which result from the combustion of our built environment (building materials, plastics, fuels, vehicles etc.) along with natural biomass. Little is known about the exposure hazards that the impacted individuals and communities face during and after a WUI fire. Our research aims to investigate the spatial distribution of heavy metals in residual soil and how that relates to distances from burnt vehicles and buildings with differing construction dates within the fire footprint. In this study, we evaluated 63 homes/businesses across the Lahaina/Kula area. For each property, we collected historical information including but not limited to construction dates, renovation dates and plans, and square footages. At each sample location, we collected geospatial data using a Global Navigation Satellite System (GNSS) receiver, took field soil screening measurements utilizing a handheld X-ray fluorescence (XRF) analyzer, and collected a physical core of the top 3 inches of soil at each sample location. For homogeneity, we filtered the soil samples through a sieve and analyzed them for metals via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Target metals included chromium, copper, arsenic, nickel and lead from burnt vehicles and buildings that were built prior to regulatory changes and were likely contaminated with metals. We utilized ArcGIS historical imagery to identify vehicles that had been burned during the August 2023 Lahaina wildfires and spatially compared those locations to our sample locations. Our analysis showed that locations inside of the burn footprint had elevated levels for heavy metals and that closer proximity to the location of a burnt vehicle was associated with higher levels of our target metals. We found that these closer locations can show an increase in heavy metal concentrations by 2.5-5 times the levels seen in unimpacted or distant sampling locations. These results support our hypothesis that elevated levels of heavy metals in soil correlate with proximity to locations of specific fire fuels. The findings from this project can help future first responders to screen sites more rapidly and to more clearly delineate “clean” sites from “dirty” sites.

4.20.P-We-060 Determination of 161 parent and alkylated polycyclic aromatic hydrocarbons by gas chromatography–tandem mass spectrometry using dynamic multiple reaction monitoring mode

Ricky Scott and Kim Anderson, Oregon State University

Polycyclic aromatic hydrocarbons (PAHs) remain a key concern in environmental monitoring due to their persistence, bioaccumulation potential, and toxicity. Yet, despite over a century of study, routine analyses often focus narrowly on the 16 unsubstituted priority PAHs designated by the U.S. Environmental Protection Agency. Thousands of PAH derivatives—especially alkylated species—are commonly overlooked, despite their prevalence and potential toxicity. Prior to this work, few analytical methods had quantified more than 50 PAHs. Alkylated PAHs often dominate PAH mixtures at contaminated sites and have been associated with toxicity to aquatic organisms. At a recent creosote-impacted site, over 80% of the detected PAHs were alkylated species—highlighting the urgent need for broader analytical coverage. For this project, we developed and validated a single, high-resolution analytical method capable of identifying and quantifying 161 PAHs, including 48 parent and 113 alkylated species, using optimized dynamic multiple reaction monitoring (dynamic MRM). The method achieves resolution of key isomers, including methyl and ethyl substitutions. Analytical performance was robust, with a quantitation range of 0.001–2 µg/mL and an average calibration R^2 of 0.999 (range: 0.995–1.000). Method detection limits averaged 0.002 µg/mL (range: 0.00007–0.02 µg/mL). Recovery studies demonstrated consistent performance across diverse matrices: 75% in a NIST-certified petroleum product, 81% in NIST-certified freeze-dried mussel tissue, 88% in spiked crayfish tails, and 98–110% in passive samplers. This single-method approach enables comprehensive monitoring of PAHs in environmental and biological

matrices—supporting improved exposure assessments, enhanced risk characterization, and the detection of emerging PAHs of concern.

4.20.P-We-061 In-line extraction of volatile and semi-volatile per- and polyfluoroalkyl substances from silicone wristbands and gauze wipes using purge-and-trap gas chromatography–mass spectrometry

Ricky Scott, Caoilinn Haggerty and Kim Anderson, Oregon State University

As scientific understanding of per- and polyfluoroalkyl substances (PFAS) continues to evolve, concern is increasing over their persistence, bioaccumulation potential, and toxicity. While most research has focused on persistent end products such as PFOS and PFOA, attention is shifting toward precursor and other end-use compounds found across a wide range of environmental matrices. Volatile and semi-volatile PFAS precursors—including fluorotelomer alcohols (FTOHs), methacrylates (FTMACs), amides (FTACs), sulfonamidoethanols (FOSEs), and sulfonamides (FOSAs)—are less frequently monitored due to the field's reliance on liquid chromatography (LC), which is not ideal for volatile analytes. To address this gap, we developed a robust, high-throughput in-line extraction method for volatile and semi-volatile PFAS using a Teledyne Tekmar Atomx XYZ purge-and-trap autosampler coupled with an Agilent 8890 gas chromatograph and 5977 mass spectrometer. This 215 combines the sensitivity and clean sample introduction of purge-and-trap with the ruggedness and simplicity of GC-MS, enabling efficient PFAS precursor screening. To date, over 30 fluorinated compounds have been evaluated. Calibration curves demonstrated coefficients of determination (R^2) ≥ 0.98 , using either linear (≥ 4 points) or quadratic (≥ 5 points) regression, with near-zero intercepts. Compounds were required to show $\geq 60\%$ extraction efficiency from silicone wristbands or gauze matrices—representing dermal and atmospheric exposure pathways—with relative standard deviations (RSDs) $< 40\%$. Analytical validation included certified standards ($\geq 97\%$ purity), over-spiked matrices, and replicate testing across multiple days to determine method detection limits. This method provides a viable tool for monitoring airborne and surface-associated PFAS precursors and enhances analytical capacity for characterizing PFAS exposure in environmental health studies.

4.20.P-We-062 Per- and polyfluoroalkyl substances (PFAS) in sediments collected from urban watersheds across California

Bushra Khan, University of California, Davis

The Stream Pollution Trends Monitoring program (SPoT) is a statewide program initiated in 2008 under the Surface Water Ambient Monitoring Program of the California State Water Resources Control Board. The program provides critical information about watershed health and identifies trends in sediment toxicity and contaminants in the context of land use across California. Per- and polyfluoroalkyl substances (PFAS) were recently added to the monitoring design and so far, two years of data have been collected at 40 sites influenced by urban land use (USGS's National Land Cover Database categories 21, 22, 23 and 24). Given the ubiquity, high persistence, bioaccumulative potential and toxicity of PFAS, it is important to identify their spatiotemporal patterns to prioritize monitoring efforts in areas of concern. A suite of 40 PFAS including perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAAs), precursors and replacement PFAS were measured in surface sediment samples. Total PFAS were calculated by summing reported concentrations of all 40 PFAS. Percent contributions to total PFAS burdens at each site as well as detection frequencies across sites were calculated for individual compounds. Sites with three highest PFAS sums as well as diversity belong to Region 2 (San Francisco Bay), Region 8 (Santa Ana) and Region 4 (Los Angeles) and each reflects $> 75\%$ urban land use. The highest detection frequency across 40 sites was observed for perfluorooctanesulfonic acid (PFOS, 88% and 90% in 2023 and 2024, respectively). Further, sites with higher sums were often dominated by PFOS in both years. Long-chain PFCAs ($\geq C8$) were reported at more sites than short-chain ($< C8$).

Perfluorooctanesulfonamide (PFOSA) and perfluorooctane sulfonamido acetic acids (FOSAAs) were also found at several sites in both years. Despite these similarities across two years of data collection, notable differences were observed in some urban areas. Sum PFAS ranged from 0–14 ng/g and 0–40 ng/g dry sediment in 2023 and 2024, respectively, representing a shift in PFAS concentrations and diversity. These data provide valuable

information on sediment contaminant profiles across California watersheds indicating presence of complex PFAS mixtures that need to be further monitored. Ongoing work will identify trends at regional and statewide levels and assist in prioritizing monitoring strategies.

4.20.P-We-063 Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in various aquatic environments including sewage treatment plant effluent, tap water and groundwater in Japan

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Pharmaceuticals and personal care products (PPCPs) are widely detected in aquatic environments. However, recent studies investigating the environmental occurrence of currently used PPCPs are lacking in Japan. In this study, a nationwide monitoring initiative focusing on PPCPs was undertaken to investigate the occurrence and fate of PPCPs in aquatic environments in Japan. Seven hundred samples were collected and analyzed from 2018 to 2022. Ninety-one PPCPs were detected in the analyzed samples. Three PPCPs (DEET, salicylic acid, and crotamiton) were detected at high frequencies, and they were detected in more than 99% of the analyzed samples. Seasonal variations were observed for several PPCPs across multiple rivers, with concentrations generally increasing during fall/winter and decreasing during spring/summer (except DEET) throughout the sampling period. The detection frequencies and concentrations were higher in PPCPs with higher domestic prescription amounts. PPCPs, such as acetylsalicylic acid exhibiting a low frequency/concentration despite the high domestic prescription amounts may have transformed into metabolites or degradates in the aquatic environment at the time of sample collection. The contribution of sewage treatment plant effluent to the PPCP concentrations in the environment was estimated by examining the correlation between each PPCP and sucralose concentration. Sewage treatment plant effluents may significantly contribute to the majority of target PPCPs; however, DEET and some other PPCPs may originate from alternate sources. This study is the first to comprehensively explore and report the occurrence and fate of PPCPs in Japan's aquatic environment. The environmental and human health risks of these PPCPs should be assessed in the future, in addition to identifying the occurrence of metabolites or degradation products of PPCPs in the environment.

4.20.P-We-064 Advancing PFAS extraction methods for marine sediments and fish tissues

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Runoff and discharge from industrial and military facilities have introduced per- and polyfluoroalkyl substances (PFAS) into aquatic systems worldwide, raising concerns about their potential environmental impacts. PFAS demonstrate remarkable persistence and mobility in aqueous media from both freshwater and saltwater systems (although our understanding of the occurrence, fate, and transport of PFAS in freshwater systems remains more advanced). Accordingly, protocols for the extraction of PFAS from aqueous samples have been established and successfully implemented for some time. By contrast, the development of reliable/standardized methods for extracting PFAS from sediment and biological samples is still underway. This discrepancy limits the collection of data on the environmental distribution and accumulation of PFAS in non-aqueous samples. Herein, we describe our progress towards developing protocols for the reliable extraction of 13 PFAS compounds from estuarine/marine sediments and fish tissues, which will allow us to efficiently fill critical data gaps regarding point source pollution accumulation and transport in saltwater systems. We tested multiple combinations of extraction solvents, sample homogenization techniques, and solid phase extraction (SPE) protocols to develop and validate an optimized extraction method for solid environmental media. Through these efforts, we have increased analyte recovery and processing efficiency, thereby allowing us to fill data gaps on the presence of PFAS in understudied environmental compartments.

4.20.P-We-066 Raman spectroscopy and machine learning for probing acetylcholinesterase dynamics and inhibition mechanisms

Abraham Abimbola Olayeri, Ashley Newland, Jan Halamek and Lenka Halamkova, Texas Tech University

Acetylcholinesterase (AChE) plays an important role in proper nerve functioning by breaking Acetylcholine (Ach) through hydrolysis which terminates impulse transmission. The mode of action of neurotoxic compounds like organophosphates is the inhibition of AChE which results to overaccumulation of Ach. This causes overstimulation in the cholinergic receptors which leads to various neurological disorders. This study explores the use of AChE inhibition as a biomarker to assess the risk of exposure to low concentrations of Glyphosate, a phosphonate. Commonly included in herbicides, Glyphosate has sparked concerns about potential health hazards. The fact that it is used as a broad-spectrum herbicide globally accounts for its ubiquitous nature in the environment. In this study, Raman spectroscopy and machine learning (ML) is combined to determine if glyphosate binding cause specific conformational changes in AChE that can be identified through unique spectral signatures. Data from preliminary kinetic studies suggests that there is a direct link between Glyphosate and the inhibition of AChE. To validate these findings, we investigated how Glyphosate alters AChE's structure while capturing subtle spectral changes indicative of enzyme inhibition mechanisms using Raman spectroscopy. With these, we can have a clear understanding of the enzyme dynamics and mechanisms at the molecular level. This study aims to close a knowledge gap on the neurological effects of Glyphosate while also providing a useful tool for the assessment of various compounds with AChE inhibition. The broader ramifications of this research will guide regulatory policies, improve public health, and advance sustainable agricultural practices.

4.20.P-We-067 Treatment of AFFF contaminated water using ion-pair enhanced coprecipitation

Brenton Thomas Murphy and Franco Pala, Battelle

PFAS are a persistent environmental contaminant that have been attributed to an innumerable number of sources. Among the most common of sources is aqueous film forming foam, or AFFF, used in fire suppression systems in airport facilities both commercial and military. Facilities that have historically used this material are often left with vast quantities of both used and unused material stored in containers, co-occurring with other common contaminants such as PAHs and oil. Treatment and ultimate discharge of this waste stream can be extremely difficult as traditional treatment options become saturated and overloaded rapidly, meaning frequent filter change outs and expensive treatment. This work demonstrates a novel method for the treatment of AFFF-contaminated waters using an ion-pair enhanced coprecipitation technique. Initial clean matrix experiments demonstrated that by pairing anionic PFAS with a cationic surfactant dramatically improves adsorption of PFAS onto a treatment media. For our system, we chose a colloidal species of iron hydroxide due to its extremely high surface area, low toxicity, affordability, and ultimate low volume of solid material. Initial experiments resulted in over 90% sequestration of 23 of the 29 quantified PFAS, most notably demonstrating significant removal of ultrashort chain PFAS such as TFA, PFPrA, TFMS, PFEtS, and PFPrS. To our knowledge, this is the first work of its kind demonstrating and addressing the removal of these ultrashort chain compounds. Our system was applied to a true AFFF contaminated water, with the primary components of 6:2FTS at 4,800,000ng/L and PFHxA at 50,800ng/L. Treatment of this water through 3 rounds of our coprecipitation technique and a final WAX polisher resulted in 6:2FTS concentrations of 59.57ng/L and PFHxA concentrations of 32.93ng/L, well below EPA MCL criteria for drinking water. Additional experiments will continue to optimize the conditions of the coprecipitation procedure, as well as explore routes for ultimate destruction of the solid phase PFAS waste generated through this process.

4.20.P-We-068 Mapping exposure hotspots of inorganic contaminants in soil and dust in Reno-Sparks, Nevada

Olufunke Dina¹, Li Li¹, yeongkwon Son², Dingsheng Li¹, Patrick Hurbain¹, You Fu¹ and Yongcheng Li¹, (1)University of Nevada, Reno, (2)Desert Research Institute

Environmental contamination poses critical health concerns for children, who are uniquely at risk due to behaviors like frequent hand-to-mouth contact. This study aimed to evaluate the spatial distribution of inorganic contaminants in outdoor soil and household dust among children aged 6 months to 6 years and to assess their associations with proximity to highways and hazardous sites. A total of 140 children residing in the Reno-Sparks area of Nevada, United States, were recruited. Environmental samples (soil and dust) were collected and

analyzed using inductively coupled plasma mass spectrometry (ICP-MS) to quantify 32 inorganic compounds. Data on hazardous sites were obtained from the U.S. Environmental Protection Agency (EPA) via the Envirofacts database. Principal Component Analysis (PCA) was conducted which identified three dominant sources; geogenic soil characteristics- Aluminum (Al), Iron (Fe), and Manganese (Mn): (68%), traffic-related emissions- Lead (Pb), Cadmium (Cd), Zinc (Zn), Copper (Cu), Manganese (Mn), and Iron (Fe): (67%), and industrial/hazardous waste- Chromium (Cr), Arsenic (As), Molybdenum (Mo), Barium (Ba), and Vanadium (V): (50%). K-means clustering grouped participants into three distinct exposure profiles. One cluster showed elevated dust-phase Pb, Lanthanum (La), and Neodymium (Nd) reside closest to parks (2.43 km) and hazardous sites (0.31km), suggesting higher environmental exposure. Another cluster was most proximal to highway: I-80 (4.67km), while the third lived furthest from all geographical hotspots. Spatial correlations identified statistically significant associations between inorganic compounds and proximity to geographical hotspots. Notably: Strontium in soil showed a positive correlation with distance to hazardous sites ($r = 0.35$, $p = 0.000034$); Molybdenum in dust showed increasing concentrations farther away from freeways ($r = 0.33$, $p = 0.000318$); Aluminum in soil showed a significant decline with increasing distance from I-80 ($r = -0.32$, $p = 0.000111$); while barium in soil is higher closer to highway (I-80): ($r = -0.34$, $p = 0.000051$). These findings demonstrate that proximity to highways, parks, and hazardous sites is associated with elevated concentrations of inorganic contaminants in soil and dust. Further research is needed to elucidate the factors influencing the bioavailability of these inorganic compounds, as well as to identify their potential sources through detailed soil characterization.

4.20.P-We-069 Alternative methods for estimating chronic exposures to pesticides for avian and mammalian risk assessments

Clifford Habig, Compliance Services International

EPA's pesticide risk assessment procedure for birds and mammals uses the T-REX model to estimate exposure and risks to avian and mammalian species. This tool is based on EPA's interpretation of the UTAB database, which consists of measured residues of a variety of pesticides on items that represent potential food items for both mammals and birds over multiple time points after application. The majority of these potential food items are herbivorous types of food (grasses, foliage, seeds). T-REX estimated exposures for birds and mammals to pesticide residues on food items are based on upper-bound residue estimates from EPA's analysis of pesticide residues in the UTAB database, and focus only on residues from day 0 and day 1 after pesticide application for estimating both acute and chronic exposures (EECs); chronic EECs are therefore the same as acute EECs for avian and mammalian pesticide risk assessments. Chronic toxicity endpoints for risk assessment, in contrast, are derived from long-term toxicity tests. This presentation evaluates and discusses alternative approaches to estimating chronic exposures to pesticide residues on food items for birds and mammals. These alternative exposure estimates include utilizing half-life values calculated from pesticide-specific residue data rather than the default T-REX 35-day half-life value and calculating chronic exposure estimates based on actual or estimated residues over time periods consistent with the duration of the chronic toxicity testing used for risk assessment endpoints. Examples comparing standard T-REX results with results for alternative chronic EEC estimates will be provided for a conventional pesticide product and a biological pesticide product.

4.20.P-We-070 Investigating mosquitoes as bioindicators for PFAS contamination across environmental and biological systems

Isabella Beasley, Michella Paige Salvitti, Mobolaji Okulate, Joseph Pitula and Eguono Wayne Omagamre, University of Maryland, Eastern Shore

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants resistant to degradation. Their bioaccumulation in ecosystems and biomagnification in organisms pose significant toxicological and reproductive risks to wildlife. In humans, PFAS exposure is increasingly associated with damage to the liver, kidneys, and reproductive systems. The detection of PFAS in human blood serum raises public health concerns; however, comprehensive monitoring is limited by resource constraints and challenges in reaching underserved

populations. This study aims to assess the feasibility of using mosquitoes as biomonitoring tools for PFAS exposure, investigating their potential to reflect environmental and biological contamination. We evaluate whether mosquitoes acquire PFAS through blood feeding and examine the contribution of environmental exposure to their PFAS body burden. To distinguish between these pathways, mosquitoes are reared in PFAS-spiked water to adulthood to determine the extent of PFAS uptake from water. A separate colony, reared in uncontaminated water, is fed blood meals spiked with the same PFAS mixture to assess uptake via blood feeding. Field collections of adult mosquitoes from five sites near sewage treatment plants on Maryland's eastern shore were analyzed for PFAS compounds using liquid chromatography-mass spectrometry (LC-MS) based on EPA Method 1633. Preliminary data revealed total PFAS loads ranging from 12.5 to 78.7 ppb across the sites, identifying 14 unique PFAS compounds. PFOS was the most prevalent, with an average concentration of 17.3 ppb (± 2.6). Long-chain PFAS dominated the samples, with three short-chain types also detected. Of these, eight were carboxylates, two were sulfonates, and three were fluorotelomers. Preliminary findings demonstrate that mosquitoes naturally accumulate PFAS, suggesting their potential as biomonitoring tools. By evaluating PFAS uptake through environmental exposure and blood feeding, this study lays the foundation for future investigations into mosquitoes' suitability as bioindicators of contamination in diverse settings.

4.20.P-We-071 Impact of volatile organic compounds (VOCs) & particulate matter (PM) exposure on respiratory health in Hispanic domestic cleaners in South Texas

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Domestic cleaning workers, particularly in Texas, face significant environmental and occupational health risks due to exposure to VOCs and PM found in cleaning products, increasing respiratory issues like asthma. These jobs, often held by low-income Hispanic women, expose them to harmful substances both at work and at home (i.e., "double jeopardy"), yet they lack adequate labor protections, leaving them particularly vulnerable. This pilot study aims to address gaps in research on the health impacts of VOCs and PM exposure in at-risk Hispanic women working as domestic cleaners and living in environmental justice neighborhoods. The study aims were: (1) measuring personal VOC exposure and associated inflammation biomarkers, (2) identifying potentially modifiable determinants of VOCs and PM exposure, and (3) analyzing the relationship of VOCs and PM exposure with health biomarkers. Partnering with a grassroots organization in San Antonio, we recruited 20 domestic cleaners who were part of an existing study of domestic cleaning workers. We examined factors influencing their VOCs and PM exposure, alongside inflammation biomarkers in airways (e.g., 8-isoprostane, eNO, pH) and systemically (e.g., CRP, urinary 8-OHdG), over a 72-hr sampling period. Data were collected through surveys, time-activity diaries, and biological samples (i.e., blood, urine, and exhaled breath condensate). VOCs and PM exposure levels were measured using wearable air quality Atmotube Pro monitors and 3M passive badges. Our comprehensive approach aims to reduce the burden of environmental and occupational hazards for vulnerable Hispanic women, striving to enhance safety in their workplaces within this underserved community. **Disclaimer/Disclosure:** Research Advancement, Innovation, & Discovery Award from UT Health San Antonio

4.20.P-We-072 Evaluation of data treatment methods for dioxin and furan measurements in fish tissue

Alice England, Nicholas Rose and Jeffrey Wilson, Verdantas LLC

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) are a group of toxic, bioaccumulative, and persistent organic pollutants that are produced inadvertently via various industrial and non-industrial chemical processes and are ubiquitous throughout the environment. Human exposure to PCDD/Fs is mainly through consumption of contaminated fish. To investigate the potential human health risk of consuming fish in a specific area or for a certain population, the PCDD/F concentrations in the fish species of

interest should be measured with sufficiently low laboratory detection limits. The toxicity equivalents (TEQs) are then calculated from the 17 toxic PCDD/F congener concentrations, which should be compared against any available consumption level guidelines or thresholds and should also be evaluated in the context of background conditions (TEQ levels in fish tissue at locations with no specific or known point sources). Many studies to date have characterized PCDD/F concentrations in fish tissue; however, there is variability in laboratory detection thresholds, data handling methods and reporting. This study presents a survey and evaluation of the different reporting limits for PCDD/Fs in fish tissue, the methods used to treat non-detect results in TEQ calculations, and how method blank contamination is addressed. An in-depth understanding of the data handling methods and their impact to the actual reported toxicity of the fish tissue is critical for determining the potential impacts to human health. The results of this analysis will provide perspective on appropriate reporting limits to measure PCDD/Fs in fish tissue and how the different data treatment methods introduce varying levels of uncertainty and conservatism in the reported concentrations and the associated potential human health risks.

4.20.P-We-073 Occurrence of disinfection byproducts in children's splash parks and associated exposure assessment

Isabella Rios, Jenny Kim Nguyen, Maryam Ghalamkari and Un-Jung Kim, University of Texas, Arlington
Undergoing chlorination to ensure the quality of water used in splash pad parks, many aquatic facilities test chlorine levels daily as a safety precaution for young children. However, many facilities overlook related disinfection byproducts (DBPs) such as melamine and cyanuric acid. Linked to bladder cancer and kidney complications, exposure to these compounds poses a threat to vulnerable infants and children. This project aims to quantify the presence of disinfection byproducts in splash parks across the North Texas metroplex. Water samples were collected from splash park locations in Mansfield, Grand Prairie, and Arlington biweekly, and three times a day for select parks to analyze diurnal and daily fluctuations of DBPs. 500 mL samples were sourced from at least five different spout variations per site. Water samples were processed in the lab using solid phase extraction, isolating the aforementioned target analytes. Chemical analysis was performed using gas chromatography-mass spectrometry. The concentration of melamine and cyanuric acid will be reported, and comparisons will be made between samples collected from the three different municipalities. Other proxies such as temperature, time, and water treatment processes will also be considered in relation to DBP concentrations.

4.20.P-We-074 Using historical datasets on fish consumption advisory to assess management action effectiveness

Nathalie Lombard¹, Joel Baker² and Upal Ghosh¹, (1)University of Maryland, Baltimore County, (2)University of Washington

Historical dataset collected by the states, territories, and tribes to establish fish consumption advisories represents a valuable source of information on PCB contamination levels in the waterbodies, changes over time, and could potentially inform on the effectiveness of management actions and where more action is needed. In this study, we used 3 decades of PCB concentration measured by the Maryland Department of Environment to perform spatial and temporal analysis. The analysis was conducted using lipid normalized data or wet weight tissue from the top 3 species sampled in Maryland. Land use, toxic release inventory (TRI) basic data files from 1987 to 2021, and Superfund sites location and status were also collected for all watershed boundaries of hydrological unit 8 of Maryland. The approach enabled the identification of 6 watersheds highly impacted by PCBs out of 25, including the Upper Chesapeake Bay. Known areas of contamination were better predictors of PCB levels in fish than generic land use or toxic release information. All impacted watersheds showed slow nationally consistent natural attenuation rates ranging from 3% to 6% yr⁻¹, except one, the Gunpowder Patapsco, where concentration in fish has been without a temporal trend since 1996. The overall study showed that PCB source mitigation implemented across Maryland and surrounding States helped recoveries of moderately to highly impacted watersheds, but more efforts are needed to fully control all PCB sources (legacy or ongoing) that are still impacting the rivers and their fish.

4.20.P-We-075 Land use patterns and per- and polyfluoroalkyl substances in low order streams: Are they all down the toilet?

Heather Delaplaine, Ryan E. Casey and Christopher Salice, Towson University

Per- and polyfluoroalkyl substances (PFAS), commonly known as “forever chemicals”, are a diverse set of synthetic chemicals utilized heavily in industrial activities, consumer products, and in aqueous film-forming foams (AFFF’s). They have been recognized by the scientific and regulatory communities as a human health concern, yet there are many unknowns related to the distribution and transport of PFAS across different landscapes that may include non-point sources of PFAS contamination. In an effort to better characterize nonpoint source PFAS contamination in Maryland, this study focuses on measuring PFAS in low order streams with relatively homogeneous land covers. Stream surface water, sediment, and crayfish sampling were conducted in the Baltimore Metropolitan region across five land use categories: undeveloped, residential (served by sewer), residential (served by septic), highway/commercial, and agricultural areas. Where possible, streams were selected that drained into drinking water reservoirs. Samples were analyzed for PFAS following methods described in EPA Method 1633A. Of the five land use categories, preliminary experiments found PFOA and PFOS values at residential septic and residential sewer locations to be above April 2024 EPA established drinking water criteria of 4 ng/L (7.2 ng/L and 13.1 ng/L respectively at these sites). PFAS were below this threshold at all other locations, suggesting a positive net input of PFAS into drinking water reservoirs not related to any point source. Results of this research will provide important baseline data on PFAS contamination in low order streams and inform whether they contribute significantly to PFAS environmental risks in central Maryland.

4.20.P-We-076 Recent increases in loading of DDT to sediments of Lake Michigan, USA

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Although use of the insecticide DDT has been restricted globally for decades, monitoring of DDT has revealed complex chronological trends. In this study, concentrations of p,p’-DDT, p,p’-DDE, and p,p’-DDD were measured in eight dated sediment cores from Lake Michigan, USA. Apparently, net fluxes of p,p’-DDT deposition at most sampling locations have increased since about 1990, reversing the declining trends that began in the 1960s. The recent mass loading of DDT was estimated to be a fraction of the “legacy” accumulation, yet its ecological impact should not be ignored. Multiple potential sources may include: releases of DDT-related compounds from other pesticides, such as dicofol, that contain DDT as an impurity; import from countries where DDT is still being used; and re-mobilization of legacy DDTs accelerated by global warming and large-scale application of glyphosate. We retrospectively reconstructed the temporal trend of DDT input by fitting the observations with a model considering the alteration caused by in-situ degradation. The model succeeded in explaining 89% to 98% of variation in observed apparent net fluxes of p,p’-DDT deposition. The modeling results suggest that the recent ascending trend in mass loading of p,p’-DDT results mainly from its increased inputs in the 1990s. Compared to the “legacy” inputs, these “fresh” inputs are smaller but yield a disproportionately pronounced increase in the observed mass loading because they are more recent thus have undergone less in-situ degradation.

4.20.P-We-077 PFAS exposure and chronic kidney disease of unknown etiology: A nested case-control study

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Per- and polyfluoroalkyl substances (PFAS) are persistent synthetic chemicals widely used in food packaging, water-repellent coatings, and firefighting foams. Their extensive use results in human exposure through contaminated food, water, air, and household items. Due to their prolonged biological half-life, certain PFAS accumulate in the body, with the kidneys being primary targets due to their role in PFAS elimination. Evidence indicates that PFAS exposure is linked to impaired kidney function in adolescents and adults, likely mediated by mechanisms such as oxidative stress, disrupted vascular permeability, and renal damage. This study aims to investigate the association between serum PFAS levels and chronic kidney disease of unknown etiology (CKDu) in young adults using a nested matched case-control study design. Data will be drawn from a rural longitudinal cohort in Northwest Nicaragua, a population at high risk for CKDu. Incident cases will be identified using a Hidden Markov Model based on estimated glomerular filtration rate measurements over time, distinguishing healthy and diseased kidney states. Control participants will consist of individuals with consistently normal kidney function. Serum PFAS concentrations will be measured using targeted (liquid chromatography–high-resolution mass spectrometry) and non-developed targeted (gas chromatography–high-resolution mass spectrometry) serum analyses. Associations between PFAS exposure and CKDu will be assessed using conditional logistic regression models, estimating odds ratios and 95% confidence intervals. Models will be adjusted for age, body mass index, smoking status, education level, and occupational environment. Our study design will provide robust insights into the role of PFAS exposure in the CKDu etiology, elucidating an environmental risk factor. These results will have significant public health implications, providing new evidence to support targeted prevention strategies, regulatory action, and early interventions in at-risk populations.

4.20.P-We-078 Chemical characterization of non-polar organic compounds transported by methane ebullition in an oil sands Pit Lake

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Base Mine Lake Demonstration (BML) is the first full-scale commercial demonstration pit lake in the Alberta oil sands region. Active microbial methane generation within the tailings underlying the lake results in gas ebullition that transports residual bitumen-associated organic compounds to the lake surface. However, the chemical composition of bubble transported organic compounds, the mechanisms of association with the bubbles, and the sources from which they are derived have yet to be well constrained. Here we addressed these questions by analyzing volatile organic compounds collected in bubble traps deployed at several sites, as well as organic compounds associated with gas bubbles trapped within ice cores from eight different locations in BML. Results showed the gas collected in bubble traps was dominated by methane gas (79.3-90.5%). No C2-C4 compounds were observed, while several C5-C7 compounds were present at concentrations from 10 to 200 $\mu\text{mol/mol}$ CH₄. Several >C7 hydrocarbons were also detected but fell below the quantification limit. Bubble trap samples collected from different sites at different times showed differentiated low molecular weight compounds profiles, likely due to the heterogeneous distribution of these compounds within the tailings layer, and/or the dynamic ebullition paths where rising bubbles can contact various bitumen materials. Sampling of each ice core collected bubbles associated with bitumen and those with no visible bitumen in minimum ice volumes. Total lipids extract (TLE) content of collected ice samples ranged from <0.01 to 2.90 g/core for bitumen containing samples and negligible for non-bitumen samples, indicating the amount transported varied across sites. Chemical fingerprints of the GC-amendable non-polar fraction of transported bitumen by carbon number, and the fingerprints of selected well-resolved aromatic compounds showed consistency in all ice cores, implying the bitumen is derived from a similar source within BML. Greater consistency of the bubble associated chemical fingerprints with fingerprints of bitumen mats versus bitumen in fluid tailings indicated that bubble associated bitumen is primarily derived from bitumen mats rather than the diffused bitumen present within the tailings. These results imply that transport of bitumen to an oil sands pit lake may be able to be minimized by prevention, removal or isolation of bitumen mats within the system.

4.20.P-We-079 Modelling of biocide and veterinary medicinal product dispersion from Atlantic salmon hatcheries using computational fluid dynamics

Tom Hargreaves, *Blue Frog Scientific Limited, United Kingdom*

Atlantic salmon hatcheries use a range of biocides and veterinary medicinal products (VMPs) for hygiene and disease control. These substances are typically dosed intermittently into freshwater and discharged into nearby tidal coastal waters. The receiving environment is complex, influenced by tidal currents (which vary with time and depth) and adjacent freshwater inflows from rivers. Understanding the fate and transport of such discharges is essential for environmental risk assessment (ERA), particularly when regulatory decisions depend on acceptable exposure predictions. Currently, accepted dispersion models for these systems are limited, often lacking the ability to account for key physical processes such as buoyancy-driven mixing or variable hydrodynamics. This study applies computational fluid dynamics (CFD), using the open-source software OpenFOAM and the twoLiquidMixingFoam solver, to simulate the dispersion of freshwater discharges into saline coastal systems. The model captures density-driven flow, tidal variability, and freshwater influence, providing insight into key dilution mechanisms and allowing delineation of mixing zones against environmental quality standards. This approach enables site-specific exposure estimation and reduces uncertainty in ERA, offering a path toward more realistic and scientifically robust assessments in regulatory contexts.

4.20.V General: Chemistry and Exposure Assessment

4.20.V-01 Strengths and weaknesses of different source apportionment models for PM_{2.5}: A comparison of simulations using CMB, PMF and AERMOD models

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High PM_{2.5} (particulate matter with a diameter less than 2.5 µm) concentrations pose significant health risks. To develop effective pollution control strategies, it is important to identify the sources of pollutants and to quantify their relative contributions. This study compares the strengths and weaknesses of different source apportionment models, including Positive Matrix Factorization (PMF), Chemical Mass Balance (CMB), and AERMOD (American Meteorological Society and EPA Regulatory Model) models. These models were used to study PM_{2.5} in Pinehurst of northern Idaho, where the PM_{2.5} concentrations were elevated. The results of all these models are in good agreement showing that biomass burning is the dominant source, and traffic is the second largest source of PM_{2.5} in Pinehurst. The simulations of all these models also show that the contributions of biomass burning have a seasonal pattern with the highest levels in winter, leading to the highest PM_{2.5} levels. The PMF model further decomposed biomass burning to residential wood combustion and prescribed and other outdoor burning based on their seasonal patterns, whereas the CMB model cannot separate them. In addition, the PMF results provided information about both secondary organic aerosols (SOA) and secondary inorganic aerosols (SIA), which is lacking in the AERMOD and CMB results. The AERMOD model provided information about the spatial distribution of PM_{2.5} concentrations, which is lacking in the CMB and PMF simulations. With different strengths and weaknesses, these models can complement each other and help us better understand the sources and dispersion of PM_{2.5}. The results of this study were used to develop control measures to reduce PM_{2.5} concentrations in the area. This work is also useful for source apportionment and pollution control in many other places.

4.20.V-02 Toxicity as a determinant of life history evolution

Jorge Herkovits, *Institute of Environmental Sciences and Health, PROSAMA Foundation, Argentina*

Environmental toxicity resulting from human activities has led to a multidisciplinary understanding of the complex interrelationship between living organisms and their environment. In this contribution, the evolutionary dimension of ecotoxicology is expanded from the Anthropocene to encompass the entire evolutionary process through case studies of toxicity as a key parameter in understanding the interrelationship between living organisms and their environment throughout the evolutionary timeline. The potential for toxicity

to trigger a planetary catastrophe will be exemplified through high exposure to and mechanisms of toxicity during the K-Pg event. For adaptation, an onto-phylogenetic approach will highlight the record of environmental signatures in living species at specific ontogenetic stages and their fundamental role in evolution. As a new dating method, it allowed us to predict that multicellular organisms existed before 2 Gy, later confirmed by the fossil record. Likewise, notable changes in susceptibility to toxic agents at different developmental stages could reflect environmental conditions during various evolutionary periods of living species' ancestors. The construction of a living planet will be exemplified by the ability of free-living embryos to modify a toxic environment into one more favorable for life. This discovery completes the circle of global interrelationships between living organisms and their environment, demonstrating that, since ancient times, organisms have actively contributed to creating favorable conditions for life. Combined with the traditional concept of adaptation, this has allowed life to thrive at increasing levels of complexity, even surpassing catastrophic events like mass extinctions. Regarding the future, toxicological management is essential for the sustainability of the biosphere and for our efforts to colonize space and understand potential extraterrestrial life. The acronym EVOTOX represents the interrelation between living beings and environmental conditions related to toxicity throughout the past, present, and future, encompassing the entire evolutionary process.

4.20.V-03 Astroecotoxicology: The challenge of EVOTOX in the universe

Jorge Herkovits, Institute of Environmental Sciences and Health, PROSAMA Foundation, Argentina

The expectation of discovering extraterrestrial life and space colonization implies that one of humanity's greatest challenges is bringing environmental toxicology and chemistry from Earth to the Universe. The Earth's biosphere complexity and the capacity of living forms to adapt to changing and even "extreme" environment conditions provide clues to understanding that organisms, from microbes to humans, can thrive beyond Earth's conditions like for instance the unexpected high resistance and resilience of microbes and even vertebrate embryos to hyper-acceleration attributable to extreme gravity (Herkovits and Colombo 1982). Progress in the ability to understand environmental toxicology and chemistry from an evolutionary perspective, which on Earth covers about 3.8 Gy and where life has managed to adapt (Herkovits, 2006), build an environment friendly to life (Herkovits et al, 2015) and recover and progress despite dramatic environment changes, including those that caused mass extinctions (Herkovits 2001), represents a wealth of knowledge of high value for the extraterrestrial life exploration and colonization projects as it imply confronting with very differential environmental conditions. Whether life on Earth originated through abiogenesis or panspermia, the Universe, populated by billions of celestial bodies, offers potential conditions for life or other highly complex entities. Recent developments in thermodynamics have shown that structures of increasing complexity could evolve if basic atoms and molecules are available as building blocks (England, 2013). Thus, toxicity-based features will be of high priority in understanding alien life conditions as well as environmental conditions sustainable for a long-term space exploration. In pursuit of humanity's goal to explore the Universe, Astroecotox could address at least three main objectives: i) evaluating extraterrestrial agents/environments for their suitability for terrestrial life; ii) the construction of ecosystems that could eventually sustain humans in extraterrestrial locations; and iii) understanding the environmental limits of extraterrestrial beings of biological or other complexities, including evolutionary aspects. Ultimately, the advancement of Astroecotox will also enhance our capacity and commitment to environmental and health protection on Earth.

4.20.V-04 Microplastic fiber contamination in Lake Matoaka: Method development and baseline assessment

Kaleea Korunka, William & Mary

This study aimed to develop a reliable and reproducible methodology for detecting and quantifying microplastic fibers in the surface waters and Asian clam (*Corbicula fluminea*) populations of Lake Matoaka, a freshwater system vulnerable to pollution. Through a combination of optical microscopy, Microscope Fourier Transform Infrared Spectroscopy (M-FTIR), and logistic regression modeling, this research established the first baseline dataset of microplastic contamination in the lake. Results revealed widespread fiber contamination, with viscose

(cellophane) as the dominant polymer type, with minor contributions from synthetics such as PET and polyacrylics. Higher microplastic concentrations were found in sections of the lake adjacent to greater human activity. Asian clams demonstrated significant microplastic accumulation relative to laboratory blanks, supporting their use of suspension-feeding bivalves as bioindicators. The findings not only validate the developed methodology but also underscore the ecological risks posed by microfiber pollution and highlight the urgent need for standardized freshwater microplastic monitoring and local mitigation strategies.

4.21.P-Th Late-Breaking Science: Chemistry and Exposure Assessment

4.21.P-Th-042 A colorimetric sensor array for identifying and quantifying engineered nanomaterials based on surface chemistry

Sam Lohse, Ifunanya Ede and Rong Wang, Central Washington University

Emerging materials contaminants, including nanoplastics and engineered nanoparticles (ENMs), have now been identified in oceans, seas, freshwater, and drinking water. While detailed data on the exact quantity of nano- and micromaterials contaminants in the environment is still lacking, studies have shown that nanoplastics can now be found in drinking water sources worldwide. Recent reports, highlighting the ability of nanoplastics to cross the blood-brain barrier, have further raised concern about the ubiquitous nature of nanoscale contaminants in the environment. Detecting these emerging materials contaminants in water is critical due to their persistence and potentially harmful effects on human and environmental health. Despite this pressing need, detection methods for materials contaminants are often expensive and time-consuming, limiting widespread monitoring efforts. This research aimed to address the ENM detection challenge by developing a colorimetric sensor array capable of detecting ENMs in water, and identifying the ENM based on its surface chemistry. Comprised of an array of seven commercially available pH indicator dyes (acridine orange, bromocresol green, methyl blue, mordant orange, methyl red, methyl orange, and methylene blue), the sensor array offers a cost-effective and portable solution for identifying ENMs present in water samples. Previous work has shown that colorimetric sensor arrays of this kind can identify and quantify ENMs with different sizes, shapes, and core composition. Our project tested the sensor array's ability to distinguish between aspect ratio 4 gold nanorods (AuNRs) functionalized with different polyelectrolytes (PEs). The PEs displayed by the gold nanorod included: polyallylamine hydrochloride (PAH), polyacrylic acid (PAA), polystyrene sulfonic acid (PSS), and poly (diallyldimethylammonium chloride) (PDADMAC). The sensor's ability to distinguish between the AuNR surface chemistries was then probed using UV-vis absorbance spectroscopy. Changes in each dye's absorbance spectrum were then converted to RGB values, and using the RGB values, a colorimetric fingerprint unique (by eye) to each AuNR surface chemistry and concentration in the test library was generated. The selectivity and sensitivity of the sensor array was then quantified using Principal Component Analysis and Hierarchical Clustering Analysis.

4.21.P-Th-043 Assessment of heavy metal contamination in Atlantic Sargassum

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Pelagic Sargassum species are widespread macroalgae that form floating mats across tropical and temperate oceans, serving as vital habitats for a diverse array of marine organisms. Since 2011, however, massive influxes in the Atlantic and Caribbean have caused coastal inundation events, raising ecological and public health concerns. One emerging issue is Sargassum's high capacity to accumulate chemical contaminants, particularly heavy metals, due to its cell wall composition. This structure is rich in alginate and fucoidan, which facilitates multiple biosorption mechanisms (e.g., ion exchange, microprecipitation, chemisorption). This study investigates the accumulation of 22 heavy metals in Sargassum spp. collected from impacted coastal zones in Puerto Rico and St. Thomas. At each site, samples were collected inshore, midshore, and offshore. Samples were digested using microwave-assisted acid digestion and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) and direct mercury analysis. Fifteen metals were detected in all samples with As, Fe,

Al, Ba, and Mn occurring most frequently and As found in the highest concentrations. Preliminary results show significantly higher concentrations of Fe, Ba, Al, Se, Zn, U, and Cr in samples from Puerto Rico compared to St. Thomas. These findings underscore the role of Sargassum in the transport and potential biomagnification of trace metals through marine food webs, posing risks to both ecosystems and human health. This study contributes to baseline data for heavy metal contamination in Caribbean Sargassum and highlights the need for long-term monitoring and mitigation strategies in affected coastal regions.

4.21.P-Th-045 Defining unequivocal: Increasing confidence in the analysis and reporting of micro- and nanoplastic particles in biological matrices

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The identification of nanoplastics (<1µm) and small microplastics (<10µm) remains an analytical challenge, exacerbated in complex matrices such as environmental and human samples. Concerns regarding the potential impact of plastics on human health, has led to an increasing number of studies aiming to identify small micron and nanoplastics within human tissues. Mass based quantification methods such as pyrolysis gas chromatography mass spectrometry have seen increased application in identifying and quantifying small plastic particles in biological samples. However, this indirect analysis method is susceptible to sample matrix interference (e.g., due to the presence of lipids in biological tissues), resulting in quantification being unreliable for certain plastics such as polyethylene and polyvinyl chloride. Furthermore, no morphological information is provided, which is important toxicologically relevant information needed to inform accurate risk assessments. Optical microscopy coupled to spectroscopic methods such as Raman or Fourier-transform infrared are widely used for microplastic identification, however for smaller nano-sized particles, the resolution is not sufficient to accurately identify individual plastic particles. The accurate quantification and characterisation of particles within biological systems is critical to assess potential health risk. Therefore, this work proposes a framework to increase confidence by using multiple orthogonal techniques to measure plastics concentrations. The techniques use fundamentally different measurement approaches to measure the same physical properties of a suspected plastic particle. Three categories of techniques are proposed, based on the amount of information they provide about chemical identification, morphology, and other physicochemical properties. The application of multiple techniques is proposed to enable unequivocal identification within complex matrices. The limitations of each technique must be acknowledged and clearly communicated when data are reported within research to ensure transparency in results. The framework that reports confidence levels for identification of plastic particles will be presented, with suggested minimum data requirements listed. This framework is proposed as a starting point for future minimum standards regarding publishing human health data, which will be valuable for future risk assessment.

4.21.P-Th-047 Biotransformation of per- and polyfluoroalkyl substances during composting: Effect of aeration method

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Per- and polyfluoroalkyl substances (PFAS) have become an increasing environmental concern since their widespread adoption in the 1950s. These persistent chemicals are commonly found in consumer products including non-stick cookware, food packaging, textiles, and firefighting foams. When PFAS-containing products enter waste streams and reach composting facilities, the resulting contaminated compost creates widespread environmental problems. Application of such compost to agricultural lands and forest ecosystems leads to PFAS accumulation in plant life and wildlife, with concentrations becoming increasingly elevated

through biomagnification processes. Here, we investigated composting methods to determine whether they could reduce or mitigate the total amount of PFAS in the process, thereby reducing environmental and human health impacts. This laboratory-scale composting investigation examined how PFAS compounds undergo biotransformation when HBT mediator and laccase enzymes are introduced to composting materials. The experimental design involved compost containers with varying conditions, including different aeration levels, moisture content, aeration methods, and enzyme additions to promote chemical breakdown. Throughout the testing period, PFAS degradation was documented both within the compost matrix and in gases captured using granular activated carbon (GAC) filtration systems. Continuous airflow promoted detection of semi-volatile PFAS in the GAC and may also impact the release of degradation products into the atmosphere depending on the composting aeration method. This research aims to enhance understanding of PFAS decomposition mechanisms during composting of contaminated organic matter.

4.21.P-Th-048 Personal airborne exposome profiles reveal novel chemical associations with asthma severity and airway obstruction

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Although ambient air pollution is found to be associated with asthma, personal airborne exposome profiles are highly personalized and cannot be captured by ambient monitoring alone. Individual-level exposures to semi-volatile organic compounds (SVOCs) that could drive asthma severity and airway obstruction level remain poorly understood. We conducted a community-based study of 40 adults with asthma, stratified by severity (not severe-controlled n=16, not severe-not controlled n=12, severe-not controlled n=10) and obstruction level (mild n=20, moderate n=10, severe n=9). Participants wore silicone wristbands for 7 days to capture personal exposure to 114 SVOCs including phthalates, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and per- and polyfluoroalkyl (PFAS) substances. We assessed lung function, analyzed chemical concentrations using targeted exposomics, and applied network analysis to identify exposure clusters. Participants with severe airway obstruction level had significantly higher concentrations of butyl benzyl phthalate (2.95-fold, $p<0.1$) and PCB-132 (5.79-fold, $p<0.05$) compared to mild level. Similarly, participants with severe-not controlled asthma shows significantly higher concentrations of Diamyl phthalate (2.29-fold, $p<0.1$), PCB-33 (2.94-fold, $p<0.01$), PCB-87 (2.76-fold, $p<0.05$) and PCB-209 (2.2-fold, $p<0.1$). Among the detection of PAHs, Benzo[e]pyrene and Benzo[ghi]perylene also showed higher concentration in severe-not controlled asthma participants compare with ones in not severe-not controlled, suggesting asthma severity may influence by the different exposure pattern. Network analysis revealed three distinct co-exposure clusters and identified anthracene as strongly negatively correlated with lung function ($R=-0.70$, $p<0.001$). Di-cyclohexyl phthalate and PCB-132 showed unexpected positive associations with pulmonary function in mild airway obstruction ($R=0.46$ and $R=0.64$, respectively, $p<0.005$), suggesting complex dose-response relationships. Personal airborne exposome assessment using silicone wristbands identified novel associations between specific SVOCs exposures and asthma severity that ambient monitoring missed. These findings support shifting from ambient air quality monitoring to personal exposure assessment for identifying at-risk individuals and developing targeted interventions to reduce chemical exposures in vulnerable populations with respiratory diseases.

4.21.P-Th-049 Chemical exposomics reveals environmental contributors to lung cancer risk: A nested case-control study in the Pittsburgh lung screening study

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While 80% of lung cancers (LCs) occur in ever smokers, only 15% of ever smokers develop LC, indicating that genetics, radon, and other lifestyle and environmental factors influence LC development. Prior studies have found that environmental factors, such as air pollution, diesel exhaust, and pesticides, were associated with increased LC risk, however many of these studies relied on geospatial or self-reported data. The chemical exposome (CE), the combination of endogenous and exogenous compound exposures encountered by an individual, and untargeted exposomics are powerful approach can allow us to comprehensively evaluate the impact of individual-level chemical and metabolic exposures on LC risk. Among participants in the Pittsburgh Lung Screening Study Extension I (PLuSS-X), a cohort of heavy former and current smokers at high risk to develop LC, we conducted an “exposome-wide” analysis to identify associations between compounds detected in the CE and LC risk. High-resolution mass spectrometry was used to generate untargeted CE profiles in serum samples collected ~7 years prior to LC diagnosis from 122 case-control pairs in PLuSS-X that were matched on age, sex, smoking status, and time of sample collection; the two groups were balanced distributed, with mean age 63.8 in both groups, 73% male, 73% current smokers, a similar race distribution, and comparable pack-years at 63.4 in cases versus 60.4 in controls. CE data was processed and putatively annotated using TidyMass in R. We quantified peak areas for each compound as undetectable, below median, above median, and evaluated the association between each compound and LC risk using multivariable conditional logistic regression models. Among 2378 compounds included in our analysis, 63 and 41 compounds were associated with increased and decreased LC risk, respectively ($p < 0.05$). Notably, N-nitrosomorpholine (OR=2.04 [95% CI: 1.00,4.15]), is an IARC Group 2B carcinogens, where carcinogenicity has only been evaluated experimentally. N-acetylneuraminic acid (OR= 2.70 [95% CI: 1.02,7.13]), has been reported as a potential biomarker of lung cancer in previous study. These findings demonstrated the feasibility of using the CE to identify known and novel carcinogenic compounds in a population-based study of LC.

4.21.P-Th-050 Exploring the association between endocrine disrupting chemicals exposure in pregnant women and changes in oxidative stress related adducts

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Endocrine-disrupting chemicals (EDCs) such as per- and polyfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and more recently emerging micro- and nano- plastics (MNPs), are widespread environmental pollutants with the potential to disrupt hormonal signaling, metabolism, and neurodevelopment. EDCs exposure during pregnancy is of particular concern due to heightened maternal vulnerability and potential developmental impacts on the fetus. Oxidative stress (OS) is widely used as a health indicator as it reflects cellular damage, increased risks of certain chronic diseases, and general health status. Maternal blood (2 ml) was collected from pregnant women ($n = 60$) receiving care from Baylor College of Medicine, Houston, TX. Samples were taken during 2017 on the day of delivery, along with surveys concerning various exposure parameters and socioeconomic status of study participants (e.g., household income, race, and occupation, etc.). 45 mono- to nona- PCBs and 13 PFAS (9 carboxylates and 4 sulfonates) were analyzed through solid-phase extraction followed by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography–tandem mass spectrometry (LC/MS/MS), respectively. Five environmentally abundant MNP polymers (PS, PC, PP, PET, PMMA) and their oligomeric profiles were analyzed by sample homogenization, microfiltration (pore size: 0.7 μm) and pyrolysis–GC/MS. 16 Oxidative stress-related protein adducts (including reduced form of HSA, direct oxidation products, lipid peroxide, antioxidant-related biomarkers) and a normalizing peptide were assessed to evaluate potential relationships. Based on our initial results, PCBs were detected at concentrations ranging from 11.8 to 401 ng/ μL (average = 190 ng/ μL) with non- or mono-ortho-substituted PCBs to be 2.89 and 5.60 ng/ μL , respectively. PFAS concentrations ranged from 0.004 to 3.81 ng/ μL , with PFHxA being detected most frequently among targeted PFAS (> 80% of samples). Some PFAS compounds (e.g., PFHxA, PFHpA, PFDA) showed moderate associations with OS adducts (e.g., Cys34-S-O2), indicating a potential

relation between enhanced protein oxidation induced by PFAS. Among MNPs, polystyrene (37.1–59.2 ng/μL) was the most abundant polymer in terms of detected concentration. Statistical relationships between EDCs (e.g., MNPs and PFAS), and between EDCs exposure level and pattern and OS adducts will be presented.

4.21.P-Th-051 Pharmaceuticals in estuarine systems across a gradient of effluent contributions to base flow: Influences of population socioeconomics

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Billions of humans live within a few hundred kilometers of coastlines, but aquatic exposure information for pharmaceuticals and personal care products (PPCPs) is limited in coastal systems. This represents a key research need because PPCPs can present water quality risks, and further development and utilization of these systems for aquaculture and coastal fisheries will be required to sustainably meet future global food demand. To better understand the influence of PPCPs in effluent discharges on estuarine systems, we used a hydrological model to identify 14 estuarine systems with varying proportions of wastewater effluent contributing to base flows across the Gulf Coast of Texas, USA. We specifically utilized traditional grab sampling methods on study day 1 and study day 2, while also collecting a time-weighted composite sample over the 24-hr sampling period; all sites were sampled in the spring and the fall to examine potential seasonal variation. From these samples, we determined surface water concentrations of 30 PPCPs by isotope dilution LC-MS/MS, calculating mass loads for sites with available streamflow data. When sufficient data was available, we developed probabilistic environmental hazard assessments to examine potential exceedances of water quality thresholds. Exceedance of these values was limited for all studied compounds across both sampling seasons. We quantitated 22 target analytes in these coastal systems, including several benzodiazepines and glucocorticoids. Frequently detected compounds were identified at the highest concentrations in Houston, TX or Corpus Christi, TX, the two largest cities included in our sampling effort. Across both sampling seasons, we observed a significant ($p < 0.05$) negative relationship between the sum pharmaceutical concentration and the sampling site county health ranking, which is assigned based on a variety of community socioeconomic and health data. Similarly, a negative relationship was observed between the sum pharmaceutical mass load and county health ranking; this trend was significant ($p < 0.05$) only in the spring sampling season. These findings can inform future surface water quality monitoring efforts of PPCPs in coastal systems across gradients of urbanization, especially regarding prioritization of sites for further study.

4.21.P-Th-052 Development of a proposed temporary mercury variance criterion for a legacy contaminated site

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Human-caused conditions have resulted in mercury release and deposition in a small legacy contaminated watershed in central Arkansas near the Arkansas River. The concentrations of mercury cannot be reduced to the state human-health based water quality criterion of 0.012 μg/L total recoverable mercury. A mercury temporary variance was developed that provides a temporary mercury variance criterion for the watershed that is composed of a fish tissue criterion of 0.855 milligrams per kilogram (mg/kg) wet weight (ww) total mercury, and a corresponding translated water concentration of 0.174 μg/L total recoverable mercury. Both values serve as a single, protective chronic criterion in the mercury temporary variance. The proposed mercury temporary variance is necessary because despite efforts to reduce the discharge of mercury at the regulated outfall through operational improvements at the site, the total recoverable mercury concentrations at outfall remain out of compliance with the mercury limits imposed in the National Pollution Discharge Elimination System (NPDES) permit. The temporary variance criterion is considered temporary for the proposed duration of the temporary variance (20 years) and represents the highest attainable water quality condition (HAC) in the watershed

because these values protect the designated aquatic life use, and both fish tissue and surface water total mercury concentrations have occasionally been exceeded in the watershed, downstream and upstream of discharge from the outfall. Derivation of the temporary variance criterion relies on use of a site-specific tissue residue concentration (TRC) derived following a modification of the United States Environmental Protection Agency's (EPA's) equation to calculate the 2001 recommended TRC of 0.3 mg/kg MeHg where a conservative estimate of local fish intake was calculated by dividing the fish intake value between a conservative estimate of fish consumed from fish caught in the contaminated watershed and other local fish caught and consumed from a uncontaminated river downstream of the watershed. The site-specific TRC was translated to a water value using a site-specific bioaccumulation factor derived following EPA recommended procedures.

4.21.P-Th-053 Consistent and complete reporting of analytical methodology in micro- and nanoplastics in biota research improves confidence in and utility of data

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Multiple analytical methods are used to quantify micro- and nanoplastics (MNP) in biota; however, there has been limited examination of how well analytical method parameters are reported within studies. We surveyed 100 peer-reviewed publications that identified or quantified MNP in biota using atomic force microscopy (AFM), inductively coupled plasma (ICP), pyrolysis-gas chromatography-mass spectrometry (py-GC-MS), Fourier transform infrared spectroscopy (FTIR), Raman, or surface spectroscopy/scanning electron microscopy (SEM). Of the 100 articles data charted, the most prevalent analytical method was FTIR (40%), followed by Raman (27%), py-GC-MS (24%), surface spectroscopy/SEM (19%), ICP (-MS and -OES; 15%), and AFM (5%). Seventy-five percent included only one analytical method, 20% included two methods, and 5% included three methods. The level of detail in the description of methods was systematically evaluated, and most method-specific parameters had a low to moderate reporting rate (i.e., percent of articles that reported analytical method parameter details) in the primary and supplemental text; these findings informed the development of minimum reporting recommendations. The resulting recommendations are intended to encourage transparency and reproducibility via clear and complete reporting of analytical methods in peer-reviewed literature, which in turn will allow for the evaluation of study quality and facilitate the comparison of results within the field of MNP research, particularly MNP in biota samples. Ultimately, this will support the broader use of MNP characterization data in exposure and risk assessment activities.

4.21.P-Th-054 Bioaccumulation of 6PPD-Q in four species of adult salmonids returning to Puget Sound

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Stormwater runoff presents a range of issues for the aquatic life that are exposed to the complex mixtures of contaminants it contains. One contaminant, 6PPD-Q, is of particular interest due to its ubiquity anywhere roads are present and its acute toxicity to some species of salmonid. While 6PPD-Q is known to be widely present in freshwater systems, its occurrence and concentration in the tissues of free-ranging salmon is very poorly understood. We present results of the first study evaluating detection frequencies and concentrations of 6PPD-Q in four species of adult salmon (Chinook, coho, chum, and pink salmon) returning to different watersheds across Puget Sound. We also examine file to whole-body 6PPD-Q relationships for a subset of fish (n = 24) to assess the utility of each matrix for food web studies and monitoring efforts. Our results largely support the findings of laboratory and field studies examining other species or regions that indicate the limited bioaccumulation potential of 6PPD-Q. However, given detection frequencies were near 50% and most detections occurred at or near quantitation limits, these data indicate exposure could be relatively common throughout Puget Sound. Our results provide Sound-wide baseline data to inform exposure and risk associated with salmon consumption, for both human and marine mammal populations. Future efforts quantifying more

persistent and bioaccumulative tire-wear compounds in Puget Sound biota would greatly enhance the ability of monitoring programs to understand how widespread exposure to these chemicals is and guide stormwater mitigation efforts.

4.21.P-Th-055 Henry's Law constants and vapor pressure for per- and polyfluoroalkyl substances (PFAS): Implications for vapor intrusion

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To evaluate which subsurface contaminants have sufficient volatility to warrant consideration of exposure potential by vapor intrusion (VI), the United States Environmental Protection Agency (USEPA) has established criteria for Henry's law constant (HC) and vapor pressure (VP): greater than or equal to 10⁻⁵ atm-m³/mol or 1 mm Hg, respectively. To enable assessment of whether per- and polyfluoroalkyl substances (PFAS) pose VI pathway concerns, this study compiled a comprehensive and extensively curated dataset that includes 424 HC values and 563 VP values for 30 PFAS from seven classes, including fluorotelomer alcohols (FTOHs); perfluoroalkane sulfonamidoethanols (FASEs); perfluoroalkane sulfonamides (FASAs); perfluoroalkyl carboxylic acids (PFCAs); perfluoroalkyl sulfonic acids (PFASAs); perfluoroalkyl ether acids (PFECAs); and fluorotelomer carboxylic acids (FTCAs). The dataset was compiled using a three-step protocol: first, experimental and model-calculated data were mined from the peer-reviewed literature and widely used chemical databases such as CompTox and CAS SciFinder. Second, a semi-automated digital curation process was applied to expand the dataset and cross-validated it with data from the initial manual curation. The resulting data set was subjected to further correlation analysis, with comparison of mean and median representative values for measured and model-predicted values, older and newer data, etc. Approximately 80% of HC values and 34% of VP values exceed the USEPA VI criteria, suggesting that VI could be an important exposure pathway for PFAS.

4.21.P-Th-056 Partitioning and half-life of 6PPDq in Bellingham Bay sediments

Ayden Mudd, Jordan Perkins and Karin Lemkau, Western Washington University

One ubiquitous rubber additive used in tires is 6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine), which helps prevent breakdown due to interaction with reactive oxygen species. When 6PPD interacts with atmospheric ozone, it forms 6PPD-quinone (6PPDq). 6PPDq is a contaminant of particular interest in the Pacific Northwest due to its acute toxicity to coho salmon. Based on the octanol-water partitioning coefficient, 6PPDq is expected to sorb to the organic material present in soils and sediments, where it may be preserved and potentially re-released into the environment. While studies have been conducted on the partitioning of 6PPDq with engineered soils to mitigate exposure, there has been limited research on the partitioning of 6PPDq in natural environmental sediments. Additionally, the half-life of 6PPDq in these natural environments is not well characterized. Here, we report preliminary data on 6PPDq partitioning in natural sediments and estimates of the environmental half-life of 6PPDq in sediments collected from Bellingham Bay.

4.21.P-Th-057 Mapping polycyclic aromatic hydrocarbons (PAHs) in two Utah Cities using pine needles as passive samplers

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Public health interventions frequently require invasive methodologies to acquire information on a given area. A unique project across two different urban areas along northern Utah's Wasatch Front aims to assess community exposure to polycyclic aromatic hydrocarbons (PAHs) by combining passive and dynamic air pollution monitoring with wastewater monitoring for metabolic markers from the target compounds. This approach has the potential to shift public health monitoring towards more targeted and less invasive interventions in local communities. The initial list of targeted atmospheric PAHs include pyrene, phenanthrene, naphthalene,

fluorene, and the nitrogenated PAH 1-nitropyrene. The two targeted cities are Rose Park and Spanish Fork, two economically, demographically, and topographically different cities located near Salt Lake City, UT. Phase I of the project included the involvement of high school (Salt Lake Center for Science Education) and middle school (Spanish Fork Middle School) students with the collection of needles from random conifer trees within the targeted collection zones. Each city was divided into spatial polygons representing differing sewer sheds, in line with future planned wastewater collection locations. The students were trained in proper sample collection and tree identification techniques, and, with the help of their local instructors, obtained samples from 3-5 separate trees within each specified polygon. The laboratory analysis is nearing completion and consists of measuring the wet and dry needle weight, determination of extractable lipid content, separation of target PAHs via Accelerated Solvent Extraction (ASE), and finally quantification via Gas Chromatography with Tandem Mass Spectrometry (GC-MS-MS). Initial lipid tests of the Spanish Fork samples have shown an average lipid content (wet weight) for Blue Spruce to be 1.5% with a variance $\leq 1\%$ observed between samples and an average lipid content for the single Austrian Pine sample to be 2.75%. Past research suggests lipid fraction may be important in parameterizing the partitioning of the PAHs into the needles. The finalized data from these investigations will be compiled, mapped, and presented. As the project moves onto subsequent Phases, successful mapping of PAH “hotspots” across the communities will inform the investigators of likely locations for future dynamic atmospheric sampling campaigns (via pumped filter and sorbent collection methodologies).

4.21.P-Th-058 Tracking 6PPD-quinone concentrations in parking lot runoff during rainfall events

Jordan Perkins, Ayden Mudd and Karin Lemkau, Western Washington University

6PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine) is a chemical commonly added to tires to increase rubber durability by minimizing oxidative damage. When 6PPD interacts with atmospheric ozone, it undergoes a chemical transformation, producing 6PPD-quinone (6PPD-q), a compound now known to be highly toxic to coho salmon. Aquatic 6PPD-q concentrations are expected to increase the closer the site is to a road or parking lot and concentrations of 6PPD-q in sediments and runoff near busy roads are well documented. However, there is limited research assessing 6PPD-q concentrations in runoff throughout a rain event. Here we examine the transport of 6PPD-q following a rain event near a heavily used parking lot by collecting runoff samples before, during, and after rainfall events with the use of an autosampler. This study aims to track the concentration of 6PPD-q throughout rain events and identify when concentrations in runoff are highest.

5.01.P-Th Advances and Applications of Probabilistic Risk Assessment

5.01.P-Th-099 Probabilistic approaches for risk assessment and regulatory criteria development:

Regulatory landscape, gaps and opportunities

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Traditional ecological and human health risk assessment often relies on deterministic frameworks that preclude characterization of variability or uncertainty among input parameters that determine exposure, effects, and risk. To generate more realistic and robust risk management decisions, probabilistic risk assessment (PRA) can be used to characterize variability and uncertainty in risk evaluation parameters. Despite existing federal and state PRA regulatory guidance and the existence of multiple robust PRA methods (e.g., Monte Carlo simulations, Bayesian networks), PRA remains underutilized in risk assessment and associated regulatory frameworks. Here we discuss the current regulatory support of PRA among states and federal agencies, challenges that may limit more widespread use, and opportunities for the expanded use of PRA in regulatory areas where it is not currently applied. Taken together, we hope to promote the understanding that probabilistic methodologies provide significant advantages in their versatility for robust, transparent, data-based environmental risk assessment and standards derivation across a range of media, and that PRA approaches align with regulatory objectives to protect aquatic and terrestrial biota, human health, and vulnerable populations.

5.01.P-Th-100 Probabilistic risk assessment approaches better protect susceptible populations

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Across all of its program areas, the United States Environmental Protection Agency conducts risk assessments to evaluate the potential adverse effects of environmental hazards on ecological and human health. Traditionally, these assessments rely on deterministic methods that use point estimates for key parameters and incorporate safety factors and precautionary assumptions to account for uncertainties in data and variability in environmental conditions, exposure pathways, and population characteristics. However, these approaches are unnecessarily conservative for the general population yet fail to transparently account for the vulnerabilities of susceptible populations. Probabilistic risk assessment (PRA) offers a more refined approach that utilizes distributional data to better characterize uncertainty and response. By leveraging empirical data and probabilistic modeling, PRA allows for a more transparent, precise quantification of risk that ensures targeted protection for susceptible populations. This article examines how PRA enhances each phase of risk assessment - hazard identification, dose-response assessment, exposure assessment, and risk characterization - to produce a more scientifically robust assessment of risk for susceptible populations. We review the literature and discuss practical applications of PRA in regulatory contexts to illustrate its advantages over deterministic approaches. Finally, we discuss key implementation challenges and propose future research directions to advance risk assessment methodologies and improve policy decision-making. **Disclaimer/Disclosure:** J. Coyle, B. Barnhart, and G. Johnson are employees of NCASI.

5.01.P-Th-101 Improving risk assessment of land-applied biosolids with probabilistic approaches

J. Sain, Paul Wiegand, Brad Barnhart and Camille Flinders, NCASI Inc.

Land application of municipal and industrial wastewater biosolids for use as a fertilizer or soil conditioner is a common practice in the United States. Regulations are in place to reduce pathogens, minimize disease vectors, and limit concentrations of nutrients and some metals, but extensive assessment of the risk of biosolids-associated chemicals to human health and environmental systems is uncommon. To facilitate a more comprehensive chemical risk assessment of land-applied biosolids, the United States Environmental Protection Agency recently developed the Biosolids Tool (BST) based on a deterministic approach that utilizes conservative model inputs without regard for the variability and uncertainty inherent in environmental exposures. However, management decisions based on probabilistic risk assessment, in which variability and uncertainty are quantified and risk is linked to specific population segments, may provide a more accurate understanding of risk. To better understand how deterministic and probabilistic risk assessment methods compare for characterizing risk, the current study examined the sediment risk assessment literature and explored the application of probabilistic model inputs within the BST. The BST model results for noncancer and cancer risk outcomes associated with total ingestion of aluminum and benzo(a)pyrene in biosolids applied to pastureland indicated that probabilistic risk assessment provides a more nuanced understanding of risk than the traditionally used deterministic approach. Findings from this study and those found in the sediment risk assessment literature underscore the need for incorporating probabilistic methods into regulatory frameworks to improve the accuracy and reliability of risk assessments for biosolids land application.

5.01.T Advances and Applications of Probabilistic Risk Assessment

5.01.T-01 Why are probabilistic risk assessments so rarely used: A consequence of improved risk characterization

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United States Environmental Protection Agency (USEPA) guidance recognizes the key advantage of probabilistic risk assessments (PRAs) when assessing the risk of chemicals in the environment: a more complete, realistic and accurate characterization of potential risk. That improved characterization, compared to

characterization provided by deterministic risk assessments, should improve management of chemicals in the environment. Yet many programs that regulate chemicals in the environment almost never use PRAs to evaluate the risk of or set allowable levels of chemicals in the environment. Why? Early in the history of PRAs, limited availability of the methodologies, computer programs and put input data as well as the absence of USEPA guidance necessary to conduct PRAs precluded their widespread adoption. Those are available now and have been for decades, yet use of PRAs remains almost non-existent. Two reasons become apparent when comparing deterministic to probabilistic characterizations of risk. First, risk management decision-making becomes more challenging. Evaluating whether a risk is allowable using deterministic methods consists of comparing a point estimate of risk to an allowable risk level. When the full distribution is revealed, in most instances, the majority of the distribution is below the allowable risk level, but a portion is above. Determining whether the distribution of risk is allowable becomes substantially more complicated as it raises the question of how large a portion can be above and by how much? Second, a deterministically derived point estimate of risk hinges on several point estimate exposure and toxicity assumptions. Selection of a point estimate for each assumption affects the deterministic point estimate of risk and, thus, whether that estimate is above or below the allowable risk level. With deterministic risk assessment methods and knowing the allowable risk level, risk assessors selecting those assumptions have the power to greatly affect risk management decision-making. With PRA, risk assessors lose that power because substantially altering distributions of risk that are based on full distributions for most input assumptions, is much more difficult than selecting point estimates to increase or decrease point estimates of risk. Expanded risk management guidance is suggested as a path to expand the use of PRAs when regulating chemicals in the environment and more clearly distinguish risk assessment from risk management.

5.01.T-02 Derivation of region-specific probabilistic human health water quality criteria using a novel shiny tool

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The United States Environmental Protection Agency (USEPA) uses a deterministic, risk-based framework to derive national recommendations for ambient water quality criteria protective of human health (AWQC) through the ingestion of water and aquatic organisms. The deterministic approach has faced criticism for relying on multiple high-percentile input parameters, leading to criteria disconnected from actual risk. Probabilistic risk assessment (PRA), in particular, represents the best-available science for transparent, risk-based criteria derivation, especially when considering subpopulations which disparate aquatic organism consumption patterns. Here, we examine the influence of regiospecific fish consumption rates in AWQC derivation using the recently developed R Shiny tool, Surface Water Probabilistic Risk Online (swPRO). swPRO was developed to conduct deterministic- and probabilistic based derivation of AWQC, with a particular focus on customized distributions for key exposure input parameters, such as fish consumption rates. Leveraging swPRO, regiospecific fish consumption rate (FCR) cumulative distributions published in the USEPA's Exposure Factors Handbook were used to derive AWQC. Among probabilistic exposure scenarios, AWQC also decreased with increasing FCR, with criteria generally higher in regions where FCRs were lowest (i.e., Inland Midwest, Midwest, and the Great Lakes), and lower in regions where FCRs were highest (i.e., Pacific, Gulf of Mexico, and Atlantic regions). Standardized AWQC ranged from 0.2 to 7, with the plurality below 1.0, indicating their relative stringency compared with US EPA national recommendations. Findings from this study demonstrate that the use of the probabilistic approach for deriving AWQC using swPRO provided greater flexibility in producing AWQC that are locally relevant and consistent with US EPA's recommendations while containing the entire analysis within one analytic 215. Human health risk assessment and subsequent regulatory criteria derivation can be improved by aligning regulatory guidance with modern methodologies, such a PRA, while ensuring transparent communication of risk.

5.01.T-03 Sensitivity analysis of input variables used in human health surface water quality criteria derivation: A case study using PFOS

Betsy Ruffle¹, Gemma Kirkwood¹, Kelly Vosnakis¹, Craig Warren Davis², Paul G. Koster van Groos² and Anita

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The derivation of surface water quality criteria (SWQC) uses a risk-based methodology with inherent assumptions and approaches. The relative importance of various inputs and derivation approaches to the calculated SWQC is not well characterized and understanding of variability and uncertainty is limited. Sensitivity analysis is a useful tool for determining which variables are key sources of uncertainty and have the biggest impact on the results of an analysis. This study presents the results of a quantitative sensitivity analysis to explore which input parameters have the greatest influence on human health SWQC derivation using perfluorooctane sulfonic acid (PFOS) as a case study. Probability distributions were used for the input parameters in the equation for calculating SWQC. The probability distributions were based on published data and studies and capture the range of values used by several US states and Europe in their SWQC derivation. In addition to exposure variables, the analysis included probability distributions for the range of animal and human-based noncancer toxicity factors for PFOS. The relative importance of each input parameter to the 10th percentile SWQC was assessed using tornado diagrams. The toxicity factors used for PFOS, as well as bioaccumulation factors and fish consumption rate significantly influence the SWQC. The results of this work improve understanding of key areas of uncertainty and provide insight on data gaps and potential areas for refinement.

5.01.T-04 Requirements and applications of statistical analysis to design monitoring programs coupled to probabilistic ecological risk assessment for the adaptive management of large scale systems

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An impediment to the wider dissemination of probabilistic risk assessment to the management of the environment is the challenge of matching experimental design, monitoring programs and data analysis to the requirements that fit the dynamics of ecological systems. Ecological systems are defined as complex systems with nonlinear deterministic dynamics coupled to stochastic features. Managers are tasked with engineering such systems often at large geographic areas, for extended time periods, with external drivers (climate change) in order to meet societal demands for a variety of ecosystem services (food, water, recreation, building materials and protection of sites and species of cultural value. Examples such as the Long Term Receiving Water Study monitoring program, analysis of forestry management for the INLAS study area, the estuaries surrounding Brisbane, Australia, rivers discharging into the Salish Sea and Columbia River of Washington State, and the Upper San Francisco Estuary will be used to point out the applicability of exposure-response curve fitting, multivariate analysis tools to describe community structure (as defined by eDNA) and to evaluate causality. The use of these tools will also guide the design of monitoring programs to evaluate causal pathways, detect patterns in the dynamics of water quality. Based on the lessons learned from these studies specifications for the design of long-term monitoring programs are proposed. These approaches and tools should be part of any risk based cumulative effects monitoring-response program for adaptive management.

5.01.T-05 Advancing impact assessment: Evaluation of a probabilistic approach to incorporate emission variability in air modeling

Zachery Emerson and Tanvir Khan, NCASI Inc.

Traditional air dispersion modeling often relies on deterministic frameworks that use multiple conservative assumptions as inputs. For example, atmospheric pollutant concentrations are often overestimated by USEPA's AERMOD tool due to the use of maximum emission rates for point sources. This approach does not accurately reflect real-world conditions, particularly for scenarios with multiple sources that have variable emission rates. A better representation of variability in ambient air concentration estimates will enable stakeholders to gain a better understanding of the impact of point source emissions, relative to default assumptions. To better understand how real-world emission variability affects modeled pollutant concentrations, we have developed a probabilistic Monte Carlo screening method to develop emission rate inputs for use in a deterministic model (AERMOD). Our previous work has focused on the development of the approach and application to a 476

facility. This framework supports more informed risk assessments and may offer a pathway for improving the balance between environmental protection and realistic industrial operating conditions. This presentation will discuss the application and evaluation of our approach to the Denver-Julesburg AERMOD model evaluation database. This dataset offers a unique combination of direct measurement of variable emission rates from low elevation point sources paired with multiple downwind monitors close to the fenceline. A comparison of estimated concentrations to actual measurements and to the model results from default permitting approaches will be included in the discussion.

5.01.T-06 Monte Carlo modeling to assess variability in food web models for PFAS

Jason M. Conder, Jennifer Arblaster, Elizabeth R Nichols, Zach Pandelides, Paige A. Leitman, Caitlin Johnson and Jean Zodrow, Geosyntec Consultants, Inc.

The abundance of abiotic and tissue data at PFAS field sites that have emerged in the last few years allowed our team to complete validation of aquatic and terrestrial food web models for per- and polyfluoroalkyl substances (PFAS). This research allows us to better understand the current performance of the aquatic and terrestrial bioaccumulation models and determine whether any improvements or updates are warranted. This validation process used abiotic data for soil, sediment, and surface water and paired tissue data from high-quality field studies of PFAS in aquatic and terrestrial environments. As part of the model validation effort, the variability of the model output based on the variability of the input parameters was assessed by performing a Monte Carlo analysis. Soil PFAS concentrations, sediment PFAS concentrations, surface water PFAS concentrations, and bioaccumulation factors were assigned probabilistic distributions for the Monte Carlo analysis as part of the model results. Standard deviations for each parameter were obtained using available study data and those standard deviations were used to generate 95 percent confidence intervals for each parameter. Model agreement was robust for forage fish, with almost half of the modeled tissue concentrations within a factor of 5 of the measured values. Modeled tissue concentration were also good predictors of measured concentrations at higher PFAS concentrations that would be expected at AFFF sites. Overall, 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 for these studies. The validation effort lends further confidence to the aquatic and terrestrial PFAS food web models and its use in determining potential risk to ecological receptors at hazardous waste sites impacted by PFAS.

5.02.P-Mo Advances in Human Health Risk Assessment

5.02.P-Mo-117 Approaches for human biomonitoring in carcinogenic minerals through a biomarker of damage: The erionite case

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Erionite exposure causes malignant pleural mesothelioma (MPM). A rural community in Guanajuato State, Mexico, has presented a high incidence of MPM, associated with erionite exposure. Erionite fibres are inhaled, and there is no way to biomonitor its exposure in blood or urine; here, we measured mesothelin and osteopontin serum levels to calculate the risk for developing MPM in healthy inhabitants, from the mentioned community. We recruited 60 participants (40 children and 20 adults) exposed to erionite and compared them with a control group paired by: sex, age and occupation (40 children and 22 adults) without exposure to the mineral. In both groups we quantified mesothelin and osteopontin serum levels. In the adult exposed group, mesothelin levels were significantly higher (23.5 ± 8.6 ng/ml) than in adult control group (15.7 ± 5.0 ng/ml; $p=0.003$); also, a strong association between serum mesothelin levels and residence place in the adult exposed group was observed ($\beta=17.6$, $p=0.005$). We did not find differences between osteopontin serum levels in both groups ($p>0.05$). For children, we did not find differences for both molecules between groups. Mesothelin has the potential for becoming an indirect endpoint for erionite biomonitoring in erionite-exposed populations.

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Secretary from Guanajuato State Government, and also we want to thank to the National Council of Science and Technology (México) for the scholarship to the graduate student (L. Ruiz-García)

5.02.P-Mo-118 Human health risk evaluation for recreating on Coeur D'Alene Lake and the Spokane River

Jeremy Johnson, Alta Science & Engineering, Inc.

Coeur d'Alene Lake is Idaho's second largest lake and a popular recreational destination, an economic catalyst for North Idaho and Eastern Washington, and the heart of the local communities. The lake is part of the CDA Tribe's indigenous Territory, and the southern third of the lake falls within their current Reservation boundary. Due to decades of historical mining activity in the "Silver Valley," millions of tons of metals-laden sediments were introduced into the CDA River and CDA Lake and remain present in the sediments on the lake bottom today. Given its location and popularity as a recreation and tourist destination, the Lake was the focus of a 2022 National Academies of Sciences, Engineering, and Medicine Study that recommended a lake-focused human health risk assessment be developed to evaluate exposures to people who frequent the lake. In response to the NAS recommendation, the Idaho Department of Environmental Quality and Alta Science and Engineering, Inc. (under contract to DEQ) have undertaken an expanded screening level risk evaluation to assess current metals concentrations and potential resulting human health risks in CDA Lake and the Spokane River. A flexible and site-specific approach was used to evaluate health risks for the unique exposure pathways present. This includes the derivation of site-specific risk-based screening levels and expanding upon the typical health screening evaluation by 1) considering site-specific bioavailability data and 2) evaluating newly collected contaminant data relative to local background soil/sediment data. The purpose of this presentation is to provide the results of the human health risk evaluation, including a summary of the sediment, soil and surface water sampling data collected in summer 2024 and the site-specific information and exposure pathways used to derive risk-based screening levels.

5.02.P-Mo-119 Are you sure you want to eat that? Insights from environmental monitoring and physiologically-based pharmacokinetic modeling of trophic transfer of PCBs from fish to humans

Sam Nuttle, Adam M. Simpson, Olivia Hodgson, Ashley Russell, Cody Wood and Jeremiah Keyes, Penn State Behrend

Documenting exposure routes and relative accumulation of legacy contaminants, such as polychlorinated biphenyls (PCBs), in humans is complicated by the ubiquity of contamination. While guidelines to limit exposure are disseminated to the public (i.e., consumption advisories), the efficacy of these guidelines in limiting exposure and accumulation are difficult to evaluate using human monitoring. Combining environmental monitoring of legacy contaminant sources with physiological-based pharmacokinetic (PBPK) modeling may provide an approachable mechanism to document accumulation based on consumption advisories. Using this novel approach, we evaluated PCB tissue concentrations in five Lake Erie fish species and used PBPK modeling to predict PCB accumulation in the tissues of men and women under different consumption scenarios according to Pennsylvania consumption advisories. Twenty-one congeners were detected between the five fish species at concentrations ranging from 56.0-411.7 ng/g. Accumulation in humans varied with tissue type, biological sex, the species consumed, and consumption rate, but following consumption advisories was expected to result in minimal adverse effects based on PCB toxic equivalencies. Therefore, our data support the utility of fish consumption advisories for limiting human exposure, if followed. Furthermore, this study demonstrates the potential of combining environmental monitoring with PBPK modeling when interested in tracking human exposure. The ability to modify model parameters based on the specific exposure scenario of interest are unlimited, such that factors that traditionally confound similar studies, such as finding willing test participants and determining the influence of multiple exposure routes on accumulation, can be avoided allowing insight into the effectiveness of existing and proposed guidelines.

5.02.P-Mo-120 Best practices when using theoretical leach test vs equilibrium partitioning to derive soil migration to groundwater levels at AFFF impacted sites

Nadia Podpora, GHD

Aqueous film-forming foam (AFFF) was introduced in the mid-1960s and underwent a number of formulation changes in the chemical makeup of fluorinated alkyls through the years. Today, the use of long-chain PFAS manufactured by the electrochemical fluorination process has been predominantly replaced by the use of short-chain PFAS manufactured using the fluorotelomerization process. When an AFFF release occurs, PFAS constituents in AFFF are dispersed to environmental media, typically soil or surface water. However, these constituents may also migrate from soil into groundwater. Using existing generic approaches in the US, soil screening levels can be calculated to ensure groundwater used as drinking water is protected in the future. In its Soil Screening Guidance, the USEPA identified multiple approaches for identifying the need for and evaluating the completeness of cleanup for constituents in soil that could leach into groundwater. The Interstate Technology and Regulatory Council (ITRC) recognized that the leach test method could be used as a theoretical worst case evaluation tool, without collecting separate samples for laboratory analysis. Therefore, both approaches can use typical characterization data collected after an AFFF release to evaluate where remediation is necessary and when remediation is complete. Specifically, the equilibrium partition and theoretical worst-case mass limited leach test methods can be used to derive soil screening levels for PFAS that would be protective of groundwater uses within the area of a release. Multiple factors must be considered when selecting a suitable method to ensure an appropriate, and defensible, screening level is used for soil. Factors include: the conceptual site model, the age and nature of the source (finite or infinite), environmental factors, and the physical chemistry properties of the constituent released. These elements will all influence the fate and transport of PFAS and must be contemplated when selecting a method to calculate soil levels. This presentation will summarize the key assumptions, benefits, and differences associated with two of USEPA's methodologies to derive soil migration to groundwater levels; the equilibrium partitioning and the theoretical leach test. The characterization of AFFF formulae and their respective behaviors in the environment will also be included to support where each method may be more appropriate based on the type of release and site-specific factors.

5.03.P-Tu Advances in Understanding Hydrocarbon Toxicity: Impacts, Mechanisms and Mitigation

5.03.P-Tu-067 A confirmatory assessment of mineral oil sediment toxicity to *Leptocheirus plumulosus*

David Daniels¹, Chris Holmes¹, Eric Litman², Aaron Redman³ and Thomas Parkerton⁴, (1)Environmental Enterprises USA, Inc., (2)NewFields, (3)ExxonMobil Biomedical Sciences, Inc., (4)EnviSci Consulting LLC

Mineral oil is commonly defined as a mixture of C15-C50 aliphatic hydrocarbons. Previous studies indicate mineral oil is too insoluble to cause toxicity to aquatic life. In contrast, exposing benthic organisms to sediment spiked with mineral oil has been shown to cause adverse effects. While existing data indicate differences in species sensitivity with LC50s ranging from ca. 1 to > 60 g/kg dry, observed toxicity is at least an order of magnitude lower than reported for polyaromatic hydrocarbons. However, in a preliminary study by Stanley et al. (2010) ET&C 29:2796, the reported LC50 for 10 d toxicity tests for mineral oil with *Leptocheirus plumulosus* were in the range of 0.1-0.2 g/kg dry. These values are lower than previous data and comparable with effects concentrations reported for polyaromatic hydrocarbons. Moreover, these results are inconsistent with lack of sensitivity observed for *Ampelisca* (LC50 ca. 20 g/kg dry), another marine benthic amphipod. To determine if these study results could be independently reproduced, a targeted project was initiated using the same heavy mineral oil. In addition, a light mineral oil from the same supplier (Sigma Aldrich) was also investigated. The heavy and light mineral oils were characterized using GC-FID analysis and were found to contain different proportions of aliphatic hydrocarbons with 68 and 94% in the C19-C36 range, respectively. To confirm these mineral oils did not cause aquatic toxicity, *L. plumulosus* were exposed in 10 d toxicity tests to water accommodated fractions prepared at 1 g/L loading. As expected, neither oil exhibited aquatic toxicity. The two mineral oils were spiked volumetrically to natural sediments at varying nominal concentrations that were confirmed analytically. Organisms were then exposed to spiked sediments to assess 10 d toxicity. Control

performance and water quality were found acceptable in all tests. Results of *L. plumulosus* sediment toxicity tests indicated significantly higher LC50s than reported by Stanley et al. 2010 and thus did not confirm early findings. The influence of oil type and sediment properties on mineral oil sediment toxicity will be discussed. The limitations of using the Stanley et al. study to support the derivation of a preliminary risk goal at contaminated sites will also be highlighted.

5.03.P-Tu-068 Assessment of OSPW toxicity using toxicity tests with rainbow trout paired with membrane introduction mass spectrometry analysis of naphthenic acids

Josh Baker¹, Howard Bailey¹, Joseph Monaghan², Dylan Steenis², John V. Headley³, Ian Vander Meulen⁴ and Erik Krogh², (1)Rise Over Run Environmental, Canada, (2)Vancouver Island University, Canada, (3)Watershed Hydrology and Ecology Research Division, Environment and Climate Change Canada, Canada, (4)Environment and Climate Change Canada, Canada

The aquatic toxicity of oil-sands process water (OSPW) is of significant interest, and understanding the toxic components of these complex mixtures is an important factor in the analysis of risk and associated regulation. While numerous studies have been conducted on the toxicity of OSPW, they have varied as much in their methodology (e.g., test species, sample fractionations), as their conclusions (e.g., magnitude of toxicity, causative agents). In this study, we build on our previous work, which demonstrated that the toxicity of OSPW samples to rainbow trout could be predicted by the concentration of classical 'O2' naphthenic acids (NAs) and, more importantly, by specifically attributing the potency associated with individual NAs as a function of their respective carbon numbers (C#). New toxicity data with rainbow trout were obtained from tests conducted with OSPW samples, OSPW sample extracts, and a commercially-available NA standard. Of particular importance, a novel application of Condensed-Phase Membrane Introduction Mass Spectrometry (CP-MIMS) coupled with high-resolution mass spectrometry enabled on-line sample clean-up for the molecular assignment of classical NAs and subsequent derivation of potency estimates. The ability of the polydimethylsiloxane membrane used in CP-MIMS to quantitatively extract the toxic components within OSPW was investigated by conducting toxicity tests on unextracted and membrane-extracted samples, and reconstituted samples of the extracted permeate. Finally, the use of the CP-MIMS method to detect the presence of extremely low concentrations of higher C# NAs was also explored, as this is a major analytical consideration due to the substantially higher potency (100X) of these compounds in comparison to the lower C# NAs. The results and future applications of this research will aid in further understanding the toxicity of naphthenic acids and oil sands process water, as well as lead to implementation of rapid throughput high resolution analytical techniques.

5.03.T Advances in Understanding Hydrocarbon Toxicity: Impacts, Mechanisms and Mitigation

5.03.T-01 Assessing the toxicity of photo-oxidized oil using biomimetic extraction and bioassays with lobster larvae

Christoph Aeppli¹, Thomas Parkerton², Caitlyn Olson¹, Benjamin Jourdan³, Danielle Philibert³ and Aaron Redman⁴, (1)Bigelow Laboratory for Ocean Sciences, (2)EnviSci Consulting LLC, (3)Huntsman Marine Science Center, Canada, (4)Exxon Mobil

Photo-oxidation alters the chemical composition and environmental behavior of petroleum, with potential implications for the toxicity to aquatic organisms. However, the relationship between photooxidative transformation and toxicity remains poorly understood. Here, we evaluated whether biomimetic extraction (BE), based on solid-phase microextraction with GC-FID (SPME-GC-FID), can detect toxicity-relevant changes in irradiated oil, and we assessed corresponding biological responses using lobster larvae (*Homarus americanus*). Two crude oils—Basrah Light and Hoover Offshore Oil Pipeline System (HOOPS)—were irradiated for up to 72 hr under two different setups: (1) “dry” irradiation on a glass surface followed by water-accommodated fraction (WAF) preparation, and (2) “wet” irradiation, in which oil was irradiated directly on the water surface during WAF generation. Inclusion of both approaches for simulating photooxidation allowed comparison of resulting transformation pathways. BE profiles, PAH and alkane concentrations, and dissolved

organic carbon (DOC) were measured. Toxicity of the resulting WAFs were assessed using 48-hr immobilization assays with lobster larvae. Irradiation caused clear chemical transformations, evidenced by altered PAH ratios and chromatographic profiles, even when overall BE signals were similar between irradiated and dark-control oils. Photooxidized oils also exhibited elevated DOC, indicating the formation of water-soluble transformation products. Despite these changes, overall toxicity was low across treatments. A slight increase in toxicity was observed for oils irradiated for 8 hr (using “dry” photooxidation), but this trend did not persist at longer irradiation times. Our findings suggest that photooxidation increases the polarity and solubility of oil components, without significantly influencing acute lobster toxicity. BE proved sensitive to compositional changes and may be a useful tool for assessing toxicity potential in weathered oils. Differences between dry and wet irradiation highlight the influence of experimental approach, with dry photooxidation progressing more rapidly due to uniform film thickness. This work advances our understanding of how weathering modulates hydrocarbon toxicity and underscores the value of integrated chemical-biological assessments for oil spill impact modeling and mitigation strategies.

5.03.T-02 Position-specific methylation drives AHR dependent fin duplication phenotype from 8-methylbenzo[a]pyrene exposure in zebrafish

Mackenzie Leigh Morshead, Lisa Truong and Robyn Leigh Tanguay, Oregon State University

Polycyclic aromatic hydrocarbons (PAHs) are an environmental contaminant class characterized by fused aromatic rings. PAHs are released through the incomplete combustion of organic materials as well as petrogenic sources like oil and gas. Benzo[a]pyrene (BaP) is one of the most well studied PAHs and is known to cause oxidative stress and cancer through the formation of DNA adducts and DNA damage. In larval zebrafish studies BaP causes behavior effects and but no observable morphological effects at 120 hr post fertilization. We have found that 8-methylbenzo[a]pyrene causes a distinct fin duplication phenotype at low concentrations and severe edema and mortality at higher concentrations. This contrasts with benzo[a]pyrenes alkyl-substituted in different positions (6-, 7-, 9-, and 10-methylBaP) which do not elicit any morphological effects. By utilizing knockout lines, we identified that the effects of 8-methylBaP are AHR dependent and Cyp1a independent while a previously identified fin duplication causing PAH (benzo[k]fluoranthene) was both AHR and Cyp1a dependent. This suggests a potentially novel mode of action for 8-methylBaP. To identify transcriptomic changes underlying the observed phenotypic effects, larvae were exposed to three concentrations each of BaP, 6-MethylBaP, and 8-MethylBaP. The RNA sequencing data was collected prior to the phenotype onset, 48 hr post-fertilization, and at the emergence of the phenotype, 72 hr post-fertilization. This systematic approach allowed us to identify the dose-dependent transcriptional responses linked to the downstream morphological phenotypes unique to BaP methylation at the eight position. This project helps fill a critical gap in our understanding of the structural dependent effects of PAHs. The results from this study have the potential to improve environmental and human health hazard assessment by identifying important structural features and mechanisms of action contributing to the toxicity of PAH mixtures in the environment. **Disclaimer/Disclosure:** This research was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under Award Numbers P42 ES016465, T32 ES007060, R35 ES031709 and P30 ES030287. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

5.03.T-03 Environmental toxicology and risk evaluation of Swift Fuels 100R unleaded aviation fuel

George Huddleston III¹, John Rodgers², Jenna Molaro¹ and Chris D'Acosta³, (1)Copperhead Environmental Consulting Inc., (2)Clemson University, (3)Swift Fuels

In 1996, the United States completed its transition to the use of unleaded gasoline in on-road automobiles. The U.S. Environmental Protection Agency orchestrated the phasing out, and eventual ban, of leaded products in on-road vehicles over concerns about lead's toxic effects on human health and the environment. To date, most general aviation fuel still contains lead. This poses the potential for long-term risk, as lead persists in the

environment and is not degraded beyond its native form. The purpose of this evaluation was to assess the relative human health and ecological risks associated with 100R unleaded aviation gasoline developed by Swift Fuels for piston-engine aircraft. This work was accomplished by developing a comparative risk framework to contrast 100R with conventional, lead-containing 100LL aviation fuel based on composition (i.e., elements and compounds) of the two fuels. Specific toxicity and fate data of components were derived from peer-reviewed literature and technical documents. The data were then analyzed relative to the components' contribution (i.e., concentration per volume) to the two fuels. The major components of 100R fuel include full-range alkylate petroleum naphtha containing butane, light alkylate petroleum naphtha, and the oxygenate ethyl tert-butyl ether to boost octane. By contrast, 100LL contains a gasoline base with additives tetraethyl lead and ethylene dibromide. Ethyl tert-butyl ether present in 100R can mobilize in surface water and groundwater if released, although the compound will degrade microbially over time. Based on carcinogenicity in humans, 100LL poses a greater cancer risk than the 100R fuel. Tetraethyl lead in the 100LL fuel has a high potential to bioconcentrate in aquatic animals, compared to the 100R components. By contrast, the organic components of 100R, if released into the environment, will degrade over time and pose less long-term risk. Based on the comparison of fuel component toxicology, the Swift Fuels 100R would pose less risk to human health and wildlife than the widely used 100LL. Tetraethyl lead and ethylene dibromide are the components primarily responsible for 100LL toxicity. If the use of 100R increases with time and replaces 100LL, risks from exposure to lead in 100LL fuel will decrease and ultimately be eliminated.

5.03.T-04 Reducing oil toxicity with chemical dispersants and herders

Kelly Marie McFarlin, Exxon Mobil

Biodegradation and combustion are the only chemical processes that can completely remove hydrocarbons from the environment. During an oil spill response, chemical dispersants and herders can enhance the efficiency of biodegradation and combustion, respectively; thereby reducing organismal exposure to toxic hydrocarbons. This presentation will introduce the science behind dispersants and herders, focusing on updated USEPA regulations on toxicity testing, significant advancements in formulations, aquatic toxicity results, and potential environmental impacts.

5.03.T-05 Modeling acute toxic effects of oil spills: Important processes and research needs

Deborah French-McCay, Tetra Tech Inc.

Hydrocarbon concentrations in aquatic systems, whether from oil spills, point source discharges, or non-point sources, are comprised of complex mixtures of compounds with varying physical-chemical and toxicological characteristics. The complexities of temporally and spatially varying compositions, concentrations, and durations of exposure need to be considered to accurately quantify toxic effects on aquatic biota. Current state-of-the-science and modeling capabilities allow for analyses that 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 than simply considering maximum total hydrocarbon concentration exposure. Oil spills typically result in short-term exposures in the water column and acute effects on biota, whereas point source discharges and non-point sources can result in chronic exposures in the receiving waters and sediments. The effects of an oil spill are determined by: 1) the nature and duration of the release; 2) geography and currents, which can confine or transport and shear the contamination; 3) turbulent energy in the environment, which entrains oil and dilutes concentrations; 4) oil properties and composition, which change with weathering processes; 5) duration of exposure, which is related to dilution rate, fate processes and behavior of the organisms in relation to the dynamics of the oil contaminant concentrations; 6) toxicity of oil components, which depends on bioavailability, the degree of sensitivity of the exposed organisms and the effect endpoint considered; and 7) environmental conditions (e.g., light exposure, temperature), which may modify toxic effects. A model system simulating oil transport and fate, organism behavior, and the additive effects of multiple component exposures via a toxic unit modeling approach can account for these processes. While it has been demonstrated that toxicity is strongly affected by exposure

durations varying from hr to days, a key research need is to calibrate the exposure duration algorithm to develop a generally applicable acute aquatic toxicity model. In addition, phototoxic effects via photosensitization would be expected to vary by duration of exposure to both light and photoactive hydrocarbons. Additional laboratory data are needed to support such model development.

5.03.T-06 Decadal impacts of oil spills in tropical marine environments: 40 years of TROPICS

Dorothy-Ellen Abigail Renegar¹, Paul Schuler², Anthony Knap³, Richard Dodge⁴, Thomas Sleeter⁵, Bernhard M. Riegl⁴, Steven Buschang⁶, Michael Hernandez⁷, Bryony Wood⁸, Ronan Jezequel⁹, Oscar Garcia-Pineda¹⁰, Austin Blakeslee⁴, Cailey Elise Dorman⁴, Kyle Pisano⁴ and Ellen Skelton⁴, (1)National Coral Reef Institute, Nova Southeastern University, (2)Clean Caribbean and Americas, (3)Texas A&M University, Geochemical and Environmental Research Group, (4)Nova Southeastern University, (5)The Atlantic Conservation Partnership, Bermuda, (6)United States, (7)Oil Spill Response Ltd., (8)Oil Spill Response Ltd., United Kingdom, (9)CEDRE, France, (10)WaterMapping, LLC

The TROPICAL Oil Pollution Investigations in Coastal Systems (TROPICS) experiment, which began in 1984 on the Caribbean coast of Panama, is one of the only well-controlled field studies that has identified and evaluated the long-term environmental trade-offs of chemical dispersant use in tropical marine and subtidal marine systems. From the initial experiment through more than three decades of study and data collection visits, the intertidal and subtidal communities have exhibited significantly different impact and recovery regimes, depending on whether the sites were exposed to crude oil only or crude oil treated with a chemical dispersant. In December of 2024, a multi-national, multi-disciplinary team of researchers returned to the experimental sites and conducted a comprehensive reassessment of mangrove, seagrass, and coral reef systems four decades after the original experimental spill, along with biomarker-based analytical characterization of remaining oil pollution in mangrove sediments at the sites. Initial results from the 2024 site visit indicate that while mangrove canopy cover has increased at all experimental sites, there are fewer live trees overall, with the largest decrease in the number of live trees at the oil only site. Mean mangrove diameter has increased over time at the reference and dispersed oil sites but has decreased over time at the oil only site. In the subtidal zone, seagrass density is similar at all sites, but sea urchin density has broadly decreased. Overall, coral cover has decreased at all of the experimental sites, with a shift to increased macroalgae cover at the dispersed oil and oil only sites. Analysis of sediment cores is in progress. By using modern and improved methods and technologies, particularly for mapping current impacts on TROPICS sites and analytical characterization of persistent oil contamination, we have generated new biological and chemical data that improves our understanding of the long-term chronic impacts of oil spills, degradation and transportation of spilled oil in this type of environment, and the environmental and situational factors that drive relative impacts and control long-term outcomes of spill response methods.

5.04.P-Mo Advancing Bioaccumulation Science: Novel Approaches and Challenges

5.04.P-Mo-055 A comprehensive screening of aquatic and terrestrial bioaccumulation potential of 800+ chemicals

Emma Mendelsohn, Bailey Campbell and Philip E Goodrum, GSI Environmental

Bioaccumulation is a critical consideration in environmental risk assessment and global chemical management, yet regulatory criteria for classifying chemicals as bioaccumulative vary across jurisdictions, and often emphasize aquatic food webs over terrestrial and air-breathing species. We screened over 800 substances from the U.S. EPA Regional Screening Level (RSL) database for bioaccumulation potential. We compiled both measured and modeled physicochemical data (e.g., Kow, Koa) and applied regulatory thresholds from the United States, Canada, and Australia to classify chemical bioaccumulation potential in aquatic and terrestrial food webs. This comparative analysis revealed inconsistencies across frameworks and highlighted data gaps. To support broader integration of bioaccumulation data into chemical assessments and prioritization, these classifications and supporting data will be shared via a publicly accessible repository (link provided in 216). As

a secondary objective, we investigated the relationship between these bioaccumulation classifications and human cancer potential using USEPA Integrated Risk Information System (IRIS) oral cancer slope factors and inhalation unit risk values. While a strong correlation would simplify screening assessments, our analysis did not support such an assumption, likely reflecting diverse mechanisms of chemical carcinogenicity. Our findings underscore the need to refine bioaccumulation assessment methods, especially for terrestrial and air-breathing species, and promote more harmonized and comprehensive chemical prioritization across ecological and human health risk contexts.

5.04.P-Mo-056 Mixture and concentration effects on bioconcentration factors and uptake of per- and poly- fluoroalkyl substances

Joseph Tucker and Alan Vajda, University of Colorado, Denver

Historical use of aqueous film-forming foams (AFFFs) for military fire training activities at Joint Base Cape Cod, Massachusetts has led to contamination of groundwater by complex mixtures of per- and polyfluoroalkyl substances (PFAS) near these fire training areas (FTAs). To evaluate the bioconcentration factors (BCFs) and uptake/depuration rates of individual PFAS existing within complex mixtures, a series of continuous-flow fish exposure experiments were conducted using on-site, purpose-built mobile laboratories. Environmental source waters were drawn from three groundwater wells, including one reference (REF) and two PFAS-contaminated water sources (FTA-1, and FTA-2). Adult male fathead minnows were exposed for up to six weeks to 100% REF, 90% REF:10% FTA-1, 100% FTA-1, 90% REF:10% FTA-2, or 100% FTA-2. After two weeks of exposure to contaminated water, half of the exposed fish were moved to additional aquaria receiving just REF water for depuration. Water and fish carcasses were sampled weekly for analysis of 40 PFAS for determination of mixture- and concentration-dependent effects on PFAS uptake rates, depuration rates, and bioconcentration factors. Preliminary results on concentration- and mixture- dependent BCFs and uptake of PFAS will be presented.

5.04.P-Mo-057 Advancing understanding of terrestrial receptor exposure to mixtures of per- and polyfluoroalkyl substances

Andrew East, University of Maryland, College Park

Per- and polyfluoroalkyl substances (PFAS) are synthetic molecules that are generally considered ubiquitous in environmental media. As such, ecological risk assessment activities near and far from PFAS release sites consistently face challenges estimating exposures. The potential for complex mixtures and food-web driven exposure concurrent with lagging data generation has left terrestrial receptor exposure estimates much worse off than aquatic receptors. This dissertation addresses several of these challenges from a largely empirical perspective. First, identification of representative mixtures from surface soil at US military installations provide a common basis for a variety of terrestrial scenarios. Secondly, dosing single and mixtures of PFAS to mice provides evidence of dose additivity in whole body and tissue compartments and in organismal effects. Thirdly, evaluation of kinetics of a food-web derived PFAS mixture in a terrestrial life stage amphibian provide capability to screen PFAS' potential to magnify in trophic transfers. Lastly, a similar evaluation of kinetics in a terrestrial herbivore expand capability to screen trophic magnification potential across multiple taxa. One of the broad conclusions of this work is that screening or protective approaches that are relatively simple (not complex!) may address exposure estimates for many of the majority PFAS and taxa of interest. Results of the individual studies will be reported, but the broad contribution to the improvement of exposure estimation in terrestrial receptors through laboratory studies will be a key takeaway message. **Disclaimer/Disclosure:** This work was supported by the Strategic Environmental Research and Development Program through projects: ER-2627, ER19-1041, and ER22-3388.

5.04.P-Mo-058 Application of congener-specific bioaccumulation factors for dioxins and furans in ecological risk assessment

Tory Lydy, Frank Dillon and Shaun A. Roark, Jacobs

Established methods for estimating bioaccumulation of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) often rely on a single bioaccumulation factor (BAF) derived from 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). However, this approach can significantly overestimate bioaccumulation and associated ecological risk, as BAFs vary among congeners due to differences in chemical properties and environmental behavior. To improve accuracy and reduce uncertainty, approaches that incorporate congener-specific BAFs have been developed at multiple sites to support baseline ecological risk assessments (ERAs). As part of an ERA examining soils contaminated with PCDDs and PCDFs, we compared estimates of mammalian dietary toxic equivalence (TEQ) exposure derived from TCDD-based bioaccumulation with those derived from literature-based congener-specific methods. The congener-specific approach yielded TEQ exposure estimates substantially lower and presumably more accurate than those produced by single-congener TCDD models. While direct field studies to generate site-specific BAFs are ideal, they are often prohibitively expensive, time-consuming, and logistically complex. The use of literature-based congener-specific BAFs represents a more cost-effective and robust alternative for estimating bioaccumulation in terrestrial food webs and supports more accurate ecological risk characterization.

5.04.P-Mo-059 Advancing ecotoxicological testing using NAMs: Comparative evaluation of the trout liver metabolic assay (OECD TG 319 A/B) and the *Hyaella azteca* bioconcentration test (HYBIT, OECD TG 321)

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The development of reliable and relevant ecotoxicological New Approach Methodologies (NAMs) is critical for reducing reliance on whole-organism bioassays and enhancing the mechanistic understanding of chemical hazard and bioaccumulation assessments. In this study, we compare in vitro (OECD TG 319A/B), in vivo invertebrate OECD TG 321, in silico, and fish in vivo (OECD TG 305) bioaccumulation methods concentrating on the trout liver metabolic assays and *Hyaella azteca* bioconcentration tests (HYBIT). Liver S9 fractions and primary hepatocytes provide robust models that incorporate phase I and II metabolic pathways, enabling quantitative and mechanistic insights into xenobiotic biotransformation. These in vitro systems offer high-throughput capabilities and reduced animal use, supporting regulatory and ethical goals under global 3R frameworks. In contrast, HYBIT assays (OECD TG 321) and traditional fish bioaccumulation studies (OECD TG 305) provide organism-level outcomes but lack mechanistic resolution, are time- and resource-intensive, and involve significant organisms use. Our comparative evaluation demonstrates that trout liver-based assays effectively predict bioaccumulation potential and metabolic clearance while providing metabolic rate constants (k_{met}) that can be integrated with in silico models. In contrast, in vivo methods, while ecologically relevant, showed limited sensitivity to sub-lethal metabolic alterations and offer lower throughput. The integration of metabolic rate data from in vitro systems into computational models enhances the prediction of bioconcentration factors (BCFs), supporting read-across and chemical prioritization efforts. These findings strongly support the adoption of an Integrated Approach to Testing and Assessment (IATA) for bioaccumulation, which combines NAMs, in silico tools, and targeted in vivo validation. Such a strategy ensures scientifically sound, efficient, and ethically responsible chemical hazard assessment aligned with regulatory needs and environmental protection goals.

5.04.T Advancing Bioaccumulation Science: Novel Approaches and Challenges

5.04.T-01 Of tides and skins: Dragonfly exuviae as novel biomonitors of total mercury along the nontidal and tidal freshwater Potomac River

Doreen Sechak Peters, George Mason University

Our study investigates total mercury (THg) concentrations in sediment and porewater of a large river and the potential for dragonfly exuviae (shed skins) to serve as a novel THg biomonitor. Water quality monitoring methods are needed to provide reliable quantitative data without contributing to further declines in global insect populations. Our study objectives are to: 1) determine how THg concentrations in sediment, porewater and dragonfly exuviae vary among sites along the nontidal and tidal freshwater Potomac River, and 2) identify the relationships between concentrations of THg in sediment, porewater and dragonfly exuviae. We collected samples from 5 nontidal and 4 tidal freshwater sites along the Potomac River in Maryland, Virginia, and Washington, DC. We collected dragonfly exuviae manually by hand, we collected surficial sediment (top 2 cm) from three points at each site using a scoop and compositing, and we collected porewater using a PushPoint sampler. All samples were analyzed for THg using U.S. Geological Survey or U.S. EPA methods. Variations and relationships were explored between THg concentrations and sites grouped by tidal influence. Although we found no difference in THg concentrations based on tidal influence, there is a strong direct/positive correlation between THg concentrations and sediment particle size. In addition, we found that dragonfly exuviae bioaccumulate THg at detectable concentrations, which reflect a positive relationship with THg concentrations in sediment and porewater. Aquatic bioaccumulation assessment approach methodologies often result in capture stress or mortality to the target organisms. Monitoring methods are needed that provide reliable quantitative data without negatively impacting insect populations, which are in decline. Devoid of life but not of purpose, dragonfly exuviae act as passive samplers when used as biomonitors and offer a novel, non-lethal means to quantitatively measure bioaccumulation of mercury in the riverine environment without negatively impacting insect populations. **Disclaimer/Disclosure:** Our research was supported by the Dragonfly Society of the Americas (DSA) 2021 Odonate Research Grants Program.

5.04.T-02 Bioaccumulation and risks associated with PFAS in bivalves at AFFF sites

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The presence of PFAS in shellfish, such as mollusk bivalves, and the potential for risks to the humans or wildlife that consume bivalves has not been well studied. A literature review was conducted to characterize the nature and extent of PFAS in bivalve tissues at sites receiving PFAS from release of aqueous film-forming foam (AFFF) to identify bioaccumulation metrics (e.g., bioaccumulation factors [BAFs]) that could be used in food web modeling in support of site-specific risk assessments. Case studies from AFFF-impacted sites indicated that bivalves accumulate lower concentrations of PFAS than fish. Assuming generic bivalve and fish consumption rates for humans and wildlife, dietary exposure from bivalve consumption is also lower than exposure associated with fish consumption. For example, PFOS dietary exposure from bivalve consumption was a factor of 3 to 10 lower than PFOS exposure from fish consumption. Concentrations of PFOS in bivalves in all case studies exceeded potential human health consumption screening levels, but none of the wildlife-based tissue screening levels were exceeded. Site-specific AFFF risk assessments in aquatic ecosystems may wish to consider the bivalve consumption pathway, however, at sites with both bivalve and fish consumption exposure pathways, exposures associated with fish consumption will likely be higher. Eight robust laboratory exposure studies were identified as sources of bioaccumulation metrics, and they corroborated observations from the field that PFAS are less bioaccumulative in bivalves than in fish. Additionally, bioaccumulation was generally higher for PFAS with longer perfluoroalkyl chains. For example, a significant regression between the freshwater bivalves BAFs (L/kg, wet weight) and the number of perfluorinated carbons was found for perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFASs), and fluorotelomer sulfonic acid (FTSs) ($r^2 = 0.91$, together, regardless of family). Overall, this review demonstrates that bivalves have the potential to accumulate PFAS at AFFF sites to concentrations that may require additional risk-based evaluation, and the bioaccumulation metrics provided will be helpful in food web modeling. However, compared to bivalve consumption, fish consumption at AFFF sites is more likely to drive risk based decision making.

5.04.T-03 Bioaccumulation and depuration of microplastics in eastern Oysters, *Crassostrea virginica*: Influence of temperature on toxicokinetic parameters

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Microplastics, plastic particles 5mm or less in size, are a contaminant of emerging concern and ubiquitous in aquatic systems. Due to their small size, microplastics are easily accumulated by organisms. This is especially true of filter feeders such as bivalves, who also face the ever-present threat of climate change. Despite these dual threats, little is known about the impact of sea water temperature on microplastic accumulation in bivalves. The present study examined the kinetics of microplastic bioaccumulation in the Eastern oyster (*Crassostrea virginica*), an ecologically and economically vital species. Oysters were exposed to a 5000 particles/L microplastic solution made of equal parts crumb rubber, fragments, and fibers (1,667 particles/L each) at one of three temperatures: 20°C, 24°C, or 28°C. Individuals were then sacrificed at 0, 24, 48, or 96 hr of uptake. Remaining oysters were allowed to depurate in microplastic-free sea water for 24, 48, or 96 hr. The results of these exposures were then used to create toxicokinetic models. Greater total microplastic accumulation was observed at both 24°C and 28°C than at 20°C ($p < 0.05$). Additionally, crumb rubber was the most highly accumulated particle type across all three tested temperatures. Conditional uptake rate constants (k_u) for total microplastics were estimated to be 0.010 ± 0.005 mL/gw.w.*h (mean $\pm$ SE) at 20°C, 0.008 ± 0.002 mL/gw.w.*h at 24°C, and 0.007 ± 0.001 mL/gw.w.*h at 28°C. Uptake at 20°C followed a hyperbolic pattern, with apparent steady state achieved at 96 hr at a concentration of 1.19 ± 0.737 particles/gw.w. (mean $\pm$ SE). Uptake at 24°C and 28°C continued linearly, with total microplastic concentrations at 96 hr of 3.03 ± 0.918 particles/gw.w. and 3.23 ± 0.674 particles/gw.w., respectively. Conditional depuration rate constants (k_d) for total microplastics were estimated as 0.040 ± 0.022 /h (mean $\pm$ SE) at 20°C, 0.001 ± 0.003 /h at 24°C, and 0.003 ± 0.003 /h at 28°C. Total microplastic reduction after 96 hr of depuration was 64.7% at 20°C, 29.1% at 24°C, and 16.2% at 28°C. These results suggest that warmer temperatures increase microplastic uptake while reducing depuration, leading to greater overall bioaccumulation and prolonged retention of microplastics in tissues. Among the particles tested, crumb rubber (used as a proxy for tire and road wear particles) exhibited the highest bioaccumulation potential, underscoring the need for further research into their toxicological effects in oysters.

5.04.T-04 Uptake and depuration of per- and polyfluoroalkyl substances (PFAS) in the polychaeta *Perineris Wilsoni* through seawater exposure

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There is a lack of experimental data on the bioaccumulation kinetics of PFAS in invertebrates such as polychaetas. This study investigates the kinetics of respiratory uptake and depuration of PFAS in a polychaeta sandworm species, *Perineris wilsoni* by seawater exposure experiment. *P. wilsoni* is found in intertidal reef flats and rocky shores in East Asia. It is a common food for fish, crabs, and shellfish and is widely used as fishing bait and plays a crucial role in the marine food web. The seawater was spiked with a mixture of perfluoroalkyl carboxylic acids (PFCAs) with carbon chain lengths from C3 to C9 and perfluoroalkyl sulphonates (PFSAs) with carbon chain length C4, C6 and C8 along with five alternative PFAS. The sandworms were exposed to spiked seawater for 7 days and then were depurated in clean seawater for 27 days. Water and sandworm samples were analyzed for PFAS using liquid chromatography connected to triple quadrupole tandem mass spectrometry. The uptake and depuration rate constants were calculated using first-order kinetic equations considering the whole-body concentration of the test chemicals in sandworms. The uptake efficiency for the PFCAs was found to be higher than that for PFSAs. The uptake efficiency relative to O2 ranged from 0.17% in hexafluoropropylene oxide dimer acid (HFPO-DA) to 113% in perfluorodecanoic acid (PFDA). In PFCAs, no monotonic trend with carbon chain length was observed for either respiratory uptake efficiency or depuration half-life ($t_{1/2}$). Notably, PFCAs with carbon chain lengths from C5 to C8 were found to have longer $t_{1/2}$ (> 100 days) compared to the longer-chain PFDA (C9), which had a $t_{1/2}$ of 67 days. Similarly, among PFSAs, the C4 and C6 analogues exhibited longer $t_{1/2}$ (> 100 days) than perfluorooctane sulfonate (PFOS, C8), which had a $t_{1/2}$ of 16 days. These findings suggest that the respiratory uptake and elimination kinetics of PFAS in

invertebrates such as polychaetas differ considerably from those in fish, indicating that kinetic models developed for fish may not be applicable to invertebrate species like *P. wilsoni*. The kinetic parameters determined in the current study will help in assessing the contribution of the polychaetas in the bioaccumulation of the PFAS in the marine food web.

5.04.T-05 Predicting biotransformation mechanisms and BCFs: Comparing the in vitro OECD 319B fish biotransformation assay to the in vivo OECD 305 study for a hydrophobic fragrance chemical

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The Log KOW is employed as an initial tier to assess potential to bioaccumulate. However, Log KOW does not provide insight into the biotransformation processes within an exposed organism. The present study describes the strong potential and capacity of the OECD 319B In vitro assay to accurately predict the bioconcentration potential of organic chemicals, but also the metabolic transformation products formed in the traditional OECD 305 In vivo fish bioconcentration study, which is still the “gold standard” test for establishing the bioconcentration factor of chemicals. Two studies were performed on a hydrophobic organic fragrance molecule with an experimentally determined Log KOW of 5.15. An OECD 305 In vivo fish bioconcentration study, followed the uptake and depuration of the 14C-radiolabelled test item in the tissue of Bluegill, and identified the major metabolite formed in the fish tissue. The second study, an In vitro biotransformation assay using Rainbow Trout liver S9 fractions according to the OECD 319B was conducted with non-radiolabelled test item. Comparison of results using the In vivo OECD 305 method and In vitro RT-S9 assay demonstrate remarkable similarity in both the derived BCFs as well as the biotransformation pathway leading to the elimination of the test chemical from the organism. A steady-state BCF of 412 L/kg, normalised to 5% lipid content was determined in the In vivo study. The predicted BCFs from the IVIVE and regression models of between 221 - 318 L/kg, were in close agreement with the In vivo result. The trans-isomer was predicted by both approaches to have a slightly higher BCF than the cis-isomer. The major metabolite, representing approximately 60% of total 14C in fish tissue in the In vivo study, was identified as the glucuronide of the hydroxylated parent chemical, and was rapidly eliminated from the fish with a tissue half-life of between 1.0 and 1.8 days. The same structure was also identified putatively as a major Phase II biotransformation metabolite in the In vitro study, confirming the utility of the OECD 319B assay as not only an efficient and fit-for-purpose screening approach for de-/prioritisation of the requirement for full In vivo testing, but also to predict similar metabolic pathways as the In vivo study. This further supports the rationale that the OECD 319B can be integrated into a systematic tiered assessment approach for evaluating bioaccumulation potential.

5.04.T-06 Microplastics-not only a micro problem: A direct comparison of micro- and nanoplastics in bottled and treated drinking water

Megan Jamison and John J Lenhart, Ohio State University

Micro- and nanoplastics (MNPs) have been identified in various sources, including drinking water, raising concerns about human exposure through ingestion. To understand the extent of this exposure requires detailed understanding of their presence in different drinking water sources. Unfortunately, there is a lack of studies applying uniform methods across different drinking water types. What does exist focused on particles >10 µm, excluding nanoplastics which have the highest potential to cross cellular barriers and accumulate in the body. To address this gap, the present study developed a method for isolating and analyzing smaller MNPs. The process involved preparing samples for analysis using scanning electron microscopy (SEM) to assess particle size, shape, and quantity, and optical photothermal infrared spectroscopy (O-PTIR) to determine polymer composition. This method was applied to treated drinking water from four water treatment plants along the Ohio shore of Lake Erie, as well as six bottled water brands. The findings revealed that both treated drinking water, and bottled water contained a higher concentration of small-sized MNPs (5000–300 nm) than previously reported. However, this increased particle count did not correspond to greater mass, as the total MNP mass in

treated drinking water remained consistent with previous studies. This result reflects nanoplastics comprised approximately 50% of detected particles, contributing little to overall mass due to their small size. This study also found that bottled water had significantly higher particle concentrations of MNPs than treated drinking water, specifically for smaller micro- and nanoplastics. This suggests that MNP exposure from bottled water is greater than tap water. Overall, this study successfully developed a method for isolating and characterizing smaller micro- and nanoplastics in environmental samples, broadening our understanding of their presence in the drinking water supply and provided guidance for reducing daily exposure to MNPs through drinking water.

5.05.P-Tu Advancing Effect Modeling for ERA: Bridging Scales, Data Gaps and Regulatory Challenges

5.05.P-Tu-069 A tiered ecological risk assessment of thiamethoxam on *Chironomus riparius* using individual- and population-level modeling

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This study presents a tiered ecological risk assessment framework for the neonicotinoid insecticide thiamethoxam (TMX) on *Chironomus riparius*, a species recognized as highly sensitive to neonicotinoids among aquatic invertebrates. The framework integrates individual-based models (IBMs) with Dynamic Energy Budget (DEB) model, and toxicokinetic-toxicodynamic (TKTD) modules to extrapolate laboratory data to real-world field scenarios. At the individual level, a DEB model, incorporating a TKTD module and temperature-dependent effects, was parameterized using laboratory experiments with varying TMX concentrations and temperatures. A moving-time-window (MTW) approach simulated *C. riparius* life cycles under various exposure scenarios, assessing survival, growth, time to emergence, and reproduction. "Survival at pupation" served as a combined sublethal and lethal endpoint, considering pupation's dependency on toxicity. Worst-case TMX exposure profiles, derived from a geographically extensive and temporally extended water monitoring program, were used to determine exposure multiplication factors (EMFs) analogous to ECx values, quantifying risk at a pre-defined effect strength. The influence of different temperature integration approaches (constant vs. dynamic) on predicting EMFs was also investigated. At the population level, a DEB-IBM model was developed, incorporating density-dependent mortality (cannibalism) observed in laboratory experiments. This density dependence partially compensates for lethal effects of TMX exposure at high population densities. The model evaluates the transferability of EMFs from the individual to the population level, integrating the effects of varying larval densities and food conditions under field conditions through relevant application examples. This integrated approach provides a more comprehensive and realistic assessment of TMX risk to populations of aquatic invertebrates in both open and closed water systems under environmentally relevant conditions, ultimately reducing uncertainty in risk characterization. **Disclaimer/Disclosure:** This work was supported by Syngenta Crop Protection. N. Galic and M. Vaugeois are employees of Syngenta.

5.05.P-Tu-070 Model risk, what it is and why we should consider it

Julann Spromberg, Getchell

Models can be an important tool in regulatory decision-making processes regarding natural resources, fisheries, and rare species. The utility of population models depends on the available species data and the alignment of the model structure with regulatory needs. Importantly, the confidence in the available data and the model rigor need to match the types of decisions to be made, the time frame for reassessment and the level of risk the regulator/agency deems appropriate. Model risk, defined as the possibility the model is wrong or the output is misapplied, may stem from data limitations, parameter estimation uncertainty, model misspecification, or inappropriate use of a model. A decision framework for considering the use of models as a line of evidence in various regulatory contexts has been proposed. The framework can assist regulators as they either work with modelers to construct new models or as they select from existing models to inform their decisions. Acknowledging and managing model risk increases the confidence of using models in regulatory contexts. As

we make progress toward utilizing models in regulatory decision-making, use of this process will ensure models fit the regulatory question, reduce model risk, and increase user confidence in applying models.

5.05.P-Tu-071 Mechanistic modeling of insecticide effects on Lepidoptera: A DEB-TKTD approach

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Current insecticide risk assessments for endangered Lepidoptera rely on safety factors often derived from honey bee data, potentially leading to inaccurate risk characterization. To address this limitation, we are developing mechanistic effect models combining Dynamic Energy Budget (DEB) theory with Toxicokinetic-Toxicodynamic (TKTD) approaches. The DEB framework models animal life cycles in terms of energy allocations for growth, development, and reproduction. TKTD submodels incorporate chemical uptake, metabolism, lethal effects and/or sublethal effects on the DEB processes. Lethal effects are simulating analogous to the widely-applied General Unified Threshold model for Survival (GUTS) whereas sublethal effects are modeled using the concept of physiological modes of action, i.e., the state of the art in DEB-TKTD models. Since there is no predefined DEB variant for Lepidoptera yet, this is developed in the course of the project and parameterized with physiological control data from literature, whereby the holometabolous life cycle of Lepidoptera will be accurately simulated. Laboratory toxicity data (acute and chronic exposures across life stages) from experiments specifically generated for this project are used for TKTD model calibration and validation. This mechanistic approach offers several advantages: extrapolate from laboratory generated toxicity data, accounting for time-variable exposures, and enabling extrapolation to untested scenarios. The resulting DEB-TKTD models will provide a refined understanding of insecticide impacts on individual Lepidoptera, forming a crucial foundation for population-level risk assessment in subsequent phases. This will support more informed regulatory decisions and enhance the scientific robustness of endangered species protection.

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5.05.P-Tu-072 Modeling host specificity and infection strategies to predict glochidia attachment success in freshwater mussels

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Freshwater mussels rely on a parasitic larval stage, glochidia, that has to successfully attach to a suitable fish host to complete metamorphosis. However, glochidia attachment success varies based on species-specific infection strategies and host specificity, factors that remain poorly understood. This research seeks to address that gap by developing a conceptual and computational model to simulate glochidia attachment outcomes across simplified host communities informed by life history and host use data. The modeling accounts for mussel infection strategy (broadcast versus targeted) and host specificity (generalist versus specialist) to evaluate how these traits influence the probability of glochidia attachment in environments with different host communities. We use data from literature and data-informed assumptions based on host compatibility to simulate the likelihood of successful glochidia attachment across different host fish species. We hypothesize that mussel species using targeted infection strategies and generalist host use will achieve higher glochidia attachment success, especially in fish communities with high species richness. In contrast, specialist species whose fish host have limited ranges may be more susceptible to changes in host availability and shifts in community composition. Model outcomes will be evaluated by analyzing attachment rates, host-specific dispersal success, and sensitivity to variations in host community structure. This research is meant to predict glochidia attachment success in understudied mussel species by linking key biological traits with host community characteristics. By identifying the conditions that enhance or constrain larval success, the model can support conservation planning efforts and help prioritize species and habitats for protection. Looking ahead, this approach could be

incorporated into a broader population-level dynamics framework, providing deeper insight into how early life-stage processes influence long term persistence of threatened mussel populations. **Disclaimer/Disclosure:** This research was funded by OURI SURF Fellowship at Florida Atlantic University and Syngenta Crop Protection. Conference participation is supported by WLW ECOS Travel Grant.

5.05.T Advancing Effect Modeling for ERA: Bridging Scales, Data Gaps and Regulatory Challenges

5.05.T-01 From sub-lethal individual-level stress to population effects—A bioenergetic individual-based model of freshwater mussels

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The central focus of this research is the development of a generalized individual-based population model (IBM) for freshwater mussels. The model is designed to be parameterized for distinct mussel life-history categories and builds upon the prior work that combined Dynamic Energy Budget (DEB) modeling with life-history theory to capture sub-lethal effects of environmental stressors at the individual level. Previous work highlighted that freshwater mussels can be categorized into three distinct groups representing different life-history strategies - opportunistic, equilibrium, and periodic - based on their life-history traits, which emerge from individual-level bioenergetic processes. The current research integrates these processes into a population-level framework, enabling assessment of how effects such as reduced growth, delayed maturation, or lower fecundity may influence long-term population dynamics. The DEB-IBM model developed in the NetLogo programming environment allows simulation of direct and indirect threats, such as pesticide exposure and sedimentation, incorporating realistic environmental drivers like temperature and food availability. It also provides a framework for comparing how different life-history strategies may buffer or exacerbate population-level consequences of stress. Because life-history characteristics drive population dynamics, population-level outcomes may differ across life-history categories even when individual-level responses appear similar. For instance, opportunistic species may prove more resilient to disturbances due to faster maturation and higher reproductive output, whereas equilibrium species may be more vulnerable given their longer generation times and lower fecundity. The oral presentation will highlight the model structure, key modeling decisions, and simulation results for selected species, followed by a discussion on how this framework can support ecological risk assessment and guide conservation strategies. **Disclaimer/Disclosure:** This research was funded by Syngenta Crop Protection and Florida Atlantic University. Conference participation is supported by the WLW ECOS Travel Grant.

5.05.T-04 Mechanistic modeling of fathead minnow populations: A comparative agent-based and integral projection modeling approach for ecological risk assessment

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Ecological risk assessment (ERA) faces challenges in extrapolating laboratory data to field populations. This study addresses these challenges by developing an agent-based model (ABM) for fathead minnows, a widely used standard test species for aquatic ERA in the USA, and by comparing it to an integral projection model (IPM). The ABM is based on Dynamic Energy Budget theory and incorporates toxicokinetic-toxicodynamic modules to represent effects of stressors on the fathead minnow metabolism. The IPM is a size-structured model that uses readily measurable variables (weight and length) to represent growth, survival, and reproduction (and their stress-driven changes) of fish in a population. Both models incorporate seasonal reproduction, density-dependence, and time-variable chemical exposure scenarios, but they represent a few life-cycle processes differently. The ABM and IPM were independently developed and parameterized using existing literature and laboratory data on FHM life history. They were applied to simulate population-level effects under the same time-varying exposure scenario. The ABM provided detailed insights into the individual-level responses to the exposure, while the IPM offered a more concise, distribution-based output to inform population-level

perspectives. The direct comparison of the ABM and IPM outputs reveals both similarities and differences in their predictions of population-level effects. It shows how modeling choices affect the final outputs and highlights strengths and limitations of each approach. Our work provides a valuable example of model cross-validation, enhancing confidence in modeling results for ERA. **Disclaimer/Disclosure:** This work was supported by Syngenta Crop Protection. M. Vaugeois is an employee of Syngenta.

5.05.T-05 Buzzing toward better risk assessment: Advancing bee effect modelling with the ICPPR bee modelling interest group

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Mechanistic pesticide exposure and effects models have the potential to improve the efficiency and accuracy of risk assessments for bees and other pollinators. Several models have been introduced or are under development, spanning organism-level effects (e.g., toxicokinetic-toxicodynamic models), colony models, and population models, for a range of taxa including honey bees, bumble bees, and non-Apis bees. However, these models are not yet well integrated into the risk assessment process, and a number of challenges remain. In response, the Bee Modelling Interest Group was established within the International Commission for Plant-Pollinator Relationships (ICPPR), with the aim of improving the acceptability and usability of exposure and effects models in bee risk assessments. The interest group provides a platform for exchange and discussion between stakeholders from different groups including regulators, industry, contract research organizations and academia. Within the interest group, three work groups have been created based on a survey of members' perceptions of the urgency and feasibility of various topics: (1) improving characterization of exposure pathways, (2) incorporation of sublethal effects, and (3) establishing a bee model inventory. In this presentation, we will introduce the aims, activities and goals of the ICPPR Bee Modelling Interest Group and its three active work groups. Through these collaborative efforts, we aim to accelerate the adoption of mechanistic models within regulatory frameworks, ultimately enabling more accurate and efficient pollinator risk assessments that better protect bee populations while supporting sustainable agriculture. **Disclaimer/Disclosure:** The views expressed in this abstract are those of the author(s) and do not necessarily represent the views or the policies of the U.S. Environmental Protection Agency.

5.06.P-Tu Almost All Things Related to Endangered Species Assessment

5.06.P-Tu-081 The data are there if one only looks: The utility of publicly available data sets to determine vulnerability of threatened and endangered species from abandoned mine land features

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Globally, active and abandoned mines pose both risks to habitat and wildlife. Across the Western United States, estimates suggest that there are more than 200,000 abandoned hardrock mine features. One million mining claims have been registered in Arizona since the Mining Law of 1872 was passed, and the Arizona State Mine Inspector's office suggests that as many as 100,000 abandoned mine features remain. Many of these features represent both physical and chemical hazards to wildlife, while others may provide opportunities for refugia. Vulnerable, Threatened and Endangered (VTE) species may be particularly at risk from exposure to legacy chemical, and particularly heavy metal, contamination from historic mining operations, even if some of the features are providing refugia especially for bats. To determine where overlap occurs between mine features and VTE species in Arizona, we searched the International Union for Conservation of Nature Red List, U.S. Fish and Wildlife Service Environmental Conservation Online System, Arizona Game and Fish Department Species of Greatest Conservation Need List, NatureServe Explorer, and other databases for currently listed vulnerable, threatened, and endangered wildlife in Arizona in addition to federally listed wildlife. We then used research grade observations from the iNaturalist Community to overlay Arizona VTE Species with the United States

Geological Survey Mineral Resources Data System locations for mine features and overlaid it with the VTE wildlife observations recorded in iNaturalist. With the caveat that VTE species exact locations are obscured in iNaturalist, the maps demonstrate proximity location overlap for many species near mine features and will help State managers coordinate activities for potential mitigation efforts in regions that may either put VTE species at risk or potentially provide protected refugia. **Disclaimer/Disclosure:** This work is funded by an Arizona Board of Regents Technology and Research Initiative Fund.

5.06.P-Tu-082 Mesocosms offer a robust higher tier test system for refined pesticide risk assessments and for refined endangered species assessments: A case study with diflufenican

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The use of higher tier studies is a common practice in pesticide risk assessments world-wide. The objective of these studies is to decrease uncertainty by accounting for environmental variability, community interactions, and the fate and behavior of active ingredients in semi-natural systems. Aquatic mesocosms are large-scale field studies that simulate exposure to still or running water systems to determine the direct and indirect effects of the test material on test communities and populations over time. Often the duration of the mesocosm tests also allows the determination of recovery to adversely impacted populations. In the United States, these studies are often considered supplemental and used only as part of a qualitative and not quantitative risk assessment. Relevant higher tier studies are of particular use in the endangered species risk assessment process in the United States given the focus on mitigations to reduce the potential for community and population-level effects. Diflufenican is used as an example of the relevance and robustness of mesocosms by demonstrating the consistency of findings from three mesocosm studies with the same test substance. Based on lower tier studies, algae were identified as the most sensitive group. The mesocosm studies focused on direct effects on phytoplankton, periphyton and indirect effects on zooplankton and grazers. Three mesocosm studies were conducted in 2007, 2010 and 2021 at three different test sites in Germany and the Netherlands. The test design and weather conditions under which the three studies were conducted varied. Even with these differences in design and seasonality, similar algae effect thresholds (NOECs) varied only by a factor of about 3. The comparison of the three studies demonstrates their scientific validity and ability to integrate environmental variability into measures of population and community-level effects. Thus, they provide more realistic results and are significantly more relevant than studies conducted in highly controlled laboratory environments. The example of these three diflufenican studies demonstrates that mesocosms are a robust and suitable tool to be used in environmental risk assessments in the US pesticide registration process including endangered species assessments and are in keeping with the requirement to use the best available data when making pesticide registration decisions including mitigations.

5.06.P-Tu-084 A new tool to calculate crop specific pollen and nectar pesticide concentrations from different application methods

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Pollinating bee dietary risk assessment for pesticide registration requires knowledge of nectar and pollen pesticide residue concentrations following different pesticide application methods to crops. The magnitude and duration of bee dietary pesticide exposures vary according to crop attractiveness to bees, physio-chemical properties, plant characteristics, application rate, method, and timing, and soil characteristics. Regulatory authorities rely on model-generated default estimates of pollinator exposure when measured food item pesticide residue data are unavailable for pesticide active ingredients. In North America, default pesticide residue

estimates for pollen and nectar are imbedded in the United States Environmental Protection Agency's BeeREX model and, depending on the application method, are derived from various model approaches and data sources. Pursuing comprehensive bee-relevant data, we compiled and analyzed pesticide residue data from nectar and pollen samples collected during numerous field studies previously submitted to the United States Environmental Protection Agency, California Department of Pesticide Regulation, Canada Pesticide Regulatory Agency, and the European Food Safety Authority by pesticide product registrants. The information was compiled into a database that is accessible through an interactive Excel® user interface termed NPRUD. The interactive file that makes up NPRUD allows the user to generate statistical estimates of pesticide residue per unit dose (RUD) values in nectar and pollen matrices for different application methods. The values can be used to calculate nectar and pollen estimated environmental concentrations (EECs) in models to assess dietary pollinator risk. The use of this database and the NPRUD tool will strengthen the dietary exposure component of pollinator pesticide risk assessments by utilizing a database of field-measured pollen and nectar residue concentrations that represent pesticide use patterns in different crops. This publication describes the procedures followed to establish a globally comprehensive nectar and pollen residue database, demonstrates the use of NPRUD and demonstrates its applicability to lower tier pollinator pesticide risk assessment. **Disclaimer/Disclosure:** The work was funded by Pollinator Research Task Force which companies includes BASF Corporation, Bayer Crop Science LP, Corteva Agriscience, Mitsui Chemicals Agro, Inc, Syngenta Crop Protection, LLC, UPL NA, Inc. and Valent U.S.A, LLC (Sumitomo Chemical). The authors declare that the financial and administrative supports were provided by Pollinator Research Task Force for this work.

5.06.P-Tu-086 Refining potential spatial overlap for species with negligible in-field exposure to agricultural insecticide products

Josh Fischer, Gerco Hoogeweg, Kurstern Anderson, Kristine LeRoy, Ashley St. Clair and Bridget O'Neill, Corteva Agriscience

In the United States, a key step in assessing potential impacts of a pesticide or plant incorporated protectant to threatened and endangered (i.e., listed) species is characterizing the potential spatial overlap between a species' geographic range and crop footprint. The development of Geographic Information System (GIS)-based tools has increased the precision of these assessments, allowing for more focused evaluations and informing potential mitigation strategies. For a row crop, the spatial overlap analysis typically compares the species' geographic range with field locations where the crop is known to be cultivated. To account for potential off-field movement of pesticide or pollen containing insecticidal proteins, off-site transport distances are incorporated around the field area, thereby expanding the area considered for the overlap analysis. However, some species may have negligible in-field exposure due to a variety of factors. Such factors may include but are not limited to species biology, biology of the crop, and the intended uses of the product under evaluation. In such scenarios, potential exposure would be limited to off-field areas. Herein, we describe a refined approach to conduct a GIS-based species spatial overlap assessment under such conditions, providing a framework to better evaluate potential risk to listed species by focusing the assessment on areas with relevant exposure.

5.06.P-Tu-087 Trait-based framework for identifying Lepidopteran surrogates for ecological risk assessment of endangered species

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As part of the Endangered Species Act (ESA), USEPA is required to assess the potential effects of pesticides to threatened and endangered species. However, effects data for threatened and endangered species is limited considering conducting laboratory experiments on these species is not feasible or even possible. For threatened and endangered pollinator and non-target arthropod species, the honey bee (*Apis mellifera*) is the model surrogate species used in ecological risk assessments. With the large discrepancy in life history traits and vast diversity of terrestrial arthropods, it raises the question of whether more appropriate surrogates could be used to

support endangered species assessments. One potential taxa where additional data would be beneficial is Lepidoptera, which represent nearly half (42%) of all threatened and endangered terrestrial arthropod species. Thus, our research focuses on developing methodologies, using comprehensive trait datasets, to identify potential Lepidoptera surrogates that can be used in laboratory toxicity tests in place of listed species. To fulfill that goal, we have compiled and integrated multiple global, continental, and national datasets encompassing physiological, life-history, geographic, ecological, and evolutionary information for thousands of Lepidopteran species into a centralized database. In collaboration with experts from government, industry, and academia, we identified the most relevant trait variables. These variables serve as inputs for unsupervised learning algorithms, enabling species clustering based on trait similarities. Our findings demonstrate that comprehensive trait data can significantly facilitate the identification and development of suitable surrogacy options for listed Lepidoptera. **Disclaimer/Disclosure:** This work was supported by Syngenta Crop Protection. E. Peterson and M. Vaugeois are employees of Syngenta.

5.06.T Almost All Things Related to Endangered Species Assessment

5.06.T-01 Move over nutrients; pesticides have formally joined the conservation game

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In 2022 the U.S. Environmental Protection Agency (USEPA) released their Endangered Species Workplan to outline a path forward to meet their obligations under the Endangered Species Act. The workplan and subsequent pesticide registration decisions utilize up-front mitigation measures to reduce risk to listed species. The new process has emphasized the use of conservation practices to reduce the off-target movement of pesticides. Historically, conservation practices have been synonymous with efforts to reduce nutrient and sediment loading, or other classes of contaminants like metals, temperature, dissolved oxygen. Those who use them, growers and applicators, are knowledgeable about the practices, but that knowledge is typically limited to contaminants like nutrients. Others in the pesticide realm (e.g., those who sell them and those who regulate them) are less familiar with these practices. To explain the new overlap between conservation practices and pesticide label mandated requirements an effort was established to use facilitated workshop style meetings. Workshops have been held in Oregon and Washington during winter 2024 and 2025. The authors will present on this workshop approach including the challenges around outreach and incentivizing participation to gather community level support for implementation. **Disclaimer/Disclosure:** Workshops funded in part through a Western Integrated Pest Management Center grant with support from Oregonians for Food and Shelter, Oregon Department of Agriculture, Compliance Services International and Washington State Department of Agriculture.

5.06.T-02 Estimating pesticide toxicity and risk of listed Lepidopteran and Coleopteran species and potential implications for ESA label language

Niranjana Krishnan, Michael Adu-Brew and Margaret Kato, University of Maryland, College Park

Since the U.S. Environmental Protection Agency's recent announcement that registration and reregistration decisions would consider the effects of pesticides on listed species and their critical habitat, there has been greater effort to estimate the toxicity and risk of old and newer classes of pesticides on listed species. This presentation focuses on insects, one of the largest listed animal groups, and on two orders that make up the vast majority of listed insects: Lepidoptera (butterflies and moths) and Coleoptera (beetles). By using surrogate species and species sensitivity distributions, we estimate the toxicity and risk of eight classes of insecticides on listed butterflies and beetles. We compare the data with honeybee data, a pollinator that is tested in the ecological risk assessment process. We will also place our findings in context of the final Insecticide Strategy and evaluate how it could impact pesticide label language.

5.06.T-03 Improving endangered species pesticide risk assessment and mitigation evaluation with geospatial aquatic exposure modeling refinement

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New strategies for efficiently conducting endangered species pesticide risk assessments and determining necessary mitigations for reducing pesticide exposure have been developed by the USEPA, including distinct strategies for herbicides, insecticides, and rodenticides. These strategies have purposefully relied upon conservative screening-level exposure modeling methods and assumptions, both for efficiency and to help ensure species protection. In the case of aquatic exposure, this high level of conservatism consequentially leads to very high levels of exposure reductions needed to meet species protection goals, often 99% (100x) or greater. These screening-level exposure estimates lead to both a mischaracterization of pesticide use risk and may misinform where and to what extent mitigations are potentially needed to protect species. An alternative approach is to incorporate aquatic exposure modeling methods that account for local environmental conditions and agronomic practices into the risk assessment process. This leads to more realistic species-specific exposure estimates and resulting risk characterization and mitigation strategies. We will demonstrate a multi-step aquatic exposure modeling methodology designed for national endangered species assessments that incorporates four key geospatial refinement elements: geographically-explicit exposure scenarios, Percent Cropped Area, pesticide use site proximity to aquatic habitat, and Percent Crop Treated. The methodology, designed to be both flexible and efficient, has been tested for the insecticide dimethoate and compared with the exposure, risk assessment, and mitigation requirement results from a dimethoate case study conducted following the USEPA's Insecticide Strategy. The new methods resulted in species-specific exposure estimates substantially lower than the generalized screening-level exposure estimates from the Insecticide Strategy, leading to more realistic risk characterization and the potential for a more effective mitigation approach. The exposure modeling methodology developed in this assessment can be readily applied to endangered species risk assessments for other pesticides and can be extended when updated datasets (e.g., crop location spatial layers or pesticide usage) become available.

5.06.T-04 Refinement of non-agricultural use data layers using publicly available data

*Gerco Hoogeweg, Raghu Vamshi, Nick Friendenberg, Sean Kenny and **John Marton**, Corteva Agriscience*

Assessing the potential exposure, effects, and risk to species listed under the Endangered Species Act (ESA) is an essential component of pesticide registration and registration review in the United States. The overlap between a species' location, based on species range or designated critical habitat, and potential pesticide use areas is a critical step in USEPA's determinations of May Affect and Likely to Adversely Affect, as well as subsequent predictions of Jeopardy and/or Adverse Modification. For non-agricultural use sites particularly, there are considerable uncertainties in the use data layers (UDLs) currently used in the overlap analysis. Some UDLs combine many different use sites and are therefore highly conservative. These UDLs are based on aggregates of historical land cover data layers, commercial location data, and some public vector data to represent sites such as golf courses, rights of ways, school grounds and parks. Using additional publicly available data from the Bureau of Census or OpenStreetMap, for example, it is feasible to generate more definitive non-agricultural UDLs. Use cases are shown for UDLs of golf courses and school grounds, providing context for a discussion of methods, advantages and challenges.

5.06.T-05 A collaborative approach to offsets for pesticides and endangered species

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Service, (5)Endangered Species, Compliance Services International, (6)Compliance Services International

There are over 25,000 pesticide products currently registered by the Environmental Protection Agency (USEPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) that will require a review by USEPA every 15 years to ensure each pesticide can carry out its intended function without creating unreasonable adverse effects to human health or the environment. This includes evaluation of potential impacts to species listed under the Endangered Species Act (ESA). The national pesticide consultations pursuant to FIFRA and ESA are extremely complex and time intensive as the risk and exposure potential of each pesticide needs to be assessed to approximately 1,800 ESA-listed species and 890 designated and proposed critical habitats. While discussions around how best to apply the mitigation sequence of avoidance, minimization, and compensatory mitigation (offsets) in pesticide registration actions have taken place for years, there is increased interest in offsets for pesticides since the release of the USEPA's Balancing Wildlife Protection and Responsible Pesticide Use workplan in early 2022. The workplan outlines the USEPA's plans to meet its ESA obligations and offsets are included as an option for mitigation; however, offsets have not been previously incorporated into the pesticide registration process. To explore the role of offsets in pesticides pesticide registration actions, pesticide registrants, Bayer and Syngenta, the USFWS, and Compliance Services International initiated a project in 2022. This project explores how, when, and why to use offsets to mitigate the impact of pesticides. This presentation will discuss the collaborative project, provide an overview of a framework for pesticides and offsets, and discuss next steps.

5.06.T-06 Reducing uncertainties in ecological risk assessments when data are limited – How population modeling can help

Valery Forbes, Florida Atlantic University

Lack of relevant data to assess the risks of chemicals to ecological systems is a pervasive problem in risk assessment. This is true for all regulatory frameworks and jurisdictions, including USEPA FIFRA pesticide registrations, European Food Safety Authority pesticide registrations, and the setting of Aquatic Life Benchmarks by the USEPA Office of Water. The problem is particularly exacerbated for endangered species risk assessments under the US Endangered Species Act, in which little may be known about the biology or ecology of the listed species, and it is not possible to conduct toxicity tests with them. Population modeling has proven to be a powerful tool to more realistically integrate exposure and effects of chemicals for species of concern, and great progress has been made in the development and standardization of good modeling practices. Nevertheless, the use of population models in regulatory risk assessment remains limited. Further acceptance of modeling would be facilitated by developing an agreed-upon toolbox of models (akin to standard core studies used for Tier 1 risk assessments), tailored to the different regulatory frameworks and jurisdictions; introducing simple population models at lower risk assessment tiers to extrapolate the individual-level endpoints measured in standard core studies to ecological impacts of concern (e.g., population size over time and space); and providing user-friendly modeling software packages that are freely available and that have been sufficiently vetted by the academic, regulatory, and industry communities. In keeping with its tripartite mission, the Society of Environmental Toxicology and Chemistry would be an ideal convener for such initiatives.

5.07.T Assessing Risks to Non-Target Terrestrial Invertebrates: Current Status and Future Refinements

5.07.T-01 Current State of the Practice for Pesticide Risk Assessments Involving Non-Target Arthropods *Dwayne R.J. Moore, Stone Environmental Inc.*

Non-target arthropods (NTAs) play a pivotal role in ecosystems including maintaining biodiversity, regulating pest populations, providing pollination services, serving as food for other organisms, nutrient cycling, and many others. Insecticides are applied to agricultural and non-agricultural landscapes to control target pest insect species and thus can affect NTAs that occur in treated areas or areas to which insecticides may be transported. Other pesticides (e.g., herbicides) can affect NTAs by reducing food availability or changing their habitats. In recent years, various jurisdictions have been developing risk assessment frameworks and methods for assessing

the risks of pesticides to potentially exposed NTAs. Assessing risk to NTAs must account for various exposure routes (e.g., contact via direct spray, foliar and soil contact, dietary and drinking water consumption, guttation) arising from where different NTAs are found and how they forage and reproduce (e.g., on or off treated areas, flying, on top of or under foliage, soil surface, sub-surface). Further, different life stages may have quite different routes of exposure (e.g., caterpillars vs butterflies, nurse bees versus forager bees). Finally, sensitivity can vary dramatically among NTAs. Currently, most pesticides only have toxicity data for a limited number of test species (honeybees, parasitoid wasp, predatory mite) and exposure routes (contact, diet). New test species and methods are being developed and have been used in some NTA assessments (e.g., bumblebee, green lacewing, seven-spot ladybird, rove beetle). Higher tier semi-field and full-field study methods are also becoming part of the risk assessment toolbox. In this presentation, I will discuss the current state of the practice for assessing direct and indirect risk of pesticides to NTAs. Recommendations for future improvements to existing frameworks and methods will also be made.

5.07.T-02 Developing a framework for identifying sentinel taxa for the non-target arthropod risk assessment using field effect studies

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Historically, the focus of Non-target arthropod (NTA) risk assessment has primarily been in Europe. However, there is growing interest to better assess and understand the risks of pesticides to NTAs in other regions such as the United States, where NTA data are considered as part of the Endangered Species Assessment. NTA field studies have the highest degree of realism, but also a high degree of variability and unlike higher tier bee studies, NTA field effect studies are conducted with naturally occurring populations of multiple species. This results in large, complex datasets involving hundreds of different taxa with differing abundances. As a result, these studies can be challenging to interpret and ascertain what is a potential effect of a plant protection product on populations and communities versus natural background and seasonal variability. In addition, the current NTA risk assessment in Europe [(ESCORT 2) and Terrestrial Guidance Document (SANCO/10329/2002 rev 2 final)] are currently undergoing revision meaning there are discussions ongoing in terms of what to protect, where to protect it, over what time period and with what degree of certainty. The authors present a project working to compile existing European NTA field effect study data into a powerful database for analysis and identification of appropriate representative "sentinel" or focal taxa and creation of an accompanying ecological traits database. A defined set of sentinel taxa, instead of an extensive set of species unique to each study, is expected to aid interpretation of studies. The identification of sentinel taxa is not only relevant for the evaluation of field effect studies but also for the development of ecological models. Considering the spatial and temporal scale of NTA field studies and the number of taxa that are observed, ecological models are expected to gain in importance in the future NTA risk assessment. As it will not be feasible to develop models for all taxa potentially present in an area, a framework to understand the key representative taxa is therefore essential. In addition, the database is valuable for analysing the spatial and temporal variability of taxa or ecological traits, thus making it a valuable resource for establishment of Normal Operating Ranges and/or Protection Goals. The approach of using ecological traits to inform ecological risk assessments and the sentinel taxa approach could also have utility for threatened or endangered species assessments. **Disclaimer/Disclosure:** project funded by CropLife Europe

5.07.T-03 Using artificial intelligence and brute force to identify diversity of laboratory non-target arthropod toxicity tests and the diversity of non-target arthropods tested

Alan Samel, Stone Environmental Inc.

Currently Non-Target Arthropod (NTA) testing in the laboratory for regulatory purposes is limited to very few terrestrial species (e.g., honey bee, bumble bees, parasitoid wasp, predatory mite). These and a few other species are used to represent the thousands of NTAs around the world. In the United States, there are

approximately 120 NTAs that are identified as endangered species. These include aquatic and terrestrial organisms that include mussels and snails, shrimps and other arthropods, aquatic insects, butterflies, bees and beetles. Exposure pathways can include direct contact, consumption of plant foliage, consumption of nectar, predation or parasitism of prey organisms that already have pesticide residues from previous exposure(s) and/or contact with soil. To determine if additional species were being evaluated in laboratory toxicity tests conducted outside the pesticide regulatory process artificial intelligence (AI) was used to determine the NTAs tested, and the types test conducted. Thorough and exhaustive literature searches were then conducted to further identify study designs and test organisms. The objective of the project was to identify laboratory toxicity tests with organisms outside the normal regulatory process that could be used applied to the endangered species risk assessment. I will discuss the process used to identify potential toxicity tests that could be used to gain a better understanding of the potential sensitivity of endangered NTAs to pesticides.

5.07.T-04 Review of toxicity test methods, exposure and species surrogacy to inform endangered species assessments for listed terrestrial arthropods: A workshop summary

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USEPA is required under the Endangered Species Act (ESA) to help ensure that pesticide registrations do not jeopardize the continued existence of federally threatened or endangered (listed) species or adversely modify their designated critical habitats. Of the 1,600 species listed as either endangered or threatened in the United States under the ESA, over 100 are terrestrial arthropod species (97 insects and 11 arachnids). Threatened and endangered arthropods represent a critical component of ESA assessments as pesticides can have direct effects on listed terrestrial arthropods or can indirectly affect other listed species which depend on these arthropods for food {e.g., birds (app. 71 species), mammals (app. 37) and fish (app. 100)} or pollination (app. 388-838 listed plants). Currently, USEPA does not have toxicity testing requirements or a risk assessment framework for assessing contact and/or oral risks of pesticides to arthropods beyond honey bees. The available acute and chronic toxicity test endpoints for larval and adult honey bees, and exposure estimates generated by the EPA risk assessment modelling tool BeeRex, is currently being used to estimate risk to arthropods when other scientifically relevant information is not available. However, there is uncertainty that honey bee toxicity data and exposure estimates are representative of toxicity and exposure for all listed terrestrial arthropod species. A multipartite workshop was held by CropLife America to discuss potential approaches for applying exposure and toxicity data from surrogate arthropod species to taxonomic groups of listed arthropods. The first day of the workshop had plenary talks focused on the state of the science of endangered insect assessments for listed arthropods, with representatives from CropLife America, USEPA's Office of Pesticide Programs, the U.S. Fish and Wildlife Service, Canada's Pest Management Regulatory Authority, and CropLife Europe all giving their perspectives. During the second day, break-out groups focused on topics related to surrogacy, hazard identification, characterization of exposure, and risk assessments for bees versus terrestrial arthropods. This presentation will summarize some of the ideas discussed in the breakout groups and the scope and nature of information needed for a direct effects assessment for a pesticide for listed arthropods.

5.08.T Beyond Direct Contact: Non-Traditional Exposure Scenarios in Human Health Risk Assessment

5.08.T-01 From farm to table: PFAS data gaps in beef and dairy

Felipe Gustavo Becker, Gregory J. Garvey, Philip E Goodrum, Kirby Tyndall and Emma Mendelsohn, GSI Environmental

Per- and polyfluoroalkyl substances (PFAS) are widely present in the environment, including human food items. Studies have demonstrated PFAS contamination in animal tissue (poultry, beef), and animal products (eggs, dairy), posing potential exposure and health risks to farming families and the general public. However, the bioaccumulative properties of PFAS in some agricultural products, such as home-produced poultry and beef, are not well characterized. Currently, risk assessments that focus on exposure to agricultural food products face

challenges in determining realistic PFAS transfer rates between soil, plants, and animals, as well as ingestion rates of animal products for under-represented populations, such as farming families. Earlier this year, USEPA published a human health risk assessment framework document for evaluating PFAS exposure from land application of biosolids. Key concepts incorporated in the exposure assessment include PFAS uptake in meat, milk, poultry, and eggs, biokinetic parameters to estimate steady-state tissue concentrations, and food ingestion rates from the USEPA's Exposure Factors Handbook (EFH). In this presentation, we identify key data gaps and highlight approaches for reducing exposure uncertainty. For example, although occurrence data are relatively abundant for some PFAS in garden produce, PFAS transfer between environmental media (e.g., soil, irrigation water), feed (locally produced and/or commercial), and animal products remains largely under-researched. In addition, while USEPA's EFH provides estimates of average daily food intake rates for the general population and farming family, further clarity is needed on how and when modifying factors are needed to address farming practices that may vary by season, region, and market sector. As the regulatory community continues to work towards developing risk-based soil screening levels for PFAS in agricultural scenarios involving land applications of biosolids, further refinements in approaches to exposure modeling are needed to reduce uncertainty in estimates of risk from consumption of beef and dairy products. We present a practical decision framework for assessing exposure and risk for such scenarios, highlighting literature and site-specific studies that can inform relevant site-specific conditions, thereby reducing uncertainty in conceptual site models and key exposure factors.

5.08.T-02 Representation of the homeless in human health risk assessment

Margaret Roy, Kestrel Tellevate Consulting

In January 2024, an estimated 771,480 people in the United States were considered homeless, an increase of 18% over 2023. Children (under the age of 18) experienced the largest increase in homelessness from 2023 to 2024. Family homelessness also increased, growing by 39% between 2023 and 2024. Further, chronic homelessness is on the rise, with nearly 152,600 people experiencing homelessness continuously for a year or more. The causes of homelessness are varied and may include displacement from disasters, families seeking asylum, lack of affordable housing, financial constraints, lack of access to shelters, insufficient shelter capacity, a need for autonomy, and mental unwellness. Human Health Risk Assessments (HHRAs) are used to assess contaminant exposure and risk to defined populations, such as residents, commercial workers, and construction workers. HHRAs can also be used to assess risk for the homeless population. It is not uncommon to find a group of homeless individuals living on contaminated sites within an encampment. These sites are often located in isolated areas and can have a surface water source onsite. The representation of the homeless in an HHRA is highly site-specific. The HHRA exposure model depends largely on the resources available at the site (e.g., water source) and understanding how the individuals living at the site are using the resources (e.g., bathing, drinking). Consistent with conducting an HHRA for other populations, care must be taken to understand the exposure scenarios and contaminants. For example, are there children within the homeless group, and are there contaminants potentially present that could adversely impact a child's health (e.g., lead)? This presentation will discuss some of the challenges associated with developing an exposure model to represent the homeless sheltering and living on a contaminated site. For example, interviews with individuals may be necessary to develop an accurate exposure model. The presentation will also discuss the importance of the field sampling crew, from observing field conditions to safety concerns.

5.08.T-03 Deriving acute soil screening values to assess remote historic mining sites: Approaches and challenges

Dana McCue¹ and Andrew Rumsby², (1)EHS Support LLC, (2)EHS Support PTY, New Zealand

Human health risk assessment and the derivation of human health screening values are typically focused on routine, daily exposure scenarios (i.e., chronic exposure). Exposure scenarios which assess acute soil exposure, defined as a single or short-term exposure event (days), are not frequently considered. As such, limited guidance or guidelines are available within the United States and Internationally, and in most cases have been

focused on the air inhalation pathway (i.e., Acute Exposure Guideline Levels [AEGLs]). This presentation will discuss the current state of science in regard to the calculation of acute soil screening criteria; describe potential receptors and exposure settings where they would apply; and, detail key sensitive assumptions related to exposure and toxicity that influence their derivation, such as appropriate soil ingestion rates for adults and children and available and applicable acute oral toxicity factors. A case study of the development of soil guideline values used to inform risk management for historic mining sites located in remote New Zealand will be provided.

5.08.T-04 Homegrown produce consumption impacted by contaminated irrigation water: An evaluation of available methodologies to calculate exposure

Alexandra Lauren Meyers and John Griffin, Integral Consulting Inc.

The United States Environmental Protection Agency (USEPA) provides standardized approaches to evaluate common chemical exposure pathways such as direct exposure to contaminants in drinking water. USEPA publishes chemical-specific Regional Screening Levels to use as benchmarks to evaluate these exposures. However, USEPA does not provide screening levels to evaluate homegrown produce consumption impacted by contaminated irrigation water. This pathway has received renewed attention due to the ubiquitous presence of emerging contaminants: 1,4-dioxane and per- and polyfluoroalkyl substances (PFAS). Our objective was to evaluate several government agency methodologies and provide recommendations on best practices for assessing this pathway. Additionally, we explored the relationship between modeled results and empirical research to evaluate model accuracy. We reviewed irrigation water screening level derivation methodologies published by the California Department of Toxic Substances Control (DTSC), Florida Department of Environmental Protection (FDEP), and Oak Ridge National Laboratory (ORNL). An approach adapted from an USEPA soil plant uptake model was also evaluated. We then calculated irrigation screening levels for 1,4-dioxane and select PFAS by applying each of these approaches using standard default exposure assumptions for a residential receptor and produce ingestion rates recommended by each agency. Bioconcentration factors (BCFs) were determined and compared to field-study derived BCFs to evaluate model estimation confidence. The DTSC, FDEP, and adapted USEPA approaches yielded relatively similar screening levels. However, the ORNL approach resulted in a screening level that was several orders of magnitude lower than the other approaches. We identified key variables and assumptions that had the greatest impact on the ORNL screening levels. Our analysis indicated that the use of default values rather than chemical-specific values in the ORNL approach yielded overly conservative results. Based on this analysis, the ORNL approach is not recommended and the DTSC, FDEP, and the adapted USEPA approaches are preferred. These approaches offer health protective outcomes; however, the estimates produced by each model are more conservative than empirical research by at least an order of magnitude. To address this concern, additional site investigations such as plant tissue sampling may be used to refine the modeled outcomes and better inform risk management decisions.

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5.08.T-05 Soil to consumer-products: Lead exposure assessment and risk reduction in former smelter community of Shymkent, Kazakhstan

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Shymkent, Kazakhstan is home to approximately 1.1 million people and the largest former lead and zinc smelter of the Soviet Union. Built in the 1930s, this refining and smelting center produced about 70% of all lead in the Soviet Union. During its more than 80 years of operation, the smelter released enormous amounts of lead-containing dust, which contaminated vast areas of the city, including residential properties. A 2011 study found

that 95% of the children tested in the city had blood lead levels (BLLs) above 10 µg/dl, the average BLL was 20 µg/dl, and the highest measured child BLL was over 100 µg/dl. Developmental delays in lead-exposed children can begin at blood lead levels (BLLs) of 1-2 µg/dL, with increasingly severe health effects at higher BLLs. International experts agree that no level of lead exposure is considered safe. In collaboration with the Environmental Health Pollution Management Institute (EHPMI) and Human Health Institute (HHI), TerraGraphics International Foundation is supporting ongoing efforts to assess heavy metal contamination in Shymkent. Handheld x-ray fluorescence (XRF) analyzers were used to quickly and reliably screen media for heavy metals. Throughout May and June 2025, additional XRF screening throughout the industrial zone will expand understanding of the extent and depth of heavy metal contamination in public spaces. Detailed site assessments of residences, including home vegetable gardens, coupled with testing of consumer products such as toys, paint, and dishes will help identify key areas for intervention and remediation in the home. Engaging residents in the screening process increases understanding of heavy metal exposures. In partnership with local government, researchers, and community members, site-specific remedial and health intervention strategies will be proposed to reduce heavy metal burdens on the most vulnerable communities. Results from the 2025 field work and analysis will be presented, along with multi-media human health risk assessment and proposed health intervention strategies. This approach may be applied to similar smelter zones and other industrial communities across the world that are affected by decades of heavy metal contamination. **Disclaimer/Disclosure:** This project is funded in part by the Sigrid Rausing Trust.

5.09.P-Mo New Approaches, Data and Solutions Regarding the Environmental Risks of Sunscreens and Cosmetics

5.09.P-Mo-121 Adverse effect thresholds in aquatic biota for organic ultraviolet filters in sunscreens: A data summary and prospectus

Daleth Del Salto, Laura M. Basirico, Kevin Armbrust and Jonathon Doering, Louisiana State University

Organic UV filters are active ingredients used in personal care products applied to the skin towards sunburn, skin cancer, early skin aging, and other risks of overexposure to ultraviolet (UV) radiation present in sunlight. Twelve organic UV filters widely used in the United States (US) are classified by the US Food and Drug Administration (FDA) as having insufficient data to determine their safety status for both humans and the environment, namely avobenzone, oxybenzone, sulisobenzene, dioxybenzone, cinoxate (CIN), ensulizole, homosalate, meradimate (MER), octinoxate, octisalate, octocrylene, and padimate-O (PDO). Organic UV filters can enter the aquatic environment during swimming and other recreational water activities or through wastewater discharge, landfill leachate, and manufacturing. As a result, the deficiency in data for these chemicals has raised concerns for the potential for impacts to the aquatic environment. The aims of this study were to: (1) review all existing toxicity data for the 12 organic UV filters, (2) compile adverse effect (AE) concentrations across aquatic taxa, (3) identify priority data gaps for future research. A total of 74 papers were found with 470 adverse effects (AE) concentrations for molecular, biochemical, sublethal, and lethal endpoints in microorganisms, plants, invertebrates, and fishes. No aquatic toxicity data was found for CIN, MER, or PDO. Fish were the most studied taxon showing 223 AE, yet only 6 of 12 UV filters were tested on them. Invertebrates (142 AE) covered 9 UV filters, plants (86 AE) covered 5, and microorganisms (28 AE) covered 6. The 9 UV filters with existing aquatic toxicity data had similar adverse effect concentrations across taxa. For acute exposures, fish had the greatest sensitivity displaying AE beginning at 0.01 µg/L followed by invertebrates (0.1 µg/L), microorganisms (10 µg/L), and plants (100 µg/L). Under chronic exposures, microorganisms and plants had the greatest sensitivity, displaying AE beginning at 0.01 µg/L, followed by invertebrates (0.1 µg/L) and fish (1 µg/L). This compilation of existing aquatic toxicity data suggests that all aquatic taxa exhibit AE to similar and environmentally relevant concentrations of these organic UV filters. However, research is needed for CIN, MER, and PDO that remain untested.

5.09.P-Mo-122 Relative risk index for mercury exposure from cosmetics

Connor Kasper and **Robert N Brent**, James Madison University

Mercury poisoning from cosmetics, particularly skin-lightening creams, is a growing concern globally and within United States (US) immigrant populations. Legacies of colonialism and cultural perceptions of beauty drive the sale and use of skin-lightening creams within African, Asian, Caribbean, and Middle-eastern communities. Many of these products contain elemental or organic mercury to inhibit the production of melanin in the skin. Since mercury is a known toxin, the usage of these products can lead to a variety of health complications including renal toxicity and neurological abnormalities. The US has regulations that limit the amount of mercury in cosmetics, but it is possible for these products to still enter the country through local market imports and online purchases. This means that there is a risk of U. consumers experiencing health complications due to mercury exposure from these skin-lightening products. To assess these risks, we developed a GIS-based relative risk index for US states and counties. This index was developed from a combination of US Census Bureau data on immigrant populations and Google search analytics (using the Glimpse extension) on regional frequencies of skin-lightening search terms. By combining information on the location of immigrants from certain countries of origin and the relative frequency of skin-lightening searches in those countries of origin, we were able to estimate the relative risk of health complications from mercury exposure to skin-lightening creams across the country. This analysis revealed the top five counties with highest risk as: Queens, NY; Bronx, NY; Essex, NJ; Prince Georges, MD; and Kings, NY. The top five statewide risks were in NY, MD, NJ, CT, and DE. This risk index can be useful in targeting education programs about mercury toxicity and alerting medical professionals to be watchful for the presentation of mercury health effects in high risk areas.

5.09.P-Mo-123 Risks of UV filters in UK freshwater environments

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The amounts and variety of UV filtering chemicals in skin care products is increasing and emissions to the natural environment are inevitable. While the occurrence, effects and risks of UV chemicals in marine systems have been relatively well-studied, much less is known about the occurrence, effects and potential risks of these substances in freshwater systems. This study therefore: 1) Explored the occurrence of three commonly-used UV filters (octocrylene, benzophenone-3 and homosalate) in freshwater systems using continuous monitoring over a one year period at 19 river locations across Yorkshire, England; 2) Performed laboratory-based ecotoxicity studies to assess the effects of each of the study UV filters on the growth of twelve phytoplankton and periphyton species; and 3) Compared the occurrence and laboratory effects data to establish whether these substances could be posing an unacceptable risk to primary producers in freshwater environments in the UK. UV-filters were detected at all of 19 locations monitored. Benzophenone-3 was the most frequently detected filter, detected in around half of the samples, followed by homosalate and octocrylene. Octocrylene had the highest mean concentration followed by benzophenone-3 and homosalate. The location with the highest concentrations of the UV filters was on the river Wharfe immediately downstream of a designated bathing water site. The different algal species responded very differently to exposure to the three compounds with the diatom, *Aulacoseira granulata*, being the most sensitive to all three study compounds. Octocrylene was observed to be the most toxic to the algal species tested followed by homosalate and benzophenone-3. Use of the ecotoxicity data to generate species sensitivity distributions for each substance resulted in HC5 (hazardous concentrations for 5% of species) of 0.011, 0.022 and 0.385 mg/l for octocrylene, homosalate and benzophenone-3 respectively. The measured concentrations, obtained to date, in Yorkshire's rivers were more than an order of magnitude lower than these HC5 values suggesting a low risk to primary producers in these freshwater environments. **Disclaimer/Disclosure:** This study was partly funded by Beiersdorf

5.09.P-Mo-124 How time and place define the concentration of UV filters off a recreational beach in Florida, USA

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Ultraviolet filters are a diverse group of compounds with a wide range of commercial applications, including as ingredients in sunscreens, that have been detected in coastal locations reflecting the extent of recreational activity. Despite the potential for source inputs to vary over the day monitoring programs do not typically capture this temporal variation. To investigate this 3 composite samples (each n=10 discrete samples) were collected along two 100m transects at different distances from the shore (T1; nearshore in the bathing zone and T2 ~50m from the shoreline) at multiple times over a 24-hr period. Samples were immediately processed by filtering (GFF) and solid phase extraction to prepare the 'dissolved fractions' with the GFF filters frozen and later extracted to prepare the 'particulate' fractions. All extracts were analyzed quantitatively for 12 organic UV filters using LC-MS/MS. Oxybenzone (BP-3), octocrylene (OC), homosalate, octisalate, avobenzone, octinoxate, and, OD-PABA were measured in all samples, with consistently higher levels observed in May, reflective of the increased number of recreational users. UV filter concentrations were higher closer to the shore and reduced significantly, with at least a 60% decrease just outside of the bathing zone. All UV filters followed similar temporal trends peaking mid afternoon (2-4 pm) reflective of the number of recreational users. Partitioning of the UV filters between the 'dissolved' and 'particulate' fractions matched their physio-chemical properties. Higher concentrations of OC were observed in the 'particulate' versus 'dissolved' phases reflective of its lower solubility and higher logKow, whereas, BP-3 concentrations were nearly all in the 'dissolved' phase. Time-weighted measured environmental concentrations (MECs) were calculated to facilitate the consideration of temporally-explicit exposure data within environmental risk assessments (ERAs). Overall, these results indicate that aquatic organisms in popular recreational areas may encounter UV filter concentrations that are highly variable over a 24-hr period rather than a steady environmental exposure, and that concentrations rapidly decline in bathing zone after people leave. These results highlight that both location and time is important in environmental risk assessments for UV filters. Furthermore, to understand total concentrations and exposure routes it is important to assess partitioning by filtering samples.

5.09.P-Mo-125 Organic ultraviolet filters in sunscreens are cytochrome P450 inhibitors in fishes

Vann Lynne Boyte, Christopher Lowrey-Dufour, Lauren Eagon and Jonathon Doering, Louisiana State University

Organic ultraviolet (UV) filters are widely used in sunscreens and personal care products to protect the skin against harmful UV light. These compounds are frequently detected in aquatic environments due to recreational water use, wastewater effluent, and landfill leachates. Several of these UV filters are known to inhibit the activity of phase I biotransformation enzymes in mammals, including cytochrome P450 1A (CYP1A) and cytochrome P450 3A (CYP3A). However, whether organic UV filters share these effects in fishes and if there are differences in sensitivity among species was unknown. The objective of this study was to assess the inhibitory potential of 8 common UV filters on the enzyme activity of CYP1A across 3 phylogenetically diverse species of fish, namely red drum (*Sciaenops ocellatus*), American eel (*Anguilla rostrata*), and spotted gar (*Lepisosteus oculatus*). Specifically, hepatic microsomes in 96-well plates were exposed to serial concentrations of each UV filter and ethoxyresorufin-O-deethylase (EROD) activity was measured after 60 minutes. Alpha-naphthoflavone (ANF) served as a reference CYP1A inhibitor. Dose-response curves and 50% inhibitory concentrations (IC50s) were generated for each compound. The reference inhibitor, ANF, exhibited a dose-dependent inhibition of CYP1A activity in each species with IC50s ranging from 193 to 785 nM. Six of the 8 UV filters inhibited CYP1A activity in at least 1 species, with average potencies of ANF > BP4 > BP3 > BP2 > Octinoxate > BP1 > BP8. Octocrylene and homosalate did not inhibit CYP1A activity in any species. Only

octinoxate (34-fold), BP3 (39-fold), and BP4 (15-fold) had IC50s that ranged by more than an order of magnitude among the 3 species, with red drum being the most sensitive. These results confirm that several organic UV filters can inhibit CYP1A activity across diverse fish taxa in common with mammals. Overall, this research highlights the potential for organic UV filters commonly used in sunscreens and personal care products to disrupt the detoxification pathways of fishes that could result in synergistic toxicities with other pollutants, such as polycyclic aromatic hydrocarbons (PAHs) or chlorinated dioxin-like chemicals. Ongoing research is investigating the inhibition of other key biotransformation enzymes in fishes, namely CYP2A and CYP3A, and could be essential for objectively assessing the risks posed to aquatic environments from the widespread use of organic UV filters.

5.10.P-Tu Per- and Polyfluoroalkyl Substances (PFAS) in Our Food: From Farms and Fields to Streams and Shores

5.10.P-Tu-088 Spatiotemporal trends and regulatory risk of PFAS in two major Pennsylvania rivers

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This project aimed to conduct targeted analyses of per- and polyfluoroalkyl substances (PFAS) in the Delaware and Schuylkill Rivers. Water samples were collected from several locations and analyzed using the EPA 1633 method for PFAS. The results indicate the widespread presence of various PFAS compounds, with PFBA, 6:2 FTS, PFOA, and PFOS being the most abundant in the Schuylkill River, detected at nearly all sampling locations. In the Delaware River, the prevalent compounds shifted from PFTeDA, PFTrDA, and PFDS in 2019 to 6:2 FTS and PFOA in 2021. Factors such as population density, proximity to industrial sites, and other pollution sources appear to influence these distribution patterns, particularly as concentrations of targeted PFAS compounds increase from Pottstown in the Schuylkill River and Trenton in the Delaware River toward central Philadelphia. Risk assessments, based on EPA advisory levels for PFOA and PFOS, using the Risk Quotient method and proposed Hazard Index, revealed that at least 60% of samples from both rivers presented a medium risk profile according to 2016 EPA guidelines, while 70% of samples fell into the high-risk category under 2024 EPA regulations. This evaluation is crucial, as both rivers are sources of drinking water and serve multiple wastewater and water treatment facilities.

5.10.P-Tu-089 Legacy and novel per- and polyfluoroalkyl substances (PFAS) in food plants: Structural insights into bioaccumulation and exposure risk

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Since the early 2000s, global restrictions on legacy per- and polyfluoroalkyl substances (PFAS) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have led to the development of novel PFAS. These emerging compounds often feature structural modifications - such as shorter carbon chains, ether insertions, or fluorine-to-hydrogen or chlorine substitutions - aiming to reduce toxicity and persistence. However, some studies suggest that novel PFAS may match or exceed their legacy counterparts in persistence and toxicity. The reuse of treated wastewater (TWW) for agricultural irrigation supports water sustainability but may introduce PFAS into food systems, as conventional treatment methods are often ineffective at PFAS removal. While some studies have addressed the plant bioaccumulation of legacy compounds, the influence of novel PFAS structures remains poorly understood. This study aimed to: (1) investigate structural drivers of PFAS bioaccumulation in edible plants, (2) develop mechanistic models for plant accumulation based on molecular features, and (3) inform future irrigation practices to safeguard human health. Dwarf determinate tomatoes (*Solanum lycopersicum*) were hydroponically grown in nutrient solution spiked with ten PFAS (PFBA, PFBS, PFHxA, PFHxS, PFOA, PFOS, GenX, ADONA, 6:2 FTSA, and 6:2 Cl-PFESA) at 5 ng/L each. Leaves and fruits were sampled every 9 days to monitor PFAS accumulation over time, and bioaccumulation factors (BAFs) were calculated at maturity. Novel PFAS such as GenX (301.1) and ADONA (12.8) showed significantly higher BAFs in fruits than legacy PFOA (2.2). In leaves, both legacy and novel compounds

reached high concentrations, with the highest BAFs for 6:2 FTSA (409.6), ADONA (406.4), PFBA (384.9), and GenX (367.9). PFAS with smaller molecular size, ether groups, or hydrogen substitutions accumulated more readily in above-ground tissues, likely due to enhanced transport across biological barriers and along the transpiration stream. In contrast, larger compounds with chlorine substitutions showed lower BAFs, such as 6:2 Cl-PFESA (105.7 in leaves and 0.11 in fruits), possibly due to restricted mobility. These findings suggest that certain novel PFAS may pose equal or greater human exposure risks through consumption of leafy and fruit crops irrigated with TWW. Structural insights can inform safer chemical design and targeted management strategies to reduce dietary exposures from crop uptake. **Disclaimer/Disclosure:** The authors gratefully acknowledge the USDA National Institute of Food and Agriculture (2023-67019-39702) for funding support.

5.10.P-Tu-090 Per- and polyfluoroalkyl substances (PFAS) risk assessment of irrigation water applied to fodder crop fields

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Jacobs performed a risk assessment evaluating the potential for adverse effects from per- and polyfluoroalkyl substances (PFAS) in irrigation water. Irrigation water was applied on agricultural fields where alfalfa and grass are farmed and sold as livestock fodder. Grazing did not occur on the fields where the contaminated irrigation water was applied nor was the water used as a livestock drinking water source. Adjacent property fields were used for domesticated horse grazing and potentially received overspray of irrigation water. Two modelling scenarios were completed. A human health risk assessment (HHRA) evaluated potential risks from consumption of beef and milk from livestock consuming fodder growing on fields where the irrigation water was applied. An ecological risk assessment (ERA) evaluated exposure to domestic horses consuming grass and incidentally ingesting soil. Using site-specific fodder tissue concentrations, the HHRA estimated perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) uptake into livestock eating the fodder, estimated livestock blood serum levels based on modelled intake, and estimated meat and milk concentrations from the modelled blood serum levels. Chemical intake estimates from adult and child meat and milk consumption were compared with chemical-specific reference doses and the excess lifetime cancer risk (ELCR) was estimated for PFOA. Estimated non-cancer indices for meat and milk consumption of PFOS and PFOA were below the threshold of 1 on a cumulative basis and the ELCR was less than 1×10^{-6} , indicating potential risks associated with consuming meat or milk from livestock ingesting the site fodder are acceptable. The ERA incorporated site-specific tissue and soil concentrations and relied on toxicity values from Argonne National Laboratory and life history data for domestic horse from the Texas Commission on Environmental Quality webpage for developing protective concentration levels. The ERA evaluated risk from exposure to PFOS, PFOA, and six other PFAS. Conservative modelling within the ERA indicated the likelihood of adverse effects to a domestic horse from PFAS at concentrations measured was de minimis. Though PFAS guidance values for soils, feedstock, livestock protection, or livestock consumption are limited, available data sources and tools were successfully used to support a risk management decision. Similar assessments may help agricultural landowners evaluate risk from exposure to PFAS on their lands.

5.10.P-Tu-091 PFAS examined in 14 frequently consumed fish species in the Cape Fear River, North Carolina

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In North Carolina (NC), there is a point source of PFAS contamination into the Cape Fear River – a river that runs over 300 kilometers (km) in length, serves as an important drinking water resource for NC residents, and is widely used by commercial and recreational anglers throughout the watershed. As a result of point-source contamination, the Cape Fear River contains a unique mixture of PFAS with specifically high concentrations of emerging PFAS that were first detected in the watershed in 2015. Due to the recent discovery of these chemicals, there remain many unanswered questions regarding the chemical toxicity, environmental accumulation, and impacts resulting from exposures both to wildlife and local communities. Therefore, our

study aims to answer some of these questions by assessing environmental accumulation/detection of 56 PFAS, both legacy and emerging, across freshwater and saltwater environments in fillets of the most frequently caught fish species in the lower Cape Fear River. This design is meant to yield information about accumulation of these PFAS in local wildlife, present information about human exposure through diet, and allow the assessment of spatial distributions across the river system. To achieve these aims, a fish and water collection project was conducted by the NC Department of Environmental Quality (NCDEQ) from June – August 2022 with supplemental sampling in October 2023. Over 250 fish across 14 species were collected from the Cape Fear River, starting below the point source and ending at the Atlantic Ocean. The fish species were identified through public surveys as the species that are most frequently recreationally caught and consumed in the Cape Fear Region. The fish were analyzed alongside water samples collected in situ for 56 different PFAS to support bioaccumulation factor (BAF) calculations across trophic levels in NC. The BAFs were used in tandem with reference doses (RfDs) to support NCDEQ standards development to protect public health and NC's water resources. Similarly, concentrations of PFAS in collected fish tissue were analyzed by the NCDHHS for the development of PFAS-specific fish consumption advisories. Altogether, the data collected in this study have informed many current activities at NCDHHS and NCDEQ and have provided a comprehensive data set to inform additional PFAS fish studies beyond NC.

5.10.T Per- and Polyfluoroalkyl Substances (PFAS) in Our Food: From Farms and Fields to Streams and Shores

5.10.T-01 Species-specific characteristics influence PFAS bioaccumulation in fish

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Little is known about species-specific bioaccumulation of per- and polyfluoroalkyl substances (PFAS) compounds in fishes. General trends with length, lipid content, and diet that characterize other contaminants, such as mercury and polychlorinated biphenyls, are not well described for PFAS in fishes. Here, we make use of a large PFAS database provided by the New York State Department of Environmental Conservation (NYSDEC) that includes 85 unique waterbodies and 53 unique fish species across New York State (n = 2511 individuals). Using both whole-body and fillet preparation results, we tested general assumptions regarding PFAS bioaccumulation to deduce trends among species and families that are useful for future sampling and policy decisions. Bayesian mixed effects models were built to rank order species and remove the effect of waterbody contamination. We found little predictive power on PFAS concentrations from length and lipid content. However, we reliably ranked species and family by PFOS, PFDA, PFUnA, and PFDoA concentrations. General diet among all waterbody types was a significant predictor for PFOS and PFDA, with benthic specialists, such as Ictaluridae, ranked consistently lower than other families. Some species performed consistently across PFAS with Blacknose Dace (*Rhinichthys atratulus*) and Brown Trout (*Salmo trutta*) among the highest ranked species for all four compounds. These results provide context for bioaccumulation patterns and may potentially be extrapolated to species that are not tested here but are in the same families. Our findings help advance our understanding of PFAS bioaccumulation in aquatic life to inform environmental monitoring and risk assessments. We recommend a subset of species to sample in future survey efforts including Brown Trout and Blacknose Dace. The modeling framework we have created can also be used for PFAS and contaminants of emerging concern not described here to achieve similar results.

5.10.T-02 Grain or fiber? Investigating plant uptake for the sustainable management of PFAS contaminated land

Ariana Lazo, Chancellor Clark, Jianxin Ma, Emily Whittington, Marguerite Bolt and Linda S. Lee, Purdue University

The land application of biosolids has historically benefited agriculture by increasing crop yields, improving soil health, and providing a sustainable alternative for managing wastewater treatment residuals and industrial byproducts. However, the land application of biosolids can also introduce contaminants of concern such as Per- and Polyfluoroalkyl Substances (PFAS) into the soil; creating an avenue for the contamination of agricultural crops through plant uptake. Two greenhouse studies were initiated to investigate the potential of PFAS accumulation from contaminated agricultural soils: (i) multi-variety soybean greenhouse study (ii) grain vs. fiber industrial hemp greenhouse study. In the multi-variety soybean study, six soybean varieties were selected for their drought tolerance mechanisms and protein/oil contents to explore factors influencing plant uptake in soybean. Soybeans were grown in PFAS contaminated agricultural soil predominated by PFOS and EtFOSAA (Total PFAS = 2866 ug/kg). For one genetic variety, an additional treatment of wood ash biosorbent was included. Soybean grain had negligible concentrations of bioaccumulative PFAS, such as PFOS, but had elevated concentrations of short-chain PFCAs. Soybean leaves had the greatest PFAS uptake followed by stems, beans, and beanpods. Soybean genetics played a role in PFAS uptake as the two soybean varieties optimized for yield had greater PFAS concentrations in leaf tissues. Wood ash carbon sorbent has potential for reducing PFAS accumulation in soybean seeds. In the industrial hemp study, the monoecious cultivar Bialobrzieski was grown under two cultivation strategies: grain vs. fiber production; to evaluate if PFAS uptake varies with management to explore economically viable land use strategies. The experiment was replicated in a soil treatment containing a wood ash biosorbent to determine if the incorporation of sorbent material can reduce the plant uptake of PFAS into industrial hemp. Hemp plant tissues- bast(phloem), hurd (xylem), leaves, pollen, and grain will be harvested separately to examine tissue-specific PFAS partitioning and decipher the mechanisms of plant uptake. The results of this research provide insights on the mechanisms of PFAS plant uptake, wood ash biosorbent amendments, and economically viable land-management practices for PFAS-contaminated agricultural systems. **Disclaimer/Disclosure:** This work is funded by the USDA Cooperative Agreement # FSA23CPT0013449

5.10.T-03 Food safety evaluation of PFAS in home-raised livestock in Washington State

Barbara Morrissey, Emerson Christie and Claire Nitsche, Washington Department of Health

Per- and poly-fluoroalkyl substances (PFAS) are currently impacting 100's of drinking water wells across WA state, including in rural areas. The same well water that people drink is often used to water livestock raised for personal consumption. Studies in other states and countries have shown that livestock with dietary exposure to PFAS can have contaminated meat, milk, and eggs. Residents in two rural communities with PFAS-contaminated well water (East Selah, Yakima County and West Plains, Spokane County) asked the WA Department of Health (WDOH) to further evaluate the safety of consuming their home-raised livestock. Between December 2023 and April 2025, WDOH collected over 70 samples of home-raised livestock products for food safety evaluation. Samples were analyzed for 16-18 PFAS by the United States Department of Agriculture using their method CLG-PFAS 2.04, or by Enthalpy Analytical lab using EPA method 1633. All 35 participating households had PFAS contamination in their drinking water wells. Concentration of PFAS in well water ranged from less than 10 ppt to over 1000 ppt. Results: PFOS and PFHxS were frequently detected in chicken and duck eggs and less frequently in poultry meat, pork and beef. WDOH developed public health consumption advice for each family tailored to the household members who regularly consumed the foods. The majority of participating households were advised to reduce their average weekly consumption of at least one home-raised food based on the PFAS testing results. We also offered retesting of foods after the animals were switched to alternate drinking water. Retesting often showed that switching to clean water reduced levels of PFAS in the foods but also that further investigation and action will be needed to fully restore the safety of these foods for regular human consumption. Our project demonstrates that home-raised meats and eggs can be an important secondary exposure to people living with PFAS contamination in their drinking water supply. There is an urgent need to investigate and mitigate these exposures where PFAS contaminate rural drinking water supplies. **Disclaimer/Disclosure:** This work was partially supported by laboratory assistance from the United States Department of Agriculture Food Safety and Inspection Service Laboratory in St. Louis.

5.10.T-04 Field-scale versus lab-scale evaluation of high carbon wood ash in mitigating PFAS uptake into feed grasses

Elijah Ojo Openiyi¹, Linda S. Lee¹, Andrew Carpenter² and Romy Carpenter², (1)*Purdue University*, (2)*Northern Tilt LLC*

Per- and polyfluoroalkyl substances (PFAS), is a family of synthetic chemicals, that have been found to be recalcitrant, bioaccumulative, and linked to adverse health effects. PFAS get into agricultural soil primarily through wet/dry deposition, irrigation with PFAS-contaminated water, and land application of biosolids. Given the persistence, mobility, and increasing occurrence of PFAS in agricultural landscapes above well above background levels, developing effective field-scale mitigation strategies is essential to reduce environmental loading and minimize transfer into the food chain. In this study, we evaluated the mitigation effect of high carbon wood ash in reducing PFAS into feed grasses over multiple harvests grown in a yearlong greenhouse and 2-year field study. The PFAS-contaminated soils used in the greenhouse study were taken from the same agricultural field as the field plots. Wood ash was mixed at 1.5, 3.0 and 6.0 wt% with PFAS-contaminated soils in the greenhouse study and at 2.0 and 6.0 wt% in the field plots. Our greenhouse findings indicate that the wood ash significantly reduced ($P < 0.05$) grass uptake of several short- and long-chain PFAS in each harvest cycle. Meanwhile, the wood ash was less effective at mitigating PFAS uptake in the field plots as greater amounts of PFAS were taken up by the grass leaves in the two harvest cycles of the first year. The discrepancies between the greenhouse and field results may be attributed to factors inherently associated with field conditions, including spatial variability in PFAS concentrations across plots, fluctuating weather patterns, thus soil moisture, and atmospheric deposition, all of which are either absent or tightly controlled in a greenhouse setting. However, consistent across both the greenhouse and field study, more PFAS were detected in the grass leaves with increasing harvest circles. A comprehensive assessment of the field-scale efficacy requires second-year harvest data, which is currently being processed, as PFAS uptake is influenced by both the initial concentration in the soil and the duration of plant root exposure.

5.10.T-06 Beyond the tap: Rethinking PFAS exposure through food

Noelle DeStefano, *Metiri Group*

PFAS contamination is most often discussed in the context of drinking water, but food is increasingly recognized as a meaningful contributor to overall exposure, particularly in areas where water contamination is well-managed or absent. Despite this, dietary pathways remain under-characterized in both scientific research and regulatory policy. This presentation takes a systems-level look at how PFAS can enter the food supply: through crops irrigated with contaminated water or grown in biosolid-amended soils, livestock exposed via feed and drinking water, and migration from food packaging or processing equipment. These routes are often interconnected and, in many cases, fall outside the scope of current monitoring and risk evaluation efforts. To map these exposure pathways, I synthesized findings from peer-reviewed studies, federal data, and environmental casework. The goal is to outline what's currently known, highlight the blind spots, and explore why certain exposure routes continue to be underestimated or overlooked. What emerges is a patchwork of fragmented data and mismatched regulatory oversight. Methods vary by matrix, detection limits remain inconsistent, and there is little alignment between environmental and food safety standards. By putting these pieces together, this work offers a foundation for more coordinated risk assessments, improved monitoring strategies, and clearer communication between scientists, agencies, and the public. It also raises a core question: if we're only measuring PFAS in water, are we missing the bigger picture?

5.10.V Per- and Polyfluoroalkyl Substances (PFAS) in Our Food: From Farms and Fields to Streams and Shores

5.10.V-01 PFAS in our food—A growing threat from farm to table in Europe—Case study Germany

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Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals. Due to their distinctive characteristics, they are used in a variety of industries and consumer products, including food packaging. However, there is growing evidence that raises significant concerns about the environmental and health impacts of PFAS, primarily due to their persistence, bioaccumulation, and toxicity. This study examined the presence of PFAS in the European food supply, using Germany as a case study. The research looked at how contaminants move from environmental sources through agriculture to processed foods and ascertains the impact of PFAS on public health and future food security in Europe. We performed a comprehensive review of scientific literature, reports from environmental and food safety organizations (e.g. the European Food Safety Authority [EFSA] and the World Health Organization)) and official data from German authorities). The study investigated the sources of PFAS, their behavior and uptake into agricultural systems (soil, water, plants, animals) and the complex challenges of regulating and managing PFAS in agricultural lands. Soils in crop fields and groundwater used for drinking water in agricultural communities or for livestock water were a source of direct exposure and a key transport medium for secondary exposure to PFAS. The primary focus of the study was to ascertain the impact of these chemicals on public health and future food security in Europe. This included an evaluation of the risks to human health posed by the consumption of PFAS containing foods, with reference to the findings of EFSA. Levels reported by EFSA were in the range of levels indicative of a significant concern regarding immune system dysfunction. The utilization of authentic case studies, exemplified by the analysis of PFAS in wild boar from Brandenburg and the outcomes of market basket monitoring, served as a demonstrative medium for elucidating the extent of contamination in diverse food products (meat, fish, vegetables). This study examined the extant research on this subject in Europe, with a particular focus on that conducted in Germany. This study elucidates the prevailing knowledge gap in this field and provides a cogent rationale for the imperative of mitigating human exposure to PFAS, thereby ensuring the safety and sustainability of the European food supply. **Disclaimer/Disclosure:** I hereby confirm that this work was completed in collaboration with my colleague C. McCarthy and that only the indicated sources and aids were used. For the literature research, Google Scholar, relevant legal regulations, reports of food safety organizations, and publications of other relevant authorities and organizations were used. Copilot was used to support the linguistic elaboration and structuring processes.

5.11.P-Mo Pharmaceuticals in the Environment—Global Innovations in Risk Assessment, Regulation and Science

5.11.P-Mo-127 Influence of site on the uptake of anti-inflammatory pharmaceuticals in *Lampsilis siliquoidea*

Kathleen Mayer¹ and **Austin D. Gray²**, (1)Department of Biological Sciences, Virginia Tech, (2)Virginia Tech
Pharmaceuticals have been increasingly detected globally in a variety of environmental matrices, including surface water and sediments. The presence of these compounds in the environment presents a risk to aquatic organisms. Bioaccumulation of commonly detected anti-inflammatory pharmaceuticals (AIPs) such as ibuprofen, acetaminophen, and aspirin are of particular interest in filter-feeding organisms such as freshwater mussels. They are highly biodiverse and valuable to aquatic ecosystems; however, the decline in their biodiversity and populations emphasizes the importance of investigating the potential impacts of pharmaceuticals found within their habitats. To understand how different habitat characteristics (e.g. sediment particle size, water chemistry etc.) influence uptake of pharmaceuticals, we exposed Fatmucket mussels (*Lampsilis siliquoidea*) to relevant and above relevant concentrations of AIPs in mixture for 21 days with different sediment types, and used liquid chromatography-mass spectrometry (LC-MS) analysis to investigate concentrations across time from different compartments (i.e. water, sediment, and tissue). Our surface water data revealed differences in concentration that follows a dose-dependent relationship. Acetaminophen and ibuprofen were present in water at ranges of 0 to 34.8 ug/L and 0.51 to 75.6 ug/L respectively, while aspirin was found to be at a range of 0 to 24.2 ug/L. Aspirin was not detectable in tissue (suspected to be from possible biotransformation and excretion). However, both acetaminophen and ibuprofen were detected at a maximum of

10 ng/g and 203 ng/g of wet weight, respectively. We found that site had a significant effect on water and tissue concentrations ($p = <0.001$ for both compartments), suggesting an environmental influence on AIP partitioning. Understanding these relationships can provide researchers with greater insight into how the environment can influence uptake in freshwater systems. Our findings also warrant further investigation into sublethal responses that may impose different physiological responses for freshwater mussels, gaining a more holistic view into factors influencing their decline.

5.11.P-Mo-128 Degradation products of siRNAs following incubation in sewage sludge

Laura Garcia Barcia, Harvey Andersen, Alec Bell, Ryan Hill, Christopher Wiethoff and Alison Nimrod Perkins, Eli Lilly & Company

Small interfering RNAs (siRNAs) are an emerging class of genetic medicines that are able to selectively silence target genes. siRNAs are duplexes consisting of a sense strand and an antisense strand and are chemically modified to reduce human metabolism. To understand the fate of siRNAs in sewage sludge in order to assess their potential entry to the environment, we conducted a degradation study using sewage sludge prepared according to OECD 314B. The siRNA tested is one that targets hypoxanthine-guanine phosphoribosyltransferase (HPRT) and contains common siRNA structural modifications such as 2'-O-methyl- and 2'-fluoro- substitutions on the nucleosides and phosphorothioate linkages in the backbone. The siRNA duplex and the antisense strand were independently incubated in sludge in non-aerated 2-mL microcentrifuge tubes for 7 days. To monitor nuclease activity during incubation, we used fluorescent probes designed to detect endonuclease and exonuclease activity. Results with the fluorescent probes indicated that both endo- and exonuclease activity was present in sewage sludge throughout the 7-day incubation. High-resolution mass spectrometry revealed that the parent siRNA duplex incubated in biotic sludge degraded by 93.6% within 24 hr, 99.7% by Day 4, and was undetectable on Day 7. The antisense strand showed similar degradation in biotic sludge: 98.2% on Day 1, 99.9% by Day 4, and was also undetectable on Day 7. Metabolite identification using Mass-MetaSite software detected 25 degradation products ranging from 5 to 23 nucleotides. The results demonstrate that GalNAc-conjugated siRNAs can be rapidly and extensively degraded in sewage sludge, resulting in small oligonucleotide fragments.

5.11.P-Mo-129 What's new with active pharmaceutical ingredients (APIs) in the EU? Understanding the requirements and risk assessment of APIs according to the new EMA ERA guideline

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Active pharmaceutical ingredients (APIs) with approved medicinal uses offer benefits to society, aiming to improve the quality of life in humans. However, concerns persist about their potential effects on the environment from continuous release through excretion and waste disposal. In response to the advancement of scientific understanding and regulatory needs, the European Medicines Agency (EMA) published an updated environmental risk assessment (ERA) guideline, which became effective in late 2024. While the prior guideline provides a basic ERA framework and shares a Phased approach, the updated guideline provides a more structured and comprehensive ERA methodology. Key updates to the ERA guideline are reflective of the regulatory developments in the European Union (EU) with an emphasis on the evaluation of endocrine active substances (EAS) and substances with persistent, bioaccumulative, and toxic (PBT) or very persistent and very bioaccumulative (vPvB) properties. Notable updates in the ERA guideline include the addition of physical-chemical properties and sediment-water toxicity data in the initial Phase II dataset, while removing sediment fate data. The 2024 guideline also expands the scope of the ERA, including (if necessary) assessments of groundwater via porewater and secondary poisoning in aquatic and terrestrial food chains. Regarding hazard assessment, both the 2006 and 2024 EMA guidelines required screening and definitive PBT/vPvB assessments. However, the 2024 guideline introduces a requirement to calculate and report ion-corrected log Dow values as a potential trigger for definitive PBT/vPvB assessments for ionizable APIs. Additionally, the derivation of the predicted no-effect concentration (PNEC) for groundwater assessment was revised. Previously, it was based on chronic toxicity endpoints in daphnia but is now based on the lowest PNEC from any aquatic toxicity endpoint,

making groundwater a likely driver of the risk assessment. This revision is significant as the predicted environmental concentration in groundwater is 10-fold lower compared to surface water according to the 2024 Guideline calculations. In summary, this presentation highlights the substantial changes in the 2024 EMA guideline compared to its 2006 predecessor and evaluates the important implications for the testing and regulatory requirements of the pharmaceutical industry concerning marketing drug products in the EU.

5.12.P-Th Potential Environmental Impacts of CO₂ Releases From Carbon Capture and Storage (CCS) Activities

5.12.P-Th-111 Human health, environmental, physicochemical and regulatory information for amine solvents, degradation products, and impurities associated with post-combustion CO₂ capture: A screening assessment

Ari Lewis¹, Tatiana Manidis¹, Robert Trautz², Annette Rohr² and Abhoyjit Bhowan², (1)Gradient, (2)EPRI Carbon dioxide (CO₂) capture and storage, which has been used commercially on a large scale for over 70 years, is expected to increase as industries start to decarbonize. Emissions and waste streams from post-combustion CO₂ capture (PCC) facilities in commercial environments, however, are poorly characterized. To better understand the health, safety, and environmental features of PCC, Gradient, in collaboration with EPRI, undertook a screening assessment of available human health, environmental, and physical hazard information, as well as regulatory information, for chemicals associated with PCC, including chemicals used in PCC process, their degradation compounds, and other constituents in PCC waste streams. First, we identified 76 compounds present in, or expected to be present in, waste streams from PCC using two key 2022 reports by EPRI and the UK EA. We then documented key information on human health, environmental, and physical hazards classified by different regulatory programs under the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and other selected regulatory determinations and limits. Since a regulatory agency had not yet assessed many of the compounds identified, we used several toxicity prediction tools to estimate potential noncarcinogenic systemic toxicity, mutagenicity, and nongenotoxic carcinogenicity. We also assessed endocrine disruption, which is of growing interest to regulatory agencies. All information was compiled in a "heat map" using a color scheme to highlight the compounds with high, intermediate, or low human health and/or environmental risk potential. Thirty-three compounds were classified as potential carcinogens, mutagens, reproductive toxicants, and/or toxicants following repeated exposure, and were prioritized for more refined human health risk evaluations. Thirteen compounds had information indicating that they have low potential for human health risk, and are unlikely to be risk drivers unless exposures are very large. Twelve compounds were identified as having high chronic and/or acute aquatic toxicity, and 27 compounds did not have adequate information to determine risk potential. Understanding and managing the potential risk of CO₂ capture will be key to the growth and acceptance of this technology. The results of this screening assessment can be used to plan for research to support human health and ecological risk assessments.

5.12.P-Th-112 Review of possible impacts to wetlands from low-level CO₂ inputs

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There is increasing interest in carbon capture and storage (CCS) technologies, as well as in possible environmental impacts associated with their implementation. To better understand possible effects of low-level subsurface CO₂ inputs on various wetland types, we reviewed literature describing contemporary studies, including those of natural analogues. It was noted that low-level CO₂ inputs may be minor relative to the range of natural CO₂ variability in wetland systems, which results from biological activity and changes in saturation, temperature, light availability, and nutrients. In cases where low-level subsurface inputs might exceed natural variability, possible abiotic and biotic impacts identified during review included: (i) shifts in the prevalence of short-chain carbon compounds (e.g., acetate), (ii) decreases in pH, (iii) changes in soil mineralogy, (iv) increases in reduced inorganic compounds, (v) shifts in microbial species (based on changes in pH and redox

level), (vi) decreases in upland plant health, and (vii) increases in eutrophication. While there are major gaps in the literature – particularly concerning biotic responses – our review suggested that zooplankton may be the most sensitive aquatic species while fungi, some insects, and higher trophic animals appeared to be more resistant. Additionally, our review suggested that possible changes that might result from low-level CO₂ inputs are unlikely to substantively impact broader wetland services (e.g., plant species may potentially shift but general flood control services would likely still function). Overall, the literature reinforced the understanding that wetlands are likely resilient to low-level CO₂ inputs, although some variation due to wetland type and location may be expected (e.g., certain types of wetlands may have greater buffering capacity than others).

5.12.T Potential Environmental Impacts of CO₂ Releases From Carbon Capture and Storage (CCS) Activities

5.12.T-01 Modeling the fate and transport of carbon dioxide pipeline ruptures

Chuck Venditti, Ryan Huntley and Hester Companies, TRC Companies

Pipelines are critical to transporting liquids and gas safely and efficiently throughout the United States. Companies have been transporting Carbon Dioxide (CO₂) via pipeline for the past several decades, primarily for use in Enhanced Oil Recovery. With efforts to decarbonize projects or reduce global emissions, thousands of miles of new CO₂ pipelines are being designed and installed to transport CO₂ emissions from the point source to permanent storage locations. Performing risk analysis and mitigation is an important part of the design and operation of any pipeline system. Understanding and predicting the risk of a CO₂ pipeline rupture event presents unique challenges due to its dense/heavier than air characteristics when released from the pipeline. Because it will exit the pipeline as a dense gas, under certain weather and topographic conditions it can remain close to the ground, following the terrain and aggregating in low areas, presenting an asphyxiation risk to those who may be in the release cloud, as well as risks to other organisms in the surrounding environment. This session will demonstrate multiple approaches to modeling CO₂ from ruptures including potential pathways through a flat terrain environment and in a topographically diverse environment. Key inputs to the process will be highlighted, as well as the potential differences in modelling results from variations in the model inputs.

5.12.T-02 Towards high-fidelity CO₂ dispersion modelling: Challenges and solutions

Ehsan Mahvelati¹, Ahmed Bayram¹ and Christian Chauvet², (1)Wood PLC, Canada, (2)Wood PLC, France

The unplanned release of CO₂ from high-pressure pipelines poses significant risks to human and animal health. Therefore, it is crucial to thoroughly investigate the atmospheric dispersion of CO₂ during the pipeline design phase to understand the associated risks and establish appropriate pipeline routes and exclusion zones. The dynamics of CO₂ release from pipelines are complex, particularly when the pipeline is buried. The release can create a crater, and the temperature drop due to the Joule-Thomson effect can lead to the formation of solid CO₂, which subsequently disperses into the atmosphere. This study presents key insights from dispersion modeling efforts on a CO₂ pipeline project, utilizing Computational Fluid Dynamics (CFD) analysis. The research involved close collaboration with experts in Technical Safety, Pipeline Engineering, and Risk Assessment. The presentation will provide an overview of current dispersion modeling approaches, their limitations, and the necessity for more advanced modeling techniques to accurately predict CO₂ dispersion. Additionally, it will highlight existing knowledge gaps and identify high-priority research and development areas essential for improving CO₂ dispersion modeling accuracy.

5.12.T-03 Modeling the fate and effect of subsurface releases of carbon dioxide

Matt Dunn¹, Jeffrey Benegar¹, Deborah French-McCay¹, Charles Faust¹, Justin Cooper¹ and Kelly Marie McFarlin², (1)Tetra Tech Inc., (2)Exxon Mobil Biomedical Sciences Inc

Carbon Capture and Storage (CCS) continues to be the focus of global efforts to minimize the amount of CO₂ that is released to the atmosphere. Proposed efforts focus on capturing CO₂ from point sources and storing it in subsurface or subsea reservoirs. Injection of CO₂ into geologic reservoirs has been proposed for long term

subsurface sequestration. It's important to understand potential environmental impacts if there is a subsurface release of CO₂ during this process. The objective of this project was to identify the best available groundwater model to predict the fate and potential environmental effects of subsurface CO₂ releases. We performed a state-of-the-science literature review to evaluate the utility of current groundwater models for these applications, as well as their near and far field behavior in representative shallow aquifer environments. Eight models were evaluated to understand their capability to characterize the release of CO₂ with single and multiphase considerations, plume morphology across space and time, and carbonate system reactions. Three models were identified as most practical for understanding the potential impact to groundwater from shallow subsurface releases: TOUGH, GEM, and USGS-PHAST. TOUGH was identified as a suitable model due to its ability to predict nearfield behavior when initialized with a release rate from a pipeline. TOUGH can also predict vertical transport of CO₂ from well releases into shallow aquifers. Additionally, TOUGH can handle multiphase transport that may occur when dense phase CO₂ expands and reaches a vapor phase that can dissolve into water or be released into the atmosphere. When provided with an aquifer's ambient buffering capacity, TOUGH can predict dissolved concentrations of CO₂ over space and time with the TOUGHREACT module which can translate released CO₂ into pH changes. Future directions of this research will explore the application of TOUGH to estimate potential groundwater impacts from representative scenarios, including a shallow subsurface pipeline breach and vertical transport through a well, in conjunction with other modeling processes such as pipeline outflow software. With defined workflows and an increased understanding of model capabilities, we aim to identify potential impacts associated with emerging CCS activities in the shallow subsurface.

5.12.T-05 Combined effects of hydrostatic pressure and acute hypercapnia on gill physiology in lumpfish (*Cyclopterus lumpus*)

Javier Santander, *Vimbai Machimbirike, Sathees Douglas and Jaime Neira, Memorial University of Newfoundland, Canada*

Carbon dioxide (CO₂) is a major focus for scientists and policymakers because it contributes to global warming. To help reduce atmospheric CO₂ levels, various efforts are underway to capture and store CO₂ beneath the seafloor. However, a collateral outcome of such strategies is the risk of accidental CO₂ leaks, which could expose marine life to high levels of CO₂. In addition to anthropogenic sources, high CO₂ levels can also originate from natural tectonic activities such as volcanoes and seeps. Fish molecular and physiological response to short-term CO₂ exposure in combination with pressure remains largely unexplored. This knowledge gap is partially due to the technical challenges of studying biological response while animals are under pressure. Lumpfish (*Cyclopterus lumpus*) is an ecologically important marine species found across both sides of the North Atlantic Ocean. Although they are typically recorded within the upper 60 meters of the water column, they have also been observed at depths reaching 800 m. The gills, as the primary respiratory organ, are especially vulnerable to changes in the marine environment. Therefore, in this study, we assessed the effects of short-term, high-intensity CO₂ exposure under increasing pressure (at 6°C) on lumpfish gill physiology. Lumpfish (n=4) were placed in hyperbaric chambers and exposed to different pressures (up to 100 bar, equivalent to 1000 m in depth) under both low and high CO₂ concentrations (0.8-98 mg/L) for 24 h. Gill tissues were then subjected to histological and transcriptomic analyses to assess the physiological impacts of CO₂ and pressure.

5.12.T-06 Modeling releases of carbon dioxide from subsea pipelines

Deborah French-McCay¹, *Melissa Gloekler*¹, *Matt Dunn*¹, *Cindy Wang*¹, *Scott Socolofsky*² and *Kelly Marie McFarlin*³, (1)*Tetra Tech Inc.*, (2)*Texas A&M University*, (3)*ExxonMobil Biomedical Sciences, Inc.*

Carbon Capture and Storage (CCS) technology includes use of pipelines to carry carbon dioxide (CO₂) to subsea storage locations. We evaluated approaches and tools to model potential releases of CO₂ from subsea pipelines. Evaluating the behavior and fate of CO₂ released into the marine environment requires a hierarchy of

models: 1) an outflow model capable of accounting for depressurization and phase change as CO₂ is released from subsea containment (such as a pipeline); 2) a nearfield model to predict the physical state and movements of CO₂ bubbles or droplet plume based on momentum and buoyancy of the release (e.g., TAMOC); 3) a far-field model to predict the transport, dispersion and fate of the dissolved fraction of the CO₂ plume (e.g., CHEMMAP); 4) a carbonate chemistry model to quantify potential changes (i.e., decreases) in water column pH resulting from added CO₂; and 5) an aquatic toxicity model and supporting data to identify thresholds for potential effects due to ephemeral perturbations of pH. The operating conditions and outflow rate from a subsea pipeline can greatly affect the relative buoyancy of the plume and its dilution rate in the far field. Several outflow models were examined for performing these calculations for subsea pipelines where ambient hydrostatic pressures are much higher than the traditionally applied terrestrial environments. Depending upon the depth of release, CO₂ released from a pipeline could rapidly form gas bubbles or remain an immiscible liquid, which could be positively or negatively buoyant compared to surrounding seawater. Based on terrestrial experiments into air, some fraction of the release may also form solid CO₂ particles. Thus, a CO₂ release would likely form a multiphase plume, some portion of which would dissolve into the entrained ambient seawater. Depending on conditions, some CO₂ could enter the atmosphere, either as a surfacing plume or as rising bubbles. Preliminary modeling indicated that an end-to-end (i.e. pipeline to atmosphere) model system is needed to evaluate the behavior and fate of CO₂ released into the marine environment. **Disclaimer/Disclosure:** Funding for this study was provided by Environmental Sciences, Toxicology and Environmental Sciences Division, ExxonMobil Biomedical Sciences, Inc. (EMBSI)

5.13.P-Tu Soil Contaminants: Fate, Bioavailability Environmental Toxicology in Ecological Risk Assessment

5.13.P-Tu-093 Surface soil PFAS mixtures dominated by PFOS: Prioritization for ecotoxicity testing and ecological risk assessment at current and former U.S. Air Force bases

Andrew East¹, Richard Hunter Anderson², Candice Duncan³ and Christopher Salice⁴, (1)University of Maryland, College Park, (2)U.S. Air Force Civil Engineer Center, (3)University of Maryland, (4)Towson University

Per- and polyfluoroalkyl substances (PFAS) detection at military installations where current and historical aqueous film-forming foam (AFFF) use has occurred drive a need for empirical derivation of environmentally relevant PFAS mixtures to facilitate toxicity testing and risk assessment efforts. We applied a formalized prioritization method to a large dataset of PFAS concentrations in surface soil at AFFF-affected sites on active and former U.S. Air Force installations. Our approach revealed several observations about PFAS at these sites. First, perfluorooctane sulfonate (PFOS) occurred most commonly and often at the highest concentration across the PFAS measured. Second, two to three PFAS contributed 86% to 91%, respectively, of the sum PFAS in any given site-specific mixture. Third, after PFOS, the most common and high concentration PFAS among target analytes were perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorooctanesulfonamide (PFOSA), and/or perfluorohexanoic acid (PFHxA), in that order. Site-specific PFAS mixtures are approximately 5% to 12% PFHxS, PFOA, PFOSA, and PFHxA behind approximately 82% PFOS. Another observation relevant to future sampling is the high concentration but inconsistent prevalence of the 6:2 and 8:2 fluorotelomer sulfonates (FTSs). An uncertainty that could also be addressed through future sampling is the detection of less abundant or yet unmeasured PFAS that have unknown or poorly characterized toxicological potency. These results support the continued importance of efforts to understand effects and exposure of PFOS but highlight the need to consider other PFAS such as PFHxS and fluorotelomers in exposure and effect estimations to support ecological risk assessments and ecotoxicological testing of PFAS mixtures.

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5.13.P-Tu-095 The relationship of soil characteristics with polycyclic aromatic hydrocarbon (PAH) and metal loading in urban soils

Hanna Mobley Hubbard and Sarah Hileman, University of Central Oklahoma

Urban environments are characterized by elevated anthropogenic activities that contribute to environmental contamination, particularly with polycyclic aromatic hydrocarbons (PAHs) and select metals. 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. Polycyclic aromatic hydrocarbons (PAHs) and select metals are generally ubiquitous in the environment and are often observed at higher levels in urban areas. These 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 (United States Environmental Protection Agency) residential soil screening level of 110 ppb. Arsenic has also been found in levels of human concern. These occurrences have been noted across the city, as well as within public elementary school playgrounds: 138 public elementary schools across 15 school districts were examined. While relationships have been previously found between a soil's total organic carbon content and the occurrence of PAHs and select metals, this relationship was not found among the playground soils tested in this study. By looking at additional soil characteristics such as soil type and pH levels, further relationships of soil characterization and contaminant load will be explored to add to our understanding of these dynamics specific to playground soils in an urban area. This type of analysis is important to inform as to what soils are more likely to retain such contaminants under such exposure settings, which could influence infrastructural decisions in areas where playgrounds may be exposed to known contamination sources like traffic.

5.13.P-Tu-096 Environmental fate and exposure of microbial Ag products part 2: The need to develop better exposure assessments for the evaluation of risk

Henry O Krueger, Eurofins Agroscience Labs

Exposure assessments are a key component of an environmental risk assessment. For biological products that fall under FIFRA there is currently a lack of methods to accurately estimate environmental concentrations of biologics for non-target organisms. Well-developed exposure models have not been developed as is the case for conventional chemicals. Risk evaluations also need to consider how application methods used in the field affect exposure. Guidance for field exposure studies also needs to be developed and standardized, so that new guidelines can be established. Additionally, having better exposure assessments can be used to design better tests for non-target organisms. Current guidelines for biologics set upper limits of test concentrations at extremely high levels that far exceed application rates. For aquatic studies, these concentrations may be relevant for products that are directly sprayed to water for mosquito control or invasive aquatic plants, but they are unrealistic for products directly sprayed to fields, soil incorporated, in-furrow applied, or seed treatments. Testing such high concentrations in water can result in opaque test solutions and excessive particles suspended in water that can interfere in the feeding of invertebrates and clog gills in fish. Aquatic testing of conventional chemicals has well defined upper limits for testing based on 100ppm or the limit of water solubility. For conventional chemicals GLP tests that include water solubility, stability, homogeneity, photolysis, and hydrolysis are used as model inputs for exposure assessments and also used to establish test concentrations for aquatic testing. As more and more biological products are being registered, it is time to modernize testing and develop models for biologics so that realistic environmental exposures can be used for testing and risk assessments.

5.13.P-Tu-097 Evaluating per- and polyfluoroalkyl substances (PFAS) leaching in biosolids-applied field plot using lysimeters

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Per- and polyfluoroalkyl substances (PFAS) are commonly used in consumer products such as cosmetics, microwave popcorn bags, and non-stick cookware. These persistent compounds can enter wastewater treatment plants (WWTPs) via sewage and accumulate in biosolids, which are often applied to agricultural fields as soil amendments. Once in the soil, PFAS may come into contact with crops and leach downward with infiltrating rainwater, raising concerns due to their environmental persistence and potential adverse effects on human health. This study evaluates the vertical transport of PFAS following a single biosolids application to a field plot. Leaching was monitored using lysimeters installed at two depths (2 and 4 feet), and percolated water was collected over one year after significant rainfall events. A total of 45 leachate post biosolid application samples were processed using solid-phase extraction (SPE), and PFAS concentrations were quantified via LC-MS/MS. Both short- and long-chain PFAS were detected at both depths, confirming vertical transport through the soil profile. Short-chain PFAS were found more frequently and at higher concentrations in the deeper lysimeters, indicating greater mobility and lower sorption. Conversely, long-chain PFAS were more abundant in the shallow lysimeters, suggesting stronger retention in the upper soil layers. These results align with the known physicochemical properties of PFAS, particularly the influence of chain length on environmental mobility. This study provides field-scale evidence of PFAS leaching after biosolids application and underscores the importance of accounting for compound-specific behavior when evaluating the environmental risks of land application. The findings inform the development of risk-based management strategies aimed at protecting groundwater quality and assessing the long-term safety of biosolids reuse. **Disclaimer/Disclosure:** Project funded by Water Research Foundation (WRF 5214)

5.13.P-Tu-098 Comparative study of bromate uptake and accumulation in agri-food systems

Raul Calderon¹ and Paulina Palma², (1)University of the Americas, Chile, (2)Ministry of Health, Chile
Bromate (BrO_3^-) is an anionic compound generated as a byproduct during disinfection process via oxidation and chlorination of drinking water. Bromate is classified as a group 2B, possible human carcinogen by the International Agency for Research on Cancer (International Agency for Research on Cancer based on sufficient evidence that bromate induces cancer in experimental animals. The United States Environmental Protection Agency (USEPA) and the World Health Organization (WHO) have proposed a tolerance limit of $10 \mu\text{g L}^{-1}$ bromate in drinking water. Bromate was determined using an Agilent 1100 series LC liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) coupled to an API 2000 electrospray ionization tandem mass spectrometry (ESI-MS/MS) triple quadrupole mass spectrometer (Applied Biosystem, Foster City, CA, USA) in negative ionization mode. The mobile phase was composed of methylamine (200 mM) in isocratic mode at a flow rate of 0.27 mL min^{-1} using an IonPac AS-21 anion exchange column ($250 \text{ mm} \times 2 \text{ mm}$; Dionex, Sunnyvale, CA, USA). Bromate concentrations in vegetables (traceable and non-traceable) were 0.15 ng g^{-1} and 2.14 ng g^{-1} , respectively. The highest bromate levels (non-traceable) occurred in leafy vegetables, such as chard, lettuce, and arugula. Leafy vegetables (traceable), such as lettuce, produced in Regions I, II and III contained bromate levels of 23.6, 9.66 and 7.68 ng g^{-1} . The EDI of bromate (DW) ranged between 58.6 and 447 $\text{ng kg}^{-1} \text{ day}$ for all age groups and regions. The EDI for all sources and age groups were lower than the RfD of $4000 \text{ ng kg}^{-1} \text{ day}$. **Disclaimer/Disclosure:** Fondecyt Regular 1230849 ANID-Chile

5.13.P-Tu-099 Assessing the habitat quality of Californian vineyard soils and how it influences the resilience of soil biodiversity to pesticide and metal

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Viticulture plays a major role in the agricultural economies of the United States and relies heavily on pesticides and mechanized soil management, leading to pollution, soil erosion, diminished carbon storage, and biodiversity loss. These environmental impacts affect the ecological and agronomic functions of vineyard soils,

posing challenges to the long-term sustainability of viticultural systems. It is important to know how vineyard soils protect soil biodiversity from the incidences of environmental contamination. To do this, we collected twenty-one vineyard soils across California ranging from conventional to organic farming systems. The soils physicochemical properties of the soil were determined. As a measure of soil biodiversity, we then extracted soil microarthropods (mites and springtails) from the soils and determined their densities and diversity. The density and diversity were then scored for each soil and both scores incorporated to determine the habitat quality of the soils. The soils were distinguished into three habitat qualities, where the highest scores had the highest habitat quality. Organic matter content and pH had positive correlation with the soil habitat qualities at 24% and 34% respectively. To determine how habitat quality influences the resilience of soil biodiversity to contaminants in the vineyard soils, we exposed the standard oribatid mite species, *Oppia nitens* to cadmium and insecticide, λ -cyhalothrin in a dose response test in the lab following the ISO 23266. The test is ongoing and the result will help to understand how these vineyard soils protect soil biodiversity from the effect of contamination through toxicokinetic or toxicodynamic processes.

5.13.T Soil Contaminants: Fate, Bioavailability Environmental Toxicology in Ecological Risk Assessment

5.13.T-01 Derivation of PFAS mammalian biomagnification factors for predicting the dietary exposure of predators that eat small mammals

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When conducting ecological risk assessments at contaminated sites, risk assessors do not usually have access to measured concentrations of chemicals in small mammals. Concentrations in small mammals are essential to estimate dietary doses of birds or mammals that prey upon small mammals. Typically, concentrations of chemicals in small mammals are predicted using food web models that use measured concentrations in soil and/or other biota as input parameters. To enable PFAS site-specific food web modeling in this fashion, we reviewed PFAS data for 11 PFAS (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFBS, PFHxS, PFHpS, PFOS, PFOSA, and 8:2 FTS) generated by a laboratory study in which concentrations of PFAS in Dutch-belted rabbits (*Oryctolagus cuniculus*) were measured while being fed plant diets containing PFAS for 28 days (SERDP project ER19-1041, Kuperman et al., 2024). Concentrations of eight PFAS (i.e., PFBA, PFPeA, PFHxA, PFOA, PFBS, PFHxS, PFOSA, and 8:2 FTS) in rabbits appeared to reach steady state within the 28-day period. Using the plant and tissue laboratory data, we calculated plant-to-rabbit (whole body) Biomagnification Factors (BMFs) by dividing the whole body concentrations in rabbits (observed during the 28-day period) by the concentration in the diets. BMFs ranged over two orders of magnitude, from 0.077 g wet weight (ww) plant/g ww rabbit whole body (PFPeA) to 7.8 g ww plant/g ww rabbit whole body (PFOSA). Log10-transformed BMFs for these 8 PFAS were significantly correlated with perfluoroalkyl chain length ($\text{Log}_{10} \text{BMF} = 0.46 \times (\text{number of perfluorinated carbons}) - 3.4$; $r^2 = 0.79$, $P = 0.00031$). For the other three PFAS (PFHpA, PFHpS, and PFOS) concentrations in rabbits did not appear to reach steady state, but increased linearly throughout the 28-day exposure. Concentrations of these three PFAS were predicted assuming 290 days of exposure (an average age assumed for a wild population of rabbits) and used to calculate BMFs (1.2, 33, and 58 g ww plant/g ww rabbit whole body, respectively). These three BMFs also increased with perfluoroalkyl chain length, although values were approximately an order of magnitude higher than BMFs for PFAS of similar perfluoroalkyl chain lengths that reached steady state within 28 days. We believe these 11 BMFs will be useful for predicting the concentrations of PFAS in small mammals and subsequent estimates of dietary exposure to predators in support of site-specific PFAS risk assessments.

5.13.T-03 Comparative chronic toxicity of field-realistic pesticide mixtures to *Eisenia fetida* and native European Lumbricids (*Lumbricus rubellus* and *Lumbricus terrestris*)

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To protect the environment, pesticide risk to soil fauna is often based on single-pesticide toxicity data and is typically assessed using the compost-dwelling earthworm *Eisenia fetida*. However, in reality, soil organisms are exposed to pesticide mixtures in the environment. As a bioindicator, *E. fetida* is typically found in composts or organic-rich soils, which are not the norm in most soils. To generate more realistic toxicity data, we compared the chronic toxicity of 11 field-realistic pesticide mixtures, formulated at measured environmental concentrations (MEC), predicted environmental concentrations (PEC), and 5× PEC, on *E. fetida*, the epigeic native species *Lumbricus rubellus*, and the anecic *Lumbricus terrestris*. Tests followed the OECD 222 guidelines for *E. fetida* and *L. rubellus* (56 and 74 days, respectively), and a 74-day glass mesocosm design for *L. terrestris*. End-points were adult survival, growth, and reproduction; for *L. terrestris*, we additionally assessed burrow volume and cast biomass. One-way ANOVA with Tukey post-hoc tests ($p < 0.05$) was used to determine the effect of the concentration on the earthworm survival, growth, reproduction, burrow volume, and cast biomass. Additionally, the sensitivities of both *E. fetida* and *L. rubellus* at each exposure concentration were assessed using a Welch's t-test. Five mixtures were hazardous to *E. fetida*, primarily via growth and reproduction. *L. rubellus* exhibited significant reproductive inhibition for five other mixtures, and *L. terrestris* showed 100% mortality or severe sub-lethal effects for two mixtures. Sub-lethal endpoints were consistently more sensitive than survival. Several mixtures produced effects, although all individual component concentrations were below their species-specific NOECs, indicating synergistic or additive mixture toxicity. Overall, eight of the eleven mixtures posed a risk to the earthworm species if considered together, compared with only five detected with the standard species. The results demonstrate that incorporating ecologically relevant earthworms alongside the standard test organism improves pesticide mixture risk detection in European soils and underscores the need to include native taxa in regulatory assessments. **Disclaimer/Disclosure:** This work was supported by the SPRINT project funded by the European Commission through Horizon 2020, the EU Research and Innovation Programme (grant agreement no. 862568)

5.13.T-04 Soil ecotoxicology studies in support of risk assessment and guideline development

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Soil ecotoxicology studies are a useful tool for risk assessment and generation of the site-specific guidelines. Background and impacted soils can provide unique information about potential toxicity modifying factors (TMFs) that may influence the bioavailability and toxicity of chemicals for a particular region and species. Recent ecotoxicology testing investigated the role of sulphate (SO₄) in agricultural lands with elevated selenium (Se), and the role of organic carbon in aged hydrocarbon-impacted boreal forest soils. The ameliorating effect of SO₄ was studied with a range of Se concentrations in coarse- and fine-textured soils tested by a battery of five plant species: *Medicago sativa*, *Hordeum vulgare*, *Cucumis sativus*, *Elymus lanceolatus*, and *Festuca rubra*. There was evidence of substantially reduced Se toxicity in presence of SO₄, starting from 250 mg/kg of SO₄. For example, the effect concentration (EC)₂₅ for *H. vulgare* (barley) plant biomass was equal to 3.0 mg/kg in fine background soils, spiked by Se with no additional SO₄. With added SO₄, the EC₂₅ for barley plant biomass increased to 20.4 mg/kg of Se for 250 mg/kg of SO₄. For barley grown in coarse soils, EC₂₅ jumped from 2 mg/kg of Se in background to 15 mg/kg of Se with 250 mg/kg SO₄ added. Similar pattern was observed in other plant species in both types of soil. The environmentally available concentrations of Se and the long-term bioaccumulation testing is part of the study. Another TMF recently investigated is organic carbon in aged hydrocarbon-impacted soils in the boreal forest. Test organisms included seven boreal forest species (*Populus tremuloides*, *Calamagrostis canadiensis*, *Solidago canadensis*, *Betula papyrifera*, *Pinus banksiana*, *Picea glauca*, and *Picea mariana*), and two soil invertebrate species (*Eisenia andrei* and *Proisotoma minuta*). Increased plant productivity was observed in soils exposed to the light extractable petroleum hydrocarbons (LEPHs). Both projects may lead to substantial reducing of potential remediation volume and associated carbon footprints. The SO₄ effect will be further evaluated to develop soil Se guidelines,

and savings on remediation efforts addressing low level Se Tier 1 (1 mg/kg) exceedances that occur naturally in Alberta. The impact of organic carbon on aged hydrocarbon-impacted soils has led to the development of site-specific LEPH guidelines, which have substantially reduced the amount of remediation work in remote boreal areas.

5.13.T-05 Fate, transport and risk assessment of dicyclopentadiene in an agricultural setting: A case study following a train derailment

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In March 2015, a train derailment occurred in a mixed farmland and woodland area outside of Minter City, MS. Several train cars overturned, including a tanker transporting Resin Oil Heavies (ROH). Based on the associated safety data sheet, the largest constituent of ROH was dicyclopentadiene (DCPD) which accounted for approximately 35% by volume; other constituents included, in lesser amounts, naphthalene, indene, styrene, ethylbenzene, and xylenes. In response to the spill, soil in the immediate area was excavated, and groundwater concentrations of DCPD and other analytes were monitored over time. In this presentation, we will discuss 1) a conceptual site model that describes the potential transport and fate of DCPD, 2) the evolution of cleanup goals and associated monitoring data at this site over time, 3) relevant literature on the phytotoxicity of DCPD to agricultural crops, and 4) results of tissue analysis of soybeans grown in remediated areas following cleanup. Both the literature review and the analytical results indicated there was no risk of DCPD-related toxicity with crops grown on the property following site remediation.

5.13.T-06 Environmental fate and exposure of microbial Ag products part 1: Developing mobility and persistence data in the field

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Global interest in biological-based products for agriculture is growing rapidly, including products based on micro-organisms. Biologicals are an expanding market globally worth \$12 billion with a 15% compound annual growth rate, with conservative estimates putting the market worth at \$27 billion by 2027. These products cover a range of functions, including crop nutritional efficiency and biocontrol. Biofertilizers are typically microbes that increase the amount or availability of nutrients e.g., nitrogen-fixing bacteria. Biostimulants improve crop yields by affecting nutrient uptake, modifying the microbiome, or stimulating root growth for water management, among other modes of action. Biocontrol products (biopesticides) are primarily fungicides and insecticides, including biochemicals or microbials. These groups of agricultural products are considered a safer alternative to conventional chemicals for a number of reasons, including that the efficacy may or may not rely on the chemistry that they produce, and aspects of pesticide regulation like food residues may be irrelevant (no crop residues). However, such products are still evaluated for safety, and there are a number of technical challenges associated with testing microbial-based products. The USEPA has published testing guidelines specific for microbial-based products. They included a guideline for environmental fate type testing, or environmental expression, and while the testing guideline describes the type of information that is sought, it does not describe how to conduct the test. In order to address questions related to environmental fate, a study was conducted to investigate the potential for persistence and mobility of both wild-type and gene-edited micro-organisms from a potential corn seed treatment use. A method with proper specificity and sensitivity is required to be able to detect microbes of interest in a complex environment. The microbes in this study were quantified using qPCR technology in soil, root, and above-ground plant tissue at various corn growth stages and time points after planting. Results demonstrated that mobility and persistence were limited from this use pattern, and the microbes did not migrate to the phyllosphere.

5.14.P-We Trifluoroacetic Acid in the Environment—Here Today, More Tomorrow

5.14.P-We-117 Potential human health and environmental impacts of trifluoroacetic acid, the ultimate transformation product of some refrigerant gases and many other fluorinated chemicals

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Trifluoroacetic acid (TFA) is the shortest chain-length perfluorinated chemical and is classified as a PFAS in some jurisdictions. TFA is used in industry as a precursor for synthesis and as a reagent in chemistry. It is also a terminal residue of some Montreal Protocol-related refrigerant gases, agrochemicals, and pharmaceuticals containing the CF₃ group. The -CF₃ group, a pseudohalogen, confers beneficial properties to these chemicals, but they could degrade to produce TFA. Molar yields of TFA for many of these potential sources have yet to be measured and would vary with the chemistry and biology of the environment (soil and water, etc.). TFA accumulates as salts in terminal sinks like the oceans and endorheic lakes with no outflows. It is globally distributed with recent inputs from human activities; however, there may be natural sources as well. The physical and chemical properties of TFA are different from longer chain PFASs, some of which are hazardous to organisms in the environment and are bioaccumulative in food-webs. This has raised concerns for potential adverse effects of TFA in the environment. Exposure assessment is complex because of release of TFA in effluents from manufacture of fluorinated chemicals, and the increasing release of pesticides and pharmaceuticals containing the -CF₃ moiety into the environment. TFA has been detected in air, fog, precipitation, soils, dust, and in plants at concentrations ranging from non-detects to 51 µg kg⁻¹, but greater values have been measured in soils and crops grown near manufacture of fluorinated chemicals. TFA-salts are inert and are accumulated in plants through evapotranspiration but rapidly excreted by animals, so do not bioaccumulate in food webs. In this talk we will show that current concentrations of TFA, and those predicted through 2100 from the degradation of compounds related to the Montreal Protocol, are of de minimis risk to ecosystem and human health, but knowledge gaps remain and need to be addressed to fully understand the long-term risks of TFA.

5.14.P-We-118 Trifluoroacetic acid in Australian human urine samples

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Trifluoroacetic acid (TFA), a potential terminal degradation product of numerous industrial chemicals, pharmaceuticals and pesticides containing C-CF₃ moieties, has been shown to be increasing in concentration in environmental media. Therefore, increasing human exposure is likely but only very limited information is available on levels and trends in humans. In this study TFA was analysed in deidentified surplus pathology urine samples from Australia, pooled by collection year, age class and gender. A dilute-and-shoot method involving direct analysis of TFA by ion chromatography (IC) coupled to tandem mass spectrometry (MS) was utilized to lower probability of sample contamination and uncertainty related to analyte losses during sample pretreatment/enrichment. TFA was also confirmed by IC-high resolution MS. TFA was detected in all urine samples (n=70 pooled samples comprising of a total of 6040 individuals) and ranged in concentration from 3.4 to 300 µg/L with a median of 24 µg/L. No differences were found between males and females. Significantly higher concentrations were found in pools of older males and females (aged >45-60 and >60 yrs) compared to younger groups (>5 to 45 yrs). TFA concentrations increased with age in children (ages <1 to 4 yrs) with a doubling time of 1.3 yrs. Mean concentrations of TFA in age classes (>5 to >60 yrs) for 2012-13, 2014-2015 and 2020-21 did not differ significantly suggesting that exposures have not changed over that seven year time period. This contrasts with the decline in concentrations of long chain PFAS in pooled serum samples of

Australians, from similar age classes as the present study, over the period 2010-2020. The results indicate high chronic exposure to TFA in Australia. This is consistent with the ubiquitous presence of TFA in food, household dust, and drinking water reported in various countries worldwide. However, many questions remain to be answered regarding trends over longer time periods, the variation with age, and the sources of TFA exposure.

5.14.P-We-119 Insights into the atmospheric sources and fate of trifluoroacetic acid from high time resolution measurements

Cora Young¹, Shira Joudan², Jessica Clouthier¹, Mayre Rodriguez¹, Trevor VandenBoer³, Ye Tao¹, Jeremy Wentzell⁴ and John Liggio⁵, (1)York University, Canada, (2)Chemistry, University of Alberta, Canada, (3)Chemistry, York University, Canada, (4)Environment & Climate Change Canada, Canada, (5)Environment and Climate Change Canada, Canada

Trifluoroacetic acid (TFA) is a persistent chemical found ubiquitously in the environment, including remote regions. While TFA could be emitted directly to the atmosphere, it can also be formed from the atmospheric oxidation of fluorinated molecules, including fluorotelomer chemicals and chlorofluorocarbon replacements. Because the atmosphere provides a rapid mechanism for long-range distribution, it is important to understand the sources and fate of TFA in this medium. To date, atmospheric measurements of TFA have been made using, such as measurements using annular denuders, sorbents, and/or filter packs with collection timescales of several hr to days. Atmospheric processes (e.g., atmospheric oxidation, gas-particle partitioning) that drive production and fate of TFA occur on faster timescales. Analyses on the timescale of seconds to one hr can give extremely valuable information on TFA temporal trends and concentrations, allowing relationships to be conclusively made with other atmospheric parameters (e.g., meteorology). Here, we describe two new approaches to high time resolution TFA atmospheric measurements, as well as their application to ambient measurements in Toronto, Canada. The ambient ion monitor-ion chromatograph-mass spectrometer (AIM-IC-MS) provides in situ hrly measurements of both gas- and particle-phase PFCAs. The time-of-flight chemical ionization mass spectrometer (ToF-CIMS) measures in situ gaseous PFCAs at a timescale of 10 seconds. Data from the AIM-IC-MS and ToF-CIMS, along with co-located meteorological and air quality parameters will be used to describe new insights into TFA sources, fate, and transport.

5.14.P-We-120 Ecotoxicity of trifluoroacetic acid: A review of current knowledge and data gaps across aquatic and terrestrial receptors

Kyle Fetters, Sydney Kruse and Phyllis Fuchsman, Ramboll

Trifluoroacetic acid (TFA) is extensively used in industrial applications and is increasingly recognized as an environmental contaminant due to its persistence, mobility, and widespread occurrence. Recent monitoring data show that TFA concentrations in global water systems have risen by 3- to 17-fold, now reaching levels exceeding those of other PFAS compounds. This trend indicates a need to evaluate potential risks to ecological receptors. Toward that end, we conducted a comprehensive review of the available toxicity literature for both aquatic and terrestrial organisms. For aquatic organisms, sufficient data exist to support a species sensitivity distribution (SSD), which was constructed to derive a protective threshold for aquatic communities. This result was compared to a 5% hazard concentration (HC5) derived using the target lipid model as calibrated for halogenated compounds. The TFA-specific HC5 is moderately lower than the model prediction for a baseline narcotic mode of action. The majority of ecotoxicological data currently available focus on aquatic species, reflecting the fact that aquatic environments are the primary sink for TFA. However, we also reviewed and summarized the limited data available for terrestrial receptors. This work highlights data gaps for terrestrial, amphibian, and reptilian species, which merit further research.

5.14.T Trifluoroacetic Acid in the Environment—Here Today, More Tomorrow

5.14.T-01 Growth in production and environmental deposition of trifluoroacetic acid due to long-lived CFC replacements and anaesthetics

Lucy Hart, Ryan Hossaini, Oliver Wild and Crispin Halsall, Lancaster University, United Kingdom

Trifluoroacetic acid (TFA) is a persistent organic chemical with potential long-term effects on the environment and on health. Recent studies based on ice core records report large increases (up to a factor of ~10) in Arctic TFA deposition since the 1970s. The ice core temporal trends suggest that CFC replacement gases introduced following the Montreal Protocol could be an important source. However, TFA is a “substance from multiple sources” and their relative importance remains poorly quantified; a challenge which needs to be addressed for the emission trend to be reversed through regulation. Here we use a chemical transport model (FRSGC/UCI-CTM) to examine the global TFA budget from the production of long-lived source gases, namely, hydrochlorofluorocarbons (HCFCs), hydrofluorocarbons (HFCs), and inhalation anaesthetics, as well as HFO-1234yf. A detailed degradation scheme describing TFA production from each precursor was added to the model and simulations performed using time-varying loadings of its major emitted precursors from 2000-2022. Model trends in TFA deposition are compared to ice core records and precipitation concentrations to infer the significance of CFC replacements as sources of TFA to the environment. Global TFA deposition from these sources increased 3.5-fold from 6.1 (5.3-6.7) Gg/yr in 2000 to 21.2 (17.9-25.0) Gg/yr in 2022, with cumulative deposition reaching 319.2 Gg. We find HCFC-123, HCFC-124, and HFC-134a account for most modelled TFA production and that long-lived CFC replacements can account for 476ly all of the observed Arctic deposition trend. TFA deposition shows a latitudinal dependence with the majority occurring in extrapolar regions. At midlatitudes, where the contribution of shorter-lived sources could be more important, our analysis supports emergence of HFO-1234yf as an important, and likely growing, component of the TFA budget. We conclude that longer-term TFA monitoring is required.

5.14.T-02 Formation of TFA from aqueous photochemistry of aryl-CF₃ agrochemicals and pharmaceuticals

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The substitution of fluorine atoms in organic molecules results in advantageous properties for applications in industrial, household, health and agricultural applications. However, once in the environment, these compounds, many of which are considered per- and polyfluoroalkyl substances (PFAS), can be persistent and cause harmful effects. In the pharmaceutical and agrochemical industries, fluorine atoms are often added either directly to an aromatic ring or as trifluoromethyl groups on the ring (aryl-CF₃). Early research reported that some aryl-CF₃ can form TFA in a competing reaction with defluorination, but yields, and the relative importance of this reaction remains uncertain. In order to understand where all of the environmental TFA comes from, this class of precursors must be better understood. Since many aryl-CF₃ end up in sunlit aquatic environments, we are interested in aquatic photochemical reactions. This presentation will report results of relative kinetics and products of aquatic photochemical reactions of a suite of aryl-CF₃ to understand how their structures impact persistence, defluorination, and formation of TFA. We systematically tested aryl-CF₃ with different substitutions on the aromatic ring, and some real use pesticides, herbicides, and pharmaceuticals. Some aryl-CF₃ form up to 10% TFA due to photochemistry, while the remaining yield undergoes defluorination to release fluoride. Conversely, some aryl-CF₃ undergo full defluorination and do not form TFA, while others are stable at the C-F bond. Understanding how structure impacts TFA yield is critical to understanding sources and fate of TFA, and the design of chemicals that do not transform to TFA.

5.14.T-03 Determination of ultrashort chain PFAS in natural water impacted from PFAS contamination

Brenton Thomas Murphy, Denise Schumitz and Franco Pala, Battelle

PFAS, or per- and polyfluoroalkyl substances, are a diverse group of environmental contaminants that pose significant concerns due to their persistence in the environment and potential health impacts. These substances differ in their carbon chain lengths and functional groups, which influence their chemical properties and

behavior in various environments. Recently, there has been increasing attention on ultrashort-chain PFAS, defined as compounds with carbon chain lengths of fewer than four. This growing focus is largely due to the rising concentrations of this subgroup in various environmental settings. The analytical challenge arises from their unique behavior, which differs from that of longer-chain PFAS due to their higher water solubility and ionic characteristics. This research investigates the distribution of ultrashort-chain PFAS, specifically trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA), trifluoromethanesulfonic acid (TFMS), perfluoroethyl sulfonate (PFEtS), and perfluoropropyl sulfonate (PFPrS). The study examines these compounds in various natural water bodies affected by aqueous film-forming foam (AFFF) and other PFAS sources, as well as in pristine water bodies. The investigation utilized a novel solid-phase extraction method (WAX SPE) combined with LC-MS/MS to ensure environmentally relevant detection limits and overcome the analytical challenges mentioned above. The correlation of ultrashort-chain PFAS with analytes (short and long-chain PFAS) identified using the EPA Method 1633 is proposed as a potential tool for environmental forensics.

5.14.T-04 Analysis of ultra-short and short-chain per/polyfluoroalkyl substances (PFAS) in human urine

Kiran Ali, Robert Pures, N.L.Dilani Perera and Carrie A McDonough, Carnegie Mellon University

Per- and polyfluoroalkyl substances (PFAS) are widespread in the environment and biota due to their persistence, aquatic mobility, and bioaccumulation potential. Due to their shorter biological residence times and lower bioaccumulation potential, short-chain (SC) and ultra-short chain (USC) PFAS were introduced as alternatives to longer-chain PFAS. However, studies have reported their presence in environmental matrices and humans. Due to their solubility, SC and USC PFAS do not accumulate to the same extent as longer-chain PFAS in blood, but they are excreted fairly readily in urine. This suggests urine may be a useful biomonitoring matrix for SC and USC PFAS. This study aims to optimize sample preparation and liquid chromatography high-resolution mass spectrometry (LC-HRMS) analysis for SC and USC PFAS in human urine using a polar-embedded analytical column. Sample preparation via liquid-liquid extraction (LLE) and solid-phase extraction (SPE) were compared for optimal recoveries and detection limits. To date, average recoveries of $100 \pm 30\%$ have been achieved for 37 and 29 out of 44 analytes in Optima water and urine samples, respectively, using LLE, and for 31 and 27 analytes, respectively, using SPE. Detection limits for LLE-prepared samples ranged from 0.007 ng/mL to 13.97 ng/mL, whereas detection limits for SPE ranged from 0.01 ng/mL to 8.38 ng/mL. Here, the analysis of real samples from occupationally-exposed individuals will be discussed.

5.14.T-05 Trifluoroacetic acid levels in firefighter serum and associations with thyroid hormone measures

Heather M. Stapleton, Kate Hoffman, Shenghui Zhang, Mackenzie Laney and Candance Vleet, Duke University

Firefighters face occupational exposure to a range of hazardous chemicals, including per- and polyfluoroalkyl substances (PFAS) used in firefighting foams and turnout gear. PFAS exposure is of growing concern due to their environmental persistence and potential health effects, including endocrine disruption. In this ongoing study, we have enrolled 470 firefighters to date and have analyzed serum PFAS concentrations to characterize exposure profiles and explore potential associations with thyroid hormone regulation. Among the PFAS analyzed, trifluoroacetic acid (TFA) was the most abundant compound in serum, with a 100% detection frequency, a median concentration of 20.1 ng/g and a maximum of 570 ng/g. The next most abundant PFAS was perfluorooctanesulfonic acid (PFOS), with a median serum concentration of 3.62 ng/g. Notably, TFA levels were not associated with participant age or years of firefighting service, in contrast to other legacy PFAS measured. TFA concentrations were positively and significantly correlated with serum perfluorotriacetic acid (PFPrA; $r = 0.6$), suggesting potential co-exposure or shared metabolic pathways. However, TFA was not significantly correlated with other measured PFAS, with the exception of a weak correlation with perfluorooctanoic acid (PFOA; $r = 0.16$). These findings indicate that TFA may have unique exposure sources or pharmacokinetics compared to other PFAS commonly studied in firefighters. To explore possible health effects associated with PFAS exposure, we conducted a pilot analysis of thyroid function biomarkers in a subset of 20 firefighters. We measured free thyroxine (T4), thyroid-stimulating hormone (TSH), and two thyroid-specific autoantibodies: thyroid peroxidase antibody (TPOab) and thyroglobulin antibody (TGAb). Three of the

20 firefighters tested positive for thyroid antibodies, suggesting potential subclinical thyroid autoimmunity. Importantly, we observed a significant and positive correlation between serum TFA and continuous TGA levels ($r = 0.54$), raising questions about a possible immunomodulatory role for TFA. These preliminary findings highlight the prominence of TFA as a novel PFAS of concern in firefighters and point to potential associations with thyroid-related biomarkers. Ongoing and future research will focus on expanding health effect analyses in this cohort to further elucidate the significance of PFAS exposures, particularly TFA, on endocrine and immune health in the firefighting workforce.

5.14.T-06 Trifluoroacetic acid contamination of UK surface waters

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Trifluoroacetic acid (TFA), an ultrashort-chain per- and polyfluoroalkyl substance (PFAS), is an ubiquitous and highly mobile environmental contaminant. Its prevalence is primarily attributed to the degradation of larger PFAS compounds utilized in a range of industrial and consumer products, including pesticides, pharmaceuticals, and f-gases. TFA has been proposed for classification as a very Persistent and very Mobile (vPvM) compound, a Persistent, Bioaccumulative, and Toxic (PBT) compound, and a Reprotoxic (R) compound. While TFA has been extensively detected in surface waters across Europe, North America, and China, a significant knowledge gap exists regarding its presence in UK surface waters. This study addressed this gap by performing the first comprehensive investigation of TFA occurrence at 54 locations across the United Kingdom, encompassing all seasons. The primary objectives were to determine the frequency and level of TFA contamination in UK surface waters and, if detected, to quantify the contamination levels relative to international findings. Our findings revealed widespread TFA contamination throughout the UK, with detections seen at 98% of the surveyed sites. The average TFA concentration observed was 2211 ng/L, positioning the UK towards the higher end of average concentrations reported globally. The highest concentration recorded in this study was 78464 ng/L, which was seen in a sample obtained from the River Kelvin in Glasgow. This value represents the second highest recorded TFA concentration in surface water worldwide. The pervasive detection of TFA across UK water samples provides compelling evidence of widespread PFAS presence in UK waterways. These data underscore the urgent need for further analysis to identify the primary drivers of TFA contamination in UK surface waters. Prioritizing strategies for source control is critical to mitigate further environmental accumulation and potential risks associated with this persistent contaminant. **Disclaimer/Disclosure:** This work was funded by Fidra

5.14.T-07 Simultaneous quantitation of ultrashort-, short- and long-chain PFAS in drinking water and beverages by a single direct injection LC-MS/MS method

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Ultrashort-chain perfluoroalkyl acids (PFAAs), such as trifluoroacetic acid (TFA), perfluoropropanoic acid (PFPrA), perfluoromethane sulfonic acid (PFMS), perfluoroethane sulfonic acid (PFEtS) and perfluoropropane sulfonic acid (PFPrS), represent a subset of PFAS that are characterized by a chain length of 1–3 perfluorinated carbons. Compared to their longer-chain counterparts, these chemicals have historically been overlooked due to their presumed lower toxicity and bioaccumulation potential. However, the high polarity, water solubility and persistence of these ultrashort-chain PFAAs can lead to accumulation in aquatic and plant environments, resulting in increased exposure for aquatic organisms and humans. In particular, TFA has been globally reported in aqueous, solid and biological matrices, often at orders of magnitude higher concentrations than those of the longer-chain PFAAs. There is limited environmental data on ultrashort-chain PFAAs due to analytical challenges. Previous evaluations of HILIC and reverse-phased chromatography have reported issues such as

poor retention and separation, matrix effects and limited analyte range (≤ 7 CF). In contrast, mixed-mode chromatography that combined reverse-phase and ion-exchange functionalities has demonstrated promising results for ultrashort-chain PFAAs; however, its application to longer-chain analytes remains limited. Here, a direct injection LC-MS/MS method based on mixed-mode chromatography enabled the simultaneous quantitation of ultrashort- (≤ 3 C-F), short- (3–6 C-F) and long-chain (≥ 6 C-F) PFAAs at ng/L to $\mu\text{g/L}$ concentrations in drinking water, fruit juices and wines.

5.15.P-Tu General: Environmental (Human and Ecological) Risk Assessment

5.15.P-Tu-101 Risk assessment under an emergency response framework

Stephanie Maggio, Joy Mcgrath and William A Schew, GHD

An environmental emergency response happens when a potentially hazardous material is released into the environment and quick actions are required to mitigate the impact to human and ecological receptors. These actions may include excavation to remove the contaminated material to prevent further impact, booming to prevent the hazardous material from spreading to other areas, evacuation of potential receptors, etc. The source of release, location of the release, and the type of released material all play a role in the response effort. Part of the response effort is a risk assessment which includes understanding which environmental media are potentially impacted, the receptors that may be present, and the fate and effects of the released material. Unlike conventional risk assessments that follow a sequential process — comprehensive site characterization, risk evaluation, and subsequent remedial action, emergency response (ER) frameworks demand immediate response and quick remediation. This presentation explores the nuances of conducting risk assessments within an ER framework by analyzing several distinct environmental releases involving varied incident types, chemicals, and response actions. For each ER release, we will discuss the initial incident and associated chemicals, the conceptual site model developed to identify potential exposure mechanisms and receptors, remedial actions, and any specific constraints that were encountered. The results of the subsequent human health and/or ecological risk evaluation, and the outcomes achieved will also be presented. The findings underscore the importance of adaptive risk assessment strategies that accommodate ER scenarios while ensuring protection of human health and the environment.

5.15.P-Tu-102 Considerations for human health and ecological risk assessments for PFAS

Stephanie Maggio, Joy Mcgrath and William A Schew, GHD

Per- and polyfluoroalkyl substances (PFAS) present unique challenges for environmental risk assessment due to the broad range of chemicals in this chemical class and the wide range in effects concentrations across the chemical class. Although human health risk assessments (HHRAs) focus on a single species—humans, ecological risk assessments (ERAs) must consider a diverse array of species across multiple trophic levels, necessitating an approach that accounts for species-specific sensitivities and site-specific ecological contexts. This complexity is further compounded by the limited toxicological data available for many PFAS compounds. In scenarios where human exposure is limited (e.g., undeveloped terrestrial areas or semi-aquatic environments), ecological risks can become the primary drivers for remedial actions. This presentation will compare media-specific screening levels and receptor-specific toxicity values for both human and ecological receptors to provide a better understanding of the shift from human exposure driven risk to ecologically driven risk for PFAS. Additionally, this presentation will delve into some challenges associated with PFAS ERAs and discuss the implications of current data gaps, which complicate the development of accurate risk characterizations at PFAS-impacted sites.

5.15.P-Tu-104 Framework for an environmental risk analysis of an unleaded piston-engine aviation fuel

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A framework was developed for an environmental risk analysis of an unleaded fuel for piston-engine aircraft,

but guidelines for conducting such an analysis did not exist. This presentation introduces the standard framework/guidelines developed to conduct the aforementioned analysis as well as future analyses. The purpose of the analysis was to characterize the relative environmental (human health and ecological) risks associated with a new unleaded 100 motor-octane aviation fuel. The fuel is being evaluated as a safer alternative to the commonly used leaded aviation gasoline, 100LL, which contains tetraethyl lead, ethylene dibromide, and variable hydrocarbon components. The new framework provides guidelines for comparing the relative risks from exposure to the two fuels in the workplace and in the environment. As suggested by the guidelines, key areas of fuel comparison in the analysis included refining and formulation, shipping, storage, usage, stability in aircraft, engine emissions, and combustion products fate and exposure. Due to the limited availability of data pertaining to the aviation fuels, per guidelines, certain assumptions regarding the environmental fate and potential toxicity of the fuels were made using current information on the fuel components and best professional judgment. Also, per framework guidelines, the environmental risk analysis was based on U.S. Environmental Protection Agency ecological and human health risk assessment methods. This framework for conducting an environmental risk analysis of unleaded aviation fuel may be formalized and applied toward evaluation of other new piston-engine aviation fuels as they are developed.

5.15.P-Tu-105 Common practices in ecological risk assessment that are inconsistent with Environmental Protection Agency guidance and may underestimate environmental risk

Amanda Maxemchuk, Karen Kracko and Lindsay Jasperse, SRC Inc.

Experienced ecological risk assessors at SRC have been tasked with reviewing ecological risk assessments (ERAs) prepared by other contractors under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), also known as Superfund, for nearly two decades. ERAs have been reviewed for accuracy, completeness, protectiveness of risk conclusions, and consistency with EPA guidance and guidelines. Across the reviews, several trends have been identified that are inconsistent with EPA guidance, resulting in incomplete risk characterization, underestimation of risk to ecological receptors, and failure to identify contaminants of potential ecological concern (COPECs) that may need further evaluation or risk management. This includes practices that justify eliminating COPECs from further evaluation that are not risk-based, the selection of toxicity reference values (TRVs) and exposure parameters for the risk evaluation that are not sufficiently protective of sensitive ecological receptors, and the use of risk management language to dismiss risk that is identified by the risk characterization. The most common errors and their impact on risk conclusions will be discussed, and the relevant EPA guidance on risk assessment practices will be presented. The objective of this work is to raise awareness of underprotective risk assessment practices and help promote greater compliance with EPA guidance and guidelines across ERAs.

5.15.P-Tu-106 Recommended hierarchy for the selection of ecological screening values for use in screening level ecological risk assessments

Lindsay Jasperse, Amanda Maxemchuk and Karen Kracko, SRC Inc.

The eight-step ecological risk assessment process outlined in the Environmental Protection Agency's Ecological Risk Assessment Guidance for Superfund begins with a screening level ecological risk assessment (SLERA), which is a simplified risk assessment that can be conducted with limited site-specific data to make a rapid determination of whether potential ecological risk at a site needs further evaluation. A SLERA conservatively evaluates the potential for adverse ecological effects from exposure of ecological receptors to site-related chemicals of interest (COIs) in environmental media. In a SLERA, the maximum measured concentration of each COI in environmental media is divided by a medium-specific risk-based ecological screening value (ESV), resulting in a hazard quotient (HQ). HQs < 1.0 indicate a high level of confidence that there is minimal or no unacceptable risk to ecological receptors from exposure to that COI and exposure medium. HQs ≥ 1.0 indicate potential risk from exposure to a COI and that further evaluation is warranted to characterize risk. The ESVs and exposure parameters used in a SLERA are biased conservative to reduce the probability of incorrectly eliminating COIs or exposure pathways from further evaluation when risk does exist. The level of confidence in

the conclusions of a SLERA is a direct result of the protectiveness of the ESVs used in the risk calculations. It is critical to select chronic no observed adverse effect levels (NOAELs) for the ESVs to ensure defensible conclusions from a SLERA. Ecological benchmark values are available from a variety of state, regional, national, and international sources, and SLERA risk conclusions can vary greatly depending on the values selected. Further, some benchmark sources include probable effect levels or acute values as “screening values”, which are not sufficiently conservative for SLERA risk calculations. A consistent approach to selecting risk-based ESVs for identifying contaminants of potential ecological concern (COPECs) in SLERAs is needed to promote consistency across ERAs and ensure that risk conclusions are protective of the environment. This 216 will discuss the importance of using conservative benchmarks to ensure that all COPECs that require further evaluation are identified at the beginning of the risk assessment process and will present a recommended hierarchy of risk-based ESVs that allows the development of a comprehensive set of ESVs that should be used in SLERAs.

5.15.P-Tu-107 Risk assessment for consumers exposed to mercury in locally harvested fish

Muhammad Saleem¹, Yuqiang Wang², David Pierce², Donald Sens¹, Seema Somji¹, Van Doze³ and Scott Garrett¹, (1)Department of Pathology, School of Medicine and Health Sciences, University of North Dakota, (2)Department of Chemistry, University of North Dakota, (3)Department of Biomedical Sciences, School of Medicine and Health Sciences, University of North Dakota

Mercury contamination in aquatic ecosystems is a worldwide issue because of the health risks associated with eating contaminated fish. The present study was carried out to assess the mercury (Hg) level in different tissues of fish, caught locally from Devil Lake, North Dakota. Fifty-two fish samples (northern pike, walleye, and white bass) were dissected, and tissue samples were individually homogenized and measured for total mercury using a Milestone DMA-80 tri-cell analyzer. The DMA-80 uses an EPA-approved method for direct analysis of solids or solutions in less than 5 min and with detection limits well below parts-per-billion. On an average basis, the highest concentration was found in liver, followed by muscle, kidney and gills. Northern pike has the highest mercury level in muscle, kidney and gills while the white bass has higher Hg concentration in liver. Correlation analysis revealed a good association between Hg concentration in muscles with weight/length of walleye and white bass. The most likely causes of mercury contamination are atmospheric deposition and bioaccumulation/biomagnification within the aquatic ecosystems. The health risk assessment was calculated by estimated daily and monthly consumption limits for Hg and target hazard quotient for each fish. The estimated daily consumption limit should be less than 0.020 kg per day for every fish and should be limited to 3 fish meals per month for adults for walleye and white bass while 2 fish meals per month for adults for northern pike. Target hazard quotient (THQ) indicated that steady long term fish consumption could pose a risk to consumers. Lastly, the mercury level should be regularly monitored, especially in piscivorous fish like northern pike for pollution control and human health.

5.15.P-Tu-108 Limitations of media-specific risk assessment in Superfund site management

Mary Looney and Lynn Woodbury, CDM Smith

At Superfund sites, dividing the site into operable units (OUs) is a common practice to facilitate site characterization, management, and remediation. OUs are often defined by geographic area, habitat, or exposure medium. While organizing by exposure medium can simplify site management, it can introduce significant uncertainty in human health risk assessments when exposure media are evaluated independently. A recent case study at a mining site showed that risks to residential receptors were underestimated when soil and groundwater were assessed separately. For lead exposure in children, the Integrated Exposure Uptake Biokinetic (IEUBK) model estimates blood lead (PbB) levels based on environmental concentrations. Risk is defined when there's a $\geq 5\%$ probability that a child's PbB exceeds a target threshold (i.e., 5 $\mu\text{g}/\text{dL}$). The default IEUBK input for lead in drinking water is 0.9 $\mu\text{g}/\text{L}$. Using this default, a soil preliminary remediation goal (PRG) of 200 mg/kg meets the risk-based remedial action objective (RAO) of 5%. However, this PRG assumes water concentrations do not exceed 0.9 $\mu\text{g}/\text{L}$. If groundwater concentrations instead reach the lead RAO and MCL of 10 $\mu\text{g}/\text{L}$, the soil PRG

must be reduced to 71 mg/kg to maintain acceptable risk. Additionally, the site backfill soil contains 89 mg/kg of lead, and groundwater must not exceed 8.7 µg/L to remain protective. The groundwater risk assessment was performed after the soil risk assessment, and groundwater concentrations often exceeded the IEUBK model default value. For non-lead chemicals, risk is typically expressed using hazard quotients (HQs) and hazard indices (HIs), with values ≥ 1 indicating unacceptable risk. When chemical concentrations in exposure media are assessed separately, cumulative risks may be missed, and properties with risks distributed across media might go unrecognized for remediation even though the total exposure indicates risk. Additionally, chemicals may also be unrecognized as chemicals of concern at a site with this approach. This case underscores the need for integrated exposure assessments. Often, site management and datasets are established well before the risk assessment process begins. As risk assessors, it is imperative to understand implications of and uncertainties from separating OUs by exposure media and to adequately characterize, present, and communicate risk to risk managers in lieu of data gaps inherited from this site management strategy.

5.15.P-Tu-109 Connecting communities and ecosystems: PFAS and other contaminants of concern in Tribal waters

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“Water is life” (Bii’ishchiiis) for the northern plains Apsáalooke (Crow) people. Water security and sustainability are issues affecting all aspects of their lives from drinking water to recreation and spiritual practices. Their connection to the rivers has been passed through generations, but due to water-quality concerns, use of the rivers for drinking, swimming, fishing, and subsistence hunting and gathering has decreased dramatically over time. To address Tribal concerns over surface-water quality, we assessed potential exposures to a broad suite of inorganic (37), organic (435) and microbial (3) contaminants in surface waters from three watersheds in southcentral Montana, United States. Further, to address Tribal concerns over the prevalence and corresponding risks of per-and polyfluoroalkyl substances (PFAS), water, sediment, biofilms, and fish were sampled at a limited number of locations in the Little Bighorn River. Water results indicated that exposures to a range of naturally occurring (e.g., arsenic) and anthropogenic contaminants (e.g., PFAS) were prevalent, and despite relatively low concentrations, cumulative risk assessments indicated chronic ecological effects and human-health secondary contact (recreation) effects at about half the sites. PFAS were also observed in fish, including whole blood and filets, and to a lesser extent in biofilms, despite few detections in water and sediment. This is the first attempt to document environmental PFAS contamination within the reservation and the potential human-health concerns for the general population from consumption of recreational/subsistence fish. Overall, this effort provided preliminary information on the contaminant mixtures present in the environment and their potential health implications. This is essential to protect community health and to support Tribal prioritization of remediation/mitigation actions to protect their natural resources.

5.15.P-Tu-111 A stable isotope approach to comparing iron status across vertebrate species

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Iron stable isotope ratios ($\delta^{56}\text{Fe}$) reflect biological iron regulation including absorption and storage, offering a promising tracer for iron metabolism. To investigate species-specific variation and its relation to storage iron (mainly ferritin-bound iron), we analyzed $\delta^{56}\text{Fe}$ in livers of 14 vertebrate species (8 mammals, 2 birds, 2 reptiles, and 2 fish) and multiple tissues from mice (*Mus musculus*, Slc:ddY, male, n = 5). Iron speciation was determined by Fe K-edge XANES spectroscopy (KEK, Japan), and trace elements quantified by ICP-MS (Agilent 7850). The $\delta^{56}\text{Fe}$ was measured using multicollector ICP-MS (MC-ICP-MS, Neptune Plus) with a

^{57}Fe - ^{58}Fe double spike. Accuracy was confirmed with certified and in-house reference materials ($\pm 0.2\%$). In mice, $\delta^{56}\text{Fe}$ varied among tissues, which is highest in the liver and spleen (-1.52% to -0.80% , and -1.58% to -1.12%), followed by muscle (-1.87% to -1.23%), testis (-1.88% to -2.07%), kidney (-2.22% to -2.03%), and red blood cells (-2.20% to -2.63%). The $\delta^{56}\text{Fe}$ of diet was -0.18% , and values in the stomach and intestinal contents were close to the dietary value. XANES revealed highest storage iron in the spleen and testis (64-89%, and 65-79%, respectively), followed by the liver (18-54%), muscle (32-33%), kidney (14-19%), and red blood cells (0%). The $\delta^{56}\text{Fe}$ was positively correlated with storage iron across tissues ($r = 0.55$, $p = 0.01$), supporting ferritin's preference for heavier isotopes. Across species, liver $\delta^{56}\text{Fe}$ was highest in mammals including mice and bovine (*Bos taurus*, -1.27% – -1.18%). Birds including Japanese quail (*Coturnix japonica*) and chicken (*Gallus gallus domesticus*) showed intermediate values (-2.11% to -1.56% , and -2.17% to -1.87% , respectively), while reptiles including Nile crocodile (*Crocodylus niloticus*) and leopard gecko (*Eublepharis macularius*) exhibited low values (-2.41% to -1.94% , and -3.44% to -3.20% , respectively). No significant difference in dietary $\delta^{56}\text{Fe}$ was observed among mouse, quail and bovine ($0.22 \pm 0.89\%$). Across species, liver $\delta^{56}\text{Fe}$ negatively correlated with storage iron ($r = -0.42$, $p = 0.01$), opposite to the trend within mouse tissues, suggesting high storage iron may reflect lower iron absorption efficiency and selective uptake of lighter isotopes. These results suggest $\delta^{56}\text{Fe}$ reflects interspecific differences in iron uptake and storage. This approach may help developing a broad, minimally invasive tool for comparative studies of iron metabolism in wild and domestic animals. **Disclaimer/Disclosure:** This study was supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) to a project on Joint Usage/Research Center - Leading Academia in Marine and Environmental Pollution Research (LaMer), JSPS KAKENHI Grant Number 24LJ0002 and JST SPRING, Grant Number JPMJSP2108.

5.15.V General: Environmental (Human and Ecological) Risk Assessment

5.15.V-01 Arsenic bioaccessibility in soils of a historical mining area

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Arsenic (As) contamination resulting from historic mining in Złoty Stok, southwestern Poland, poses a significant health risk to local populations through the incidental ingestion of contaminated soils. Human exposure to As-contaminated soil in the vicinity of the abandoned mining area of Złoty Stok and allotment gardens could lead to severe health implications. Previous studies have focused on the estimation of total As concentration (TA) and the mobile/easily soluble fraction of As, which could lead to overestimation or underestimation of human health risk assessment. The aim of this study was to determine the bioaccessible fraction of As using Simplified Bioaccessibility Extraction (SBET) methods, which is an oral in-vitro bioaccessibility assay. This method simulates the human gastrointestinal system. Soil samples were collected from a depth of 0-20cm, pretreated and sieved through a 2mm and $<250\ \mu\text{m}$ mesh sieve stainless steel sieve. SBET procedures were applied using soils with particle size of $<250\ \mu\text{m}$. It was determined that TA are around 4900 mg/kg in soils. In-vitro bioaccessible As (BA) was around 1400 mg/kg (30% of TA). The results indicate that the use of total arsenic concentration in risk assessment could lead to overestimation, as the bioaccessible fraction of arsenic in all the studied soils was below 50% of the total arsenic concentration. This shows that As fraction that could be absorbed into the human bloodstream is not 100% of the ingested concentration. Thus, the use of in vitro bioaccessibility methods is a more reliable approach for the estimation of human health risk with regard to exposure through the incidental ingestion of soils contaminated with As.

5.15.V-02 Lead in blood in a Buenos Aires slum

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Villa 2124 is located in Buenos Aires city, near the Matanza-Riachuelo, one of the most polluted rivers in Argentina. It covers approximately 70 hectares and has a population of 30,000 inhabitants exposed to industrial pollution. Considering that 40% of diseases affecting children are related to environmental factors, it was thought of interest to conduct a study of lead in the blood of children and pregnant women due to its severe and irreversible effects, especially on the nervous system, which can cause: reduction of the IQ, behavioral changes, decreased ability to concentrate, increased antisocial behavior and lower academic performance. Lead exposure can also cause anemia, hypertension, kidney, immune, and reproductive dysfunction, among other complications. In pregnant women, lead easily crosses the placental barrier and poses risks to the fetus, especially its central nervous system. There is no level of lead concentration in blood that can be considered risk-free. The study was conducted in 2019 on a total of 71 children between the ages of 0 and 10, including 7 pregnant women. Participation was voluntary, with prior consent from the caregiver or adult responsible for the disease. Blood lead determinations were performed using a portable Lead Care II lead measuring device in 50 µl of capillary blood, with a lower detection limit of 3.3 mcg/dl. The results were compared with an epidemiological questionnaire and a developmental survey. The sample consisted of 78 participants in total: 71 children under 10 years of age and 7 pregnant women; 55% of those tested had a positive capillary value, and the remaining 45% had a low value. Of the total positive results, 68% had a capillary value between 3.3 and 4.9 mcg/dl; 30% had a value between 5 and 9.9 mcg/dl. The median was 4.1 mcg/dl. The results suggest that the area of Villa 2124 analyzed in this study has a high prevalence of lead, requiring urgent action to clean up the neighborhood and assist the affected population.

5.15.V-03 Hypoxia may link toxicity to infectious and non-infectious diseases

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Oxygen is critical for energy-dependent functions. Many physical and chemical agents, such as Ni, Cd, As, Cu, Pb, atrazine, 2,4 D, bisphenol, UV-b, etc., reduce oxygen consumption and increase oxidative stress as mechanisms of toxicity. A previous contribution reported that these adverse effects could be synergistic with COVID-19 severity (https://www.academia.edu/2994-435_X/1/1/10.20935/AcadMed6106). This study highlights the potential synergy of physical and chemical agents, altering oxygen consumption and oxidative stress as widespread disruptors with potential effects on infectious and non-infectious diseases. Hypoxia-ROS inducible factors (HIFs) are transcriptional regulators that modulate the expression of hypoxia-dependent genes, including apoptosis and the suppression of cell proliferation. Hypoxic conditions occur in several diseases, such as ischemic heart disease, ischemic stroke, solid tumors, erythropoiesis, and angiogenesis, as well as inflammatory and infectious diseases. Over the last few decades, numerous studies have reported a close relationship between viral infections and hypoxia by promoting their replication or enhanced viral gene expression. Hypoxic states have been shown to aid the Epstein-Barr virus (EBV, HHV-4) and the Kaposi's Sarcoma-associated herpesvirus (KSHV, HHV-8) in switching from a latent to a lytic mode. Hypoxia has also been described to upregulate genome replication of the RNA dengue virus (DENV). The oncolytic mutant herpes simplex virus type 1 (HSV-1 G207) has been reported to exhibit increased replication under hypoxic conditions. Hypoxia, which occurs when cellular oxygen demand exceeds supply, is also a common feature during inflammation associated with bacterial infection, due to increased oxygen demand to meet the requirements of resident cells, infiltrating inflammatory cells, and, in some cases, multiplying pathogens. Furthermore, chronic inflammation associated with long-term infection leads to diminished blood supply to tissue as a result of the combination of vascular pathology and micro thrombosis, resulting in hypoxia. In summary, this contribution expands our understanding of the potential synergy of physical and chemical agents on COVID-19 severity to their adverse effects on a wide range of infectious and non-infectious diseases.

5.16.P-Th Late-Breaking Science: Environmental (Human and Ecological) Risk Assessment

5.16.P-Th-060 Detection of per- and polyfluoroalkyl substances (PFAS) in complex environmental matrices with molecularly imprinted polymer – electrochemical sensors (MIP-ES) via a ratiometric

approach

Dane Wagner¹, Mobina Masdari¹, Keeley Farmer¹, Michael Harrigan¹, Rebecca Clark¹, Larry Barber² and Jeffrey Dick¹, (1)Purdue University, (2)U.S. Geological Survey

Per- and polyfluoroalkylsubstances (PFAS) are established environmental micropollutants that need a rapid and robust sensor as a first line of defense. Molecularly imprinted polymer (MIP)-based sensors have shown great promise in electrochemically detecting PFAS. However, like all binding-based sensors, they suffer from interferent effects, especially in the absence of the target of interest. Here, we address this challenge by proposing a ratiometric sensing method. This method uses MIP-modified electrodes in two different compartments: a compartment in which we know there is no perfluorooctanesulfonate (PFOS) or perfluorooctanoic acid (PFOA), and a compartment in which we add PFOS or PFOA. A sample with the absence of PFOS or PFOA results in a ratio of electrochemical signals of ~ 1. A sample that contains PFOS or PFOA results in a ratio different from 1. We demonstrate the principle in controlled samples of lab water and Wabash River water (West Lafayette, IN). Further application is explored through the application of these molecularly imprinted polymer – electrochemical sensors (MIP-ES) in both ground water and surface water samples collected from Joint Base Cape Cod (Cape Cod, MA). The MIP-ES showed great promise in identifying PFAS containing samples down to concentrations of ~200 ppt (~0.4nM for PFOS and ~0.5nM for PFOA) for ground water and surface water sample matrices. The blank samples, not containing analyte but mimicking the sample matrices, were provided through our partnership with the United States Geological Survey (USGS). This method in both lab-controlled samples and field relevant samples is now established as a way to address interferent effects when applying MIP-ES PFAS sensing in complex sample matrices.

5.16.P-Th-061 Risk-based remediation goals for PFAS – Solving for the multimedia exposure scenarios

Philip E Goodrum, Emma Mendelsohn, Sara Hutton, Casey R Remmer and Noah Goodkind, GSI Environmental

By convention, most risk-based screening levels and preliminary remediation goals are calculated for potential exposure media independently - resulting in solutions that may not adequately address site-specific conditions. Accounting for the relative contributions of pathways tied to contaminated surface water, sediment, and soil is understood to be relevant in forward-calculations of risk, particularly for semi-aquatic avian and mammalian omnivores. However, because there is no single analytical solution to the back-calculation of multi-media remediation goals, developing cost-effective and practical remediation strategies remains a challenge. Using an example of a baseline ecological risk assessment of PFAS contamination in a coastal waterway, we illustrate how to leverage site-specific data on bioaccumulation and relative source contributions of multiple media to derive a "solution space" within which remediation goals can be optimized. We illustrate both an analytical solution and empirical solution, and demonstrate the utility of a data gap analysis that can guide sampling designs and reduce key uncertainties in risk-based action levels. Results are compared to the current approaches used by regulatory agencies to derive risk-based action levels for surface water, sediment, and soil for PFOA and PFOS.

5.16.P-Th-062 Drinking water residue testing: A case study on the UV treatment of a generic pharmaceutical compound using a collimated beam device

Yvonne Cooney, Bartek Ciszewski, **Pamela More** and Danielle van der Westhuizen, Charles River, United Kingdom

Active substances being evaluated under Regulation (EC) No 1107/2009 should have no immediate or delayed harmful effect on human or animal health, directly or through drinking water, taking into account substances resulting from water treatment. In recent years, exposure to transformation products formed during the water treatment process has emerged as a significant data gap, prompting manufacturers to demonstrate safety in this previously overlooked area. This concern has led to the issuance of new guidance by the European Food Safety Authority (EFSA) and European Chemicals Agency (ECHA) on assessing the impact of water treatment processes (e.g., chlorination, ozonation) on active substances, their metabolites and environmental

transformation products. UV irradiation is a common water treatment process in the European Union, and our poster presents a case study from the UV treatment of a generic pharmaceutical, using a collimated beam device, a technique which meets the requirements in the guidance document. This case study includes the experimental workflow, analysis of CIPAC-C water (with dissolved organic carbon) treated at a low concentration with a generic pharmaceutical, subsequent stability assessments using sensitive LC-MS/MS, non-target analysis and suspect screening by LC-HRMS with Compound Discoverer, and the process of tentative identification of transformation products.

5.16.P-Th-063 Seasonal occurrence and water quality hazards of per- and polyfluoroalkyl substances in estuarine systems across a gradient of effluent contributions to base flow

HyeongYoung Choi¹, Adam Wronski¹, Sara Kamanmalek², Jacelyn Rice-Boayue³, Jaylen Sims⁴, Kevin Stroski⁵, Chong Chen⁶, Alistair Boxall⁷, Kenneth M.Y. Leung⁶ and Bryan W. Brooks⁸, (1)Baylor University, (2)University of North Carolina, Charlotte, (3)North Carolina State University, (4)Oak Ridge Institute for Science and Education, (5)University of Antwerp, Belgium, (6)City University of Hong Kong, Hong Kong, (7)University of York, United Kingdom, (8)Environmental Science, Baylor University

Global estuaries provide diverse benefits to human populations, biodiversity and ecosystem services. Similar to other coastal systems, Gulf of Mexico estuaries in Texas, USA experience diverse anthropogenic stress, including base flows that are influenced by upstream wastewater discharges. Given the limited global information for the occurrence and water quality hazards of per- and polyfluoroalkyl substances (PFAS) in estuaries, we employed a hydrological model to examine 14 Texas estuaries across gradients of annual precipitation (~60-140 cm/yr) and wastewater effluent contribution to base flows. Conventional grab and time-weighted composite samples over a 24-hr period were collected from these 14 estuaries during wetter (Spring) and drier (Fall) seasons of 2024, the hottest year in Texas since 1895. We quantitated surface water concentrations of 24 PFAS via isotope dilution LC-MS/MS. We identified total PFAS, short-chain PFAS, perfluoroalkyl sulfonic acids (PFSA), and precursors concentrations to significantly ($p < 0.05$) differ between seasons, with higher median concentrations in fall. Concentrations of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) did not exceed United States Environmental Protection Agency water quality criteria for aquatic life during either season across the 14 estuaries examined here. However, PFOS levels in these study systems consistently exceeded European environmental quality standards and Australian and New Zealand guideline values during both seasons. These results highlight an urgent need to better understand PFAS sources, exposure scenarios, and water quality hazards in global estuarine systems across gradients of urbanization and climatic conditions.

5.16.P-Th-064 Assessing the fate of per- and polyfluoroalkyl substances within riparian food webs using the total oxidizable precursor assay

John Fast, Harmanpreet Sidhu and Karen A. Kidd, McMaster University, Canada

Wastewater treatment plant (WWTP) effluents introduce per- and polyfluoroalkyl substances (PFAS) into freshwater systems, leading to their bioaccumulation in downstream organisms. Current analytical methods that focus only on legacy PFAS can underestimate accumulation from effluent exposure and associated risks to emergent aquatic insects and their predators. Larval insects accumulate PFAS during development and then transfer them from aquatic to terrestrial food webs as adults, warranting the need for better approaches to assess the total transfer of PFAS at aquatic-riparian interfaces. The total oxidizable precursor (TOP) assay allows for semi-quantitation of both unknown PFAS intermediates and those without available reference standards in environmental samples by converting them to commonly analyzed terminal PFAS. This study will use the TOP assay to assess total PFAS accumulation and transfer within riparian food webs exposed to WWTP effluents. During insect emergence events, surface water, larval insects, and emergent adults (caddisflies, mayflies), along with riparian spiders (long-jawed orb weavers) were collected upstream and downstream of two WWTPs, with one site potentially influenced by an adjacent landfill. Collections occurred in July and August of 2025 on the Grand River, a highly urbanized watershed in Southern Ontario, Canada. The oxidation process consists of

treating water and biota extracts with potassium persulfate under basic conditions (pH ~10) at elevated temperatures (80 °C, 8 h) to convert precursors to terminal PFAS to compliment targeted analyses. We are analyzing samples for pre- and post-TOP concentrations of 39 PFAS from the EPA 1633a method, including the novel surfactants 6:2 fluorotelomer sulfonamide alkylbetaine (6:2 FTAB) and amine oxide (6:2 FTNO). Previously validated methods using a QuEChERS extraction and dispersive solid phase extraction cleanup, followed by LC-MS analysis, have achieved detection limits <1 ng/g, and quantitation and optimization of the TOP assay on extracts are ongoing. Analyzing legacy PFAS and total precursors in tandem is likely to directly address the underestimation of unknown PFAS accumulation in aquatic invertebrates and transfer to terrestrial food webs through emergent adults. These data will improve our understanding of the risks that wastewater effluents pose to aquatic-riparian ecosystems by expanding the scope of detectable PFAS in both water and biota.

5.16.P-Th-065 Optimizing ecotox data quality reviews with AI: Can ChatGPT help?

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The rapid expansion of large language models (LLMs) presents a compelling opportunity to streamline evidence appraisal in ecotoxicology. To test this potential, we examined whether a firewalled implementation of ChatGPT (RamGPT) could accelerate and standardize data-quality assessments of peer-reviewed literature. A corpus of more than 80 ecotoxicological studies was compiled, each targeting a range of chemicals, taxa and endpoints. We developed a data-quality framework comprising 23 weighted metrics distributed across seven domains: test substance, experimental design, exposure characterization, test organisms, outcome assessment, confounding-variable control and data presentation/analysis. Cumulative scores placed each study into high, medium or low quality categories. Two human reviewers independently applied this framework, while a “master prompt” enabled RamGPT to do the same, constraining hallucinations and requiring confidence estimates and in-text citations for every claim. After scoring was complete, AI and human results were compared statistically. Although the overall weighted scores differed significantly between reviewers and the LLM, the final high-, medium- and low-quality classifications rarely diverged. RamGPT completed the evaluation far more quickly than manual review, yielded more objective scores, and was generally more conservative, tending to penalize studies that omitted explicit methodological details that human experts inferred from context. This screening approach is currently being applied to environmental occurrence data with similar results. These findings suggest that LLM-assisted review can meaningfully reduce workload and increase objectivity in ecotoxicological evidence assessment, especially when paired with targeted human quality assurance and control.

5.16.P-Th-066 Climate and mineralogical controls on arsenic bioaccessibility in legacy mine tailings: Implications for human health risk assessment

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Legacy sulfide mine tailings represent long-term sources of toxic metal(loid)s, yet conventional human health risk assessments often apply conservative defaults for bioavailability that overlook site-specific climate and mineralogical controls. The objective of this study was to evaluate how climatic regime, geochemistry, and mineral hosts influence arsenic (As) bioaccessibility across a climate gradient. We examined polymetallic tailings from eleven sites across the western United States, spanning arid to humid climates. Depth-resolved sampling characterized geochemical profiles, pH, and mineralogy alongside in vitro bioaccessibility (IVBA) of arsenic (As). Molecular-scale speciation was determined using As, Fe, and S X-ray absorption spectroscopy (XAS) to identify As host phases and their transformations with depth. Mineralogy through X-ray diffraction (XRD) analyses showed depth-dependent changes reflecting oxidative weathering fronts. Climatic variation strongly influenced the downward advance of oxidation: arid sites showed shallower oxidation, while wetter sites exhibited deeper oxidative fronts. pH profiles revealed strongly acidic surface horizons at several sites

(TB, KT, MM), transitioning to higher pH at deeper reduced zones. IVBA-As varied widely with depth and climate. Surface horizons in arid/semi-arid sites (KT, MG, TB) exhibited the highest gastric IVBA-As, linked to poorly crystalline Fe phases (ferrihydrite, jarosite). In contrast, humid sites (IM, BB, FM) consistently displayed very low IVBA-As, reflecting formation of stable phases such as scorodite. Depth trends included decreases in IVBA-As at KT and TB, where arsenopyrite and sulfide minerals dominated, but elevated values in deeper humid profiles (CT, MM), possibly related to reductive dissolution and clay-associated arsenate. Speciation analysis helped to show that As bioaccessibility is governed by mineral hosts and redox transitions rather than total concentrations. Strong IVBA-As/Fe correlations at some sites suggest release from single dominant phases, while weaker relationships indicate heterogeneous hosts. Overall, this work demonstrates that climate, geochemistry, and mineralogy jointly control As lability, and that integrating IVBA with speciation advances human health risk assessments by reducing uncertainty and refining risk estimates for mining-impacted communities.

5.16.P-Th-067 Exposure driven environmental risk assessment—A case study on fragrance materials

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Fragrance materials are ubiquitous in the environment due to pervasive uses in cosmetics, consumer products, and other daily use items. Over the past 20 years, the Research Institute for Fragrance Materials (RIFM) has been the leader in conducting environmental risk assessments on more than 3,000 fragrance materials. It has been with this mission that RIFM has developed an updated three-tiered framework that utilizes exposure-based modeling for assessing and ensuring the safe use of these materials. The recent updates to this framework allow for more accurate assessment of fragrance materials by employing new approach methodologies (e.g., mode-of-action based ecological thresholds) to limit the use of animal testing, covering more geographical regions, and yielding realistic exposure concentrations (e.g., using updated IFRA Volume of Use surveys). The RIFM Environmental Risk Assessment determines exposure concentrations by using now available data on region-specific water usage, wastewater treatment models, river dilution, and measured biodegradation and ecotoxicity data. In addition, a hazard assessment is evaluated alongside the risk assessment, to ensure the safety of the fragrance material. The hazard assessment is conducted by using the current ECHA REACH (European Chemical Agency; Registration, Evaluation, Authorization, and Restriction of Chemicals) guidance document on Persistence, Bioaccumulation, and Toxicity (PBT) using available measured or modeled data. This poster highlights a case study in which the step-by-step approach of exposure-based risk assessment of a fragrance material is employed.

5.16.P-Th-068 Bridging environmental and public health: Introducing the interstate technology and regulatory council biological contaminants of emerging concern guidance

Doreen Sechak Peters, *Interstate Technology and Regulatory Council Biological Contaminants of Emerging Concern Team*

Covid-19 widened public appreciation for the interconnection between an emerging environmental hazard and health outcomes. What we learned is that we often do not have sufficient information to understand the effects between our complex environment and emerging biological contaminants, nor sufficient collaboration, communication and coordination across public health and environmental sectors to identify biological contaminants of emerging concern (BioCEC), prevent disease outbreaks, and address environmental concerns. To address the challenges that still lie ahead it is important to bridge expertise between the environmental and public health sectors. The Interstate Technology and Regulatory Council (ITRC), a state-led environmental coalition working to create innovative solutions and best management practices for the environmental sector, has expanded its chemical contaminants of emerging concern identification framework to further address environmental pathogens. ITRC defines BioCEC as, "a microbial pathogenic agent that may pose newly identified risks to humans through the environment and is found in a vector, water, soil, waste, or air." We created a BioCEC guidance document for public health and environmental professionals to broaden and deepen technical knowledge and expedite quality regulatory decision making while protecting human health and the

environment. The guidance, which is rooted in the "One Health" approach, 1) introduces the topic of BioCEC, 2) presents a process guide for identifying and addressing human health-relevant BioCEC, 3) provides information on Conceptual Exposure Models, 4) summarizes key variables that may be used to identify, evaluate, and prioritize BioCEC, 5) describes analytical methods for detecting and quantifying BioCEC in environmental matrices and vectors, 6) compiles information on existing monitoring programs, and 7) presents case studies. By providing a holistic approach to identifying, characterizing and managing BioCEC risks to human health and the environment, the BioCEC guidance can bridge environmental and public health investigations and risk management. The guidance is designed to be relevant to environmental and health professionals at state agencies who are tasked with making informed and timely decisions regarding BioCEC. The content may also be useful to environmental consultants, federal agencies, industrial representatives, tribal organizations and stakeholders with the need to manage BioCEC.

5.16.P-Th-069 Development of non-targeted analysis method for risk assessment of produced water using solid phase microextraction (SPME) and high-resolution mass spectrometry (HRMS)

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Advances in water treatment technologies have enabled the reuse of produced water (PW) generated from oil and gas operations. The raw and treated PW are chemically complex and reuse relies on characterization of the chemical and toxicological properties of treated PW. Non-targeted analysis (NTA) can be used to identify contaminants in complex environmental samples - including PW - due to its ability to detect targeted constituents using suspect screening and characterize unknown constituents. This study will start with a literature review evaluating current use of NTA of treated PW to assess the distribution and characteristics of treated produced water. This review will establish the state of science, and document key decision points in the sample preparation, analytical detection, and data processing steps to provide guidance for interpretation of NTA data. In later stages of the project, raw and treated PW from the Permian basin (TX and NM) will be collected and analyzed using high resolution mass spectrometry (HRMS) and NTA to profile chemical constituents and better understand chemical variability. The next steps will include risk based considerations using non-depletive biomimetic extraction-solid phase microextraction (BE-SPME) and liquid chromatography (LC) retention times to estimate the potential toxicity of compounds present in raw and polished desalinated PW. Based on the results of the literature search, guidance will be provided to help identify the chemical profiles of the compounds found in these samples and quantify their concentrations (when standards are available). This study will advance our understanding of compounds in PW, contributing to improved management practices for PW reuse in the oil and gas industry.

5.16.P-Th-070 Do methylsiloxanes biomagnify in marine food web? A case study of Tokyo Bay, Japan **Yuichi Horii¹, Natsuru Yasuno¹, Takahiro Nishino² and Takeo Sakurai³, (1)Center for Environmental Science in Saitama, Japan, (2)Tokyo Metropolitan Research Institute for Environmental Protection, Japan, (3)National Institute for Environmental Studies, Japan**

Methylsiloxanes (MSs), including cyclic MSs (CMSs) and linear MSs (LMSs), are deployed as intermediates for producing silicone-based polymers and are directly incorporated into a wide range of personal care products (PCPs). Japan is a major producer and consumer of silicone, with the annual sales volume of 145,000 ton. As the negative ecological effects of CMSs have become more evident, regulations have been introduced to reduce or limit their emissions in the European Union. Among bioaccumulation metrics, field-derived trophic magnification factors (TMFs) are considered an important tool to assess chemical behavior in food webs. Although a number of previous studies assessed food web biomagnification of MSs by estimating the TMFs, they mainly focused on major CMSs; D4, D5, and D6 and TMF trends in various MSs were not fully investigated. Furthermore, the previous studies reported either trophic magnification or dilution, or neither of VMSs in aquatic food webs. The objective of this study was to evaluate the trophic transfer of various MSs in the marine food web, focusing on TMF, including benthic organisms. Occurrence and distribution of MSs,

including 6 CMSs (D4–D9, where the number refers to the number of Si-O bonds), 14 LMSs (L3–L16), and 7 modified MSs were investigated in the food web samples (8 species) taken by bottom trawling from the northwestern inner Tokyo Bay in February 2024. Surface and bottom seawater, as well as sediment samples, were collected from 18 stations in Tokyo Bay during the same period. The biota samples were treated using a solvent-shaking extraction combined with a double solid-phase cartridge clean-up. Individual MSs were then identified and quantified using GC/MS. The relative trophic positions (TP) of the organisms based on their $\delta^{15}\text{N}$ values ranged from 2.0 (*Mercenaria mercenaria*) to 3.4 (*Lateolabrax japonicus*). The concentrations of ΣMS (sum of all target MSs) in aquatic organisms, surface seawater, bottom seawater, and sediment samples ranged 1.74–8.17 $\mu\text{g/g}$ lipid weight, 10.3–85.4 ng/L, 9.3–54.7 ng/L, and 72.6–3370 ng/g dry weight, respectively. The estimated TMFs for certain MSs were found to exceed a value of 1 (e.g., D4), suggesting that these substances may exhibit trophic magnification within aquatic food webs. The details of distribution characteristics, TMFs, and bioaccumulation factors (BAFs) for MSs will be discussed in the meeting.

6.01.T Case Studies in Natural Resource Damage Assessment and Restoration

6.01.T-01 Impacts from heavy metal sediment contamination to freshwater mussels in the Big River, Southeast Missouri

Andrew Roberts¹, Josh Hundley¹, Amanda Rosenberger², Kristen Bouska³, Bryan Simmons¹, Stephen McMurray⁴ and Leslie Lueckenhoff¹, (1)U.S. Fish and Wildlife Service, (2)U.S. Geological Survey, Tennessee Cooperative Fishery Research Unit, (3)U.S. Geological Survey, Upper Midwest Environmental Sciences Center, (4)Missouri Department of Conservation

The Big River in southeast Missouri drains the largest historical lead mining area in the United States. Releases of metal contaminated sediments are suspected of suppressing freshwater mussel populations. We characterized the spatial extent of contaminated sediments and evaluated its relationship with mussel populations in the Big River. Mussels and sediments were collected at 34 sites with potential metal effects and 3 reference sites. Analysis of sediment showed 1.5 to 65 times greater lead (Pb) and zinc (Zn) concentrations than background for 168 km downstream of Pb mining releases. Mussel abundance decreased acutely where sediment Pb concentrations were highest and increased gradually as Pb sediment concentrations attenuated downstream. We compared species richness with historical data from three non-mining impacted reference rivers. Big River species richness was on average half of reference populations and 70–75% lower in reaches with high median Pb concentrations. Sediment Zn and Pb, had significant negative correlations with species richness and abundance. The association of sediment Pb concentrations with mussel community metrics in otherwise high-quality habitat indicates Pb toxicity is likely responsible for depressed mussel populations observed within the Big River. Concentration-response regressions of mussel density verses sediment Pb showed that the Big River mussel community is adversely affected at sediment Pb concentrations above 166 parts per million. Based on this assessment of metals concentrations sediment and mussel fauna, our findings indicate that sediment in approximately 140 km of the Big River with suitable habitat has a toxic effect to mussels.

6.01.T-03 Establishing norms for environmental monitoring via machine learning and imagery in science-based legal processes

Emily Evenden, Industrial Economics Inc.

Natural Resource Damage Assessment (NRDA), a science-based legal process, relies on time-sensitive data to establish the type and magnitude of injury to public natural resources. Many NRDA's rely on data derived from aerial or satellite imagery to characterize baseline and/or post-event conditions at a site. Application of machine learning (ML) to aerial and satellite imagery (ASI) is an increasingly popular analytic method used in a variety of legal, economic and policy contexts, with potential relevance for NRDA. This talk will compare how ML and ASI were used to evaluate environmental and economic injury in two recent non-NRDA litigation cases and describes benefits and challenges from plaintiff and defendant perspectives. Given the rigorous standards for scientific methodologies used in NRDA's, we must establish norms for evaluating model effectiveness and

reporting certainty of results. This comparative analysis highlights the importance of reporting the training data from which ML models learn, documenting points of human decision-making and intervention in ML/ASI methodologies, and quantifying metrics of accuracy. Finally, this presentation will identify some potential applications for ML and ASI in on-going NRDAs.

6.01.T-04 Duck and otter creeks: Secret sauce ingredients for successful case settlement

Christopher Lewis¹ and **Amber Bellamy²**, (1)*Industrial Economics Inc.*, (2)*U.S. Fish and Wildlife Service*
Identifying and working cooperatively with multiple responsible parties on legacy Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) natural resource damage assessment (NRDA) cases can be challenging, especially when there is a long history of contamination from multiple sources, multiple hazardous substances, and complex corporate successorship and liability. Decades of refining and manufacturing activity and improper waste disposal practices resulted in numerous releases of various hazardous substances to Duck and Otter Creeks within the Maumee River watershed. The natural resource trustee for Duck and Otter Creeks is the U.S. Department of the Interior (DOI), acting through the U.S. Fish and Wildlife Service (USFWS). The USFWS initiated the evaluation of injuries to natural resources in 2009 and settled the case for \$7.2 million in January 2024 with five potentially responsible parties. The Duck and Otter Creeks case is unique in that, despite significant periods of stagnation, the case settled relatively quickly, based on existing data, once open communication was established with potentially responsible parties and the U.S. Department of Justice. In this talk, we explore the key factors that led to successful settlement and restoration. We provide a brief history of the case, share our approach for determining injuries, service losses, and damages, and discuss challenges and lessons learned, including the importance of leveraging existing partnerships and relationships with federal, state, and local partners.

6.01.T-05 Creative NRD settlement transforms land and lives on the banks of the Passaic River

John Bleiler¹, **J. Douglas Reid-Green²** and **William J Reese III¹**, (1)*AECOM*, (2)*BASF Corporation*
East Newark, New Jersey's (NJ) first major public park has transformed an industrial remediation site into an ecological showcase. Located in an Environmental Justice community, East Newark Riverfront Park explores human connectivity with ecological systems, with a design focused on biodiversity, pollinator gardens, meadows, wooded areas, and native plantings. East Newark is in an industrialized urban area of NJ, colloquially known as the "Gateway" region for its long history of immigration and ethnic diversity. The Borough is bordered by the Passaic River, a federal Superfund site. In 2009, the site's precious metal catalyst production facility was closed and in 2013, demolition was completed. The property was remediated under the NJ Site Remediation program. In 2022, BASF and federal trustees reached agreement on a pro-active NRD Settlement project that establishes credits to address a portion of alleged NRD sediment-related liabilities at several major NJ superfund sites. Key elements of this project include Sustainable Design; Climate Resilience and Flood Protection; Community Job Training; Student Research Engagement with Local University; Education Outreach in East Newark Public Schools; Considerate Construction. Results/Lessons Learned. First envisioned as a creative and innovative way to settle a long-term liability, this project represents a first in the nation precedent. Recognizing that a win-win nature-positive legal settlement has national implications, BASF and natural resource trustees have worked tirelessly to ensure the success of this project – it has attracted national attention, has been visited by multiple dignitaries, and establishes a precedent for fast-track transformation of remedial lands into sustainable urban green space. After more than 150 years of industrial use, this project is re-winding the clock, transforming the waterfront into an extraordinary community asset, a rich landscape providing human and ecological uplift in an overburdened community. The project truly represents regenerative remediation at its best, returning formerly contaminated land into beneficial use, providing a range of economic, environmental, and social benefits to an underserved community, and providing social value for generations to come.

6.01.T-06 What can we learn from past NRDA settlements?

Nadia Martin and Jaime Hodgdon, Industrial Economics Inc.

In the United States, Natural Resource Damage Assessment and Restoration (NRDAR) is primarily conducted under the Comprehensive Environmental Response, Compensation, and Liability Act for hazardous waste sites and the Oil Pollution Act for oil spills. Although these processes have separate regulatory requirements, they are similar and include steps to determine and quantify natural resource injuries and service losses and select and scale restoration actions to compensate the public for any losses incurred. Each NRDAR is unique and reflects the characteristics of the specific site or spill – such as the nature of the release (e.g., type of spill or industrial operations), type(s) and number of hazardous substances released, the natural resources involved, the range of ecological and human use services injured, and the number of natural resource trustees involved in the process (states, federal agencies, tribal governments). Despite the unique characteristics and challenges of each NRDAR case, there are lessons to learn from past settlements that may help inform future cases. This talk will present a database of settlements, which will be used to draw conclusions or insights from past cases, discuss trends and patterns, and provide recommendations for future settlement database analyses and future NRDAR cases. Questions examined will include: (1) Are there any trends in settlement values over time? (2) Are there any trends in the relationship between hazardous substances released, natural resources exposed, and damages values? (3) Are there patterns in settlements by region within the United States? (4) Are there patterns in the length and cost of assessments or the settlement values and the number of trustees and/or potentially responsible parties involved? (5) Are there insights to be gained from comparing the results of cooperative versus non-cooperative assessments?

6.02.T Interventions to Reduce Environmental Microplastics

6.02.T-01 Enzyme-enhanced consortia from landfill soil, sea and wastewater plants; plastic and microplastic biodegradation

Aubrey Dickson Chigwada and Memory Tekere, University of South Africa, South Africa

The recalcitrant nature of synthetic polymers makes plastic pollution a major environmental problem. Microbial consortia that can break down plastics present a promising bioremediation strategy, but their effectiveness is frequently constrained by slow rates of degradation and incomplete mineralization. Enzyme enhancement, which involves supplementing or genetically overexpressing plastic-degrading enzymes during plastic biodegradation, like PETases, cutinases, and laccases, has become a potent method to boost the performance of these consortia. This study investigates the biodegradation of polyethylene terephthalate (PET), polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC) over 7 to 60 days using enzyme-enhanced microbial consortia that were isolated from landfill soil, seawater, and wastewater treatment facilities. From the bio-prospected consortia, enhancement-related enzymes will be extracted and purified directly. The microbial consortia communities will be identified, and their function and gene expression will be characterized using shotgun metagenomics. SEM, Spectroscopic Methods (FTIR, Raman, NMR), and Gas Chromatography/Mass Spectrometry (GC/MS) were used to confirm plastic biodegradation for surface modifications, molecular weight alterations, and changes in chemical composition, respectively. Furthermore, plastic mineralization is determined by measurements of carbon dioxide and methane evolution, and byproducts of plastic biodegradation are identified using Liquid Chromatography-Mass Spectrometry (LC-MS). The purpose of this research is to determine substrate accessibility, maximize enzyme-consortia activity, and comprehend microbial synergy in plastic biodegradation after integrating microbial communities and synthetic enzymes. Sustainable plastic waste management and the growth of circular bio-economies are topics that this study can cover. Above all, this study aims to demonstrate how consortia enzyme enhancement can increase plastic biodegradation.

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6.02.T-02 How effectively do filters reduce the release of microplastics in stormwater in Oregon?

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Microplastics and microfibers that are not intentionally manufactured form from the use of tires, containers and clothing. Wind and water circulate these materials in the environment and organisms inhale and ingest them. Microplastics and microfibers have been linked to deleterious effects in a variety of terrestrial and marine organisms. Our Microplastics-Science Optimizing Solutions project tests the efficacy and ease of using filters on washing machines, dryers and storm drains to control the release of microplastics and microfibers into the environment. City staff were surveyed before and after installing filters on the storm drains. Six litres of stormwater were sampled from three drains and three outfalls in Cannon Beach and Yachats, three drains and one outfall in Pacific City and two drains and two outfalls in Depoe Bay using a 63-micron sieve during a sufficient Spring, Fall and Winter rain event before and after installing the filters. All samples included a simultaneous air fall control, and each community included a triplicate sample. Samples were density separated and filtered, dried and microplastics on the filters were analyzed using a dissecting microscope and a Bruker focal plane array Fourier Transform Infrared Spectrometer. Baseline analysis showed microfibers, tire wear particles, foam, fragments and film (in order of abundance). City staff expressed varying enthusiasm and concern for the stormwater filtration intervention. These findings can guide widespread implementation of similar strategies to reduce the release of microplastics and microfibers in stormwater.

6.02.T-03 Dryer vents versus washing machines: Quantifying sources of environmental microplastics

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Sources of environmental microplastics into urban environments include washing machines, and dryer vents. However, the extent to which these various sources contribute microplastics into our air and water have not been explicitly compared. To assess whether washing machines or dryer vents are greater contributors of environmental microplastics, we simultaneously installed and sampled filters on household washing machines and dryers across two coastal communities for a 30 day period. We quantified the dry weight of the microparticles in each of the filter types and compared within and between households. We also surveyed users about the ease of use of each filter type. Washing machine and dryer type and age affected the amount of microparticles collected in the filters. The efficacy of the washing machine and dryer vent filters at trapping microplastics and the user experience can inform policy for wider application to minimize environmental microplastics from laundry. Reductions in microfibers entering the environment is essential to reducing exposure by organisms, including humans. **Disclaimer/Disclosure:** This research was funded by federal grants from NOAA and Oregon Sea Grant

6.02.T-04 Size-based ingestion of microspheres and microfibers by two freshwater mussel species (*Dreissena bugensis* and *Elliptio complanata*): Implications for removal of microplastic pollution

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Quagga mussels (*Dreissena bugensis*) are a freshwater species of bivalve invasive to the United States. The eastern elliptio (*Elliptio complanata*) is a widely dispersed and ecologically significant freshwater bivalve species common to the eastern United States. The presence of both these species can cause ecosystem-wide effects in rivers and lakes, but their ability to process large volumes of water (up to 8 L/hr/g-dry weight) may have applications for water clarification in freshwater systems. In particular, freshwater mussels have been proposed to remove microplastics (MP) from wastewater treatment plants. Existing literature on particle feeding by freshwater mussels primarily focuses on the capture and ingestion of microalgae and bacteria. Lacking are data on the way in which *D. bugensis* and *E. complanata* handle MP including whether large microfibers and

microfragments are ingested or rejected in pseudofeces. Understanding the feeding interactions with MP has implications for the design of removal and settling systems, as diffuse pseudofeces may be more easily resuspended than denser feces. To examine this issue mussels were isolated and exposed to a known number of MP of the same size and shape. Quagga mussels were exposed to both microspheres and microfibers of 50 μ m, 100 μ m, 150 μ m, 250 μ m, and 500 μ m in diameter or length in individual experiments. Eastern elliptios were exposed to microspheres 20 μ m, 500 μ m, and 1mm in diameter, and microfibers 75 μ m, 500 μ m, and 1mm in length. Biodeposits were collected and digested using KOH to remove organics and isolate MP. The number of plastic particles rejected in pseudofeces and egested in feces was quantified to determine the proportion of each type that was rejected vs. ingested. Large microfibers and spheres (250 μ m, 500 μ m, 1mm) were rejected in high proportions up to 100%, whereas the smallest particles (50 μ m and 75 μ m fibers, 20 μ m and 50 μ m spheres) were rejected in lower proportions (ca. 20 to 75%). Suspension-feeding represents a potential avenue to remove MP from wastewater effluent. Bivalve feeding may also be used to co-concentrate MP and plastic-degrading bacteria to increase the elimination of plastic particles. Data on the size and shape of MP likely to be rejected in pseudofeces versus ingested and egested in feces will aid in the design of living systems that help mitigate release of MP and associated contaminants into the environment.

6.02.V Interventions to Reduce Environmental Microplastics

6.02.V-01 Engineered cigarette butt-derived nanocomposites for polystyrene microplastic removal: A circular economy approach to dual pollution control

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Approximately 4.5 trillion cigarette butts (CBs) are recklessly littered every year, with over one million tonnes of CBs polluting marine environment. These CBs are significant sources of cellulose acetate, a non-biodegradable polymer, another source of emerging contaminants prone to generating microplastics (MPs) (> 5mm). CBs, composed primarily of cellulose acetate fibers, were collected, cleaned, and carbonized through a controlled pyrolysis technique. Specifically, our study presents an innovative circular economy-driven solution to address improper disposal of CBs via upcycling through a slow pyrolysis at 450°C to produce char (C-450) which was later modified for the synthesis of nanocomposites using two different metal oxides (TiO₂ and Fe₂O₃) to generate TiO₂-Char (Ti-C) and Fe₂O₃-Char (Fe-C), respectively. The C-450 and corresponding nanocomposites ((Ti-C) and (Fe-C)) were characterized using SEM-EDS, FTIR, BET, and XRD to evaluate their morphology, surface chemistry, and structural properties. Batch adsorption experiments were conducted using polystyrene microplastics (1 μ m) as the model pollutant. The removal efficiency was assessed under varying conditions. A UV-Vis spectrophotometer was employed to monitor the concentration of microplastics before and after treatment with the adopted adsorbents. Among the synthesized materials, Fe-C demonstrated the highest polystyrene (PS) removal (~87 %) compared to C-450 (~70%) and Ti-C (~69%). The interesting performance reported for Fe-C can be attributed to its exhibited hierarchical pore structure and improved demonstrated crystal dislocation density, which aids in enhanced surface interactions. Thus, adsorption followed a pseudo-second-order kinetic model (PSO), indicating a chemisorption-controlled process driven by electrostatic interactions, pore filling, and hydrophobic effects. By transforming waste into a resource, this research exemplifies a practical application of circular economy principles in environmental cleanup, offering a smart and synergistic strategy to mitigate two pollutants simultaneously. Keywords: Cigarette butts, Microplastics (MPs), Polystyrene (PS), Nanocomposites, Remediation. **Disclaimer/Disclosure:** The first author gratefully acknowledges the support from the UNIPRS (Tuition Fees) and the UNRS Central Scholarship, funded by the University of Newcastle, Australia. The HDR allowance provided by the University of Newcastle is also highly appreciated.

6.02.V-02 10095 – Robust detection and characterization of microplastics in wastewater using a plant-based fluorescent dye

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Wastewater treatment plants (WWTPs) act as major conduits for microplastics (MPs) (plastic particles smaller than 5 mm) from domestic, industrial, and hospital wastewater (WW). Unfortunately, most WWTPs are not equipped to effectively filter MPs, resulting in their release into freshwater systems and terrestrial environments through treated water and sludge. Accurate detection and quantification of MPs are essential for understanding their environmental distribution and ecological risks. Among the existing techniques, fluorescent staining has emerged as a rapid, cost-effective, and reliable method for visually identifying MPs in environmental samples and biological organisms. Nile Red (NR) has been the most commonly used fluorescent dye for this purpose due to its lipophilic nature. However, its solvatochromic properties can alter fluorescence based on MP polarity, and its tendency to leach poses ecological risks. To address these limitations and align with the United Nations Sustainable Development Goals (SDGs), there is growing interest in using natural, plant-derived fluorescent dyes as eco-friendly alternatives. These biocompatible dyes offer a lower carbon footprint (SDG 13), reduce health hazards for both consumers and industrial workers (SDG 3), and help prevent secondary pollution in aquatic and terrestrial ecosystems (SDGs 6 & 14). The use of such dyes represents a sustainable innovation in MP detection, promoting safer environmental practices and supporting global efforts to combat plastic pollution. This is the first study on the application of the plant-based fluorescent dye (coded as GG) for the staining and detection of MPs in WW. Polyethylene (PE) MPs (nurdles) in the size range of 200-9900 nm and 108-212 µm were stained with the natural fluorescent dye. The stained PE-A and PE-B MPs exhibited green fluorescence under a fluorescence microscope confirming successful staining regardless of the size. A destaining test further confirmed the strong binding of curcumin onto the surfaces of MPs. In addition, Fourier transform infrared spectroscopy (FTIR) analysis of the stained MPs confirmed only electrostatic interactions existed between the dye and MPs surface. The staining procedure was also tested for the MPs recovered from real samples of WWTPs and resulted in fluorescently labelled MPs for easy tracking. NB: The name of the dye is not disclosed in the abstract as per the Intellectual Protection (IP) guidelines of York University.

6.03.P-We Natural Resource Damage Assessment: Applied Toxicology for Injury Determination

6.03.P-We-124 Integrating climate change impacts into natural resource damage assessments: Redefining baselines for a changing landscape

Amber Stojak, Carolyn Meyer and Holly McChesney, Arcadis Inc., USA

Climate change is increasingly altering the frequency, intensity, and duration of extreme weather events, including wildfires, fundamentally reshaping ecosystems and the services they provide. Wildfires driven by prolonged droughts, higher temperatures, and increased fuel loads are becoming more frequent across the U.S., particularly in the west. These changes affect the baseline conditions used in Natural Resource Damage Assessments (NRDA). Shifts in fire regimes, measured by fire return intervals (FRIs), challenge our understanding of how ecosystems and species adapt to climate change and wildfires over time. Shorter FRIs have cascading effects on habitat services, increasing the complexity of NRDA modeling. This raises the question: how can wildfires or other weather events and their interactions with climate change be incorporated into NRDA baseline to better assess damages and identify restoration needs? To address this, we employ several methods to quantify FRIs and their impacts. Historical FRIs are derived using LANDFIRE tools, while current wildfire data provide geospatial information on fire footprints and years of occurrence. Percent departure from historical FRIs (FRID) is assessed at the watershed scale using GIS-based models, and future fire frequency is projected based on rates of change and land use scenarios. State-and-transition models help explore how habitat conditions evolve under changing fire regimes, including feedback loops created by invasive species such as cheatgrass (*Bromus tectorum*) or giant reed (*Arundo donax*). These species increase fire susceptibility by supplying fine fuels that promote fire spread, recolonizing burned areas rapidly and perpetuating the fire hazard conditions. This understanding of baseline conditions is applied to NRDA models

by redefining service levels to reflect dynamic baselines impacted by wildfires and climate change. Integrating FRIs and climate change projections into NRDA models enables NRDA practitioners to dynamically assess ecosystem baseline in response to shifting fire regimes or other major weather events that are intensified due to climate change. Findings highlight the need for adaptive methodology to account for the complex interplay between climate change, fire frequency, and ecological responses. NRDA practitioners can use these insights to better estimate damages, develop long-term restoration goals, and implement measures that align with evolving environmental conditions.

6.03.P-We-125 Evaluating injury to soil invertebrates in natural resource damage assessments (NRDAs)

Joy McGrath, William A Schew and Stephanie Maggio, GHD

A critical phase in Natural Resource Damage Assessments (NRDAs) is the Injury Assessment and Restoration Planning, which aims to evaluate the environmental impact of contamination and guide efforts for resource restoration. Although a consensus-based approach exists for evaluating sediment-related natural resource damages from exposure to mixtures of chemicals, there remains a notable gap in generally accepted approaches for evaluating damages to terrestrial receptors. This case study presents an NRDA in which soil-related natural resource damage plays a central role in understanding the broader ecological consequences of contamination. We discuss a pragmatic framework linking soil concentrations to ecological service loss by comparing Site concentrations to established ecological benchmarks for soil invertebrates, a key group of terrestrial receptors. The method quantifies injury by calculating the percentage of Site soil samples that exceed benchmarks for each contaminant of concern (COC). The exceedance percentages for each COC are then averaged to determine the overall service loss for the Site. This approach provides a reproducible, transparent method for evaluating injury to soil invertebrates.

6.03.T Natural Resource Damage Assessment: Applied Toxicology for Injury Determination

6.03.T-01 Assessing injury and impairments in colonial waterbirds exposed to persistent organic pollutants: Immune, reproductive and apical endpoints

Keith Grasman, Calvin University

Wildlife injury evaluations for Natural Resource Damage Assessments (NRDAs) and impairment determinations under the Great Lakes Water Quality Agreement (at specific Areas of Concern [AOCs] or in lake-wide regions) often emphasize apical endpoints (e.g., population sizes, reproductive success) and comparison of exposure levels to toxicity reference values. The use of modern toxicological methods such as biochemical, cellular, and physiological biomarkers (i.e., steps in adverse outcome pathways [AOPs]) can enhance injury determinations and strengthen cause-effect linkages to specific contaminants. This talk will describe two studies of waterbirds exposed to polychlorinated biphenyls (PCBs), dibenzo-p-dioxins, and dibenzofurans (PCDD/Fs) in which apical endpoints (population declines and poor reproductive success) were elucidated by measuring immunological and developmental endpoints. A 15 year program monitored fish-eating birds in Saginaw Bay, which is both an NRDA site and AOC (high PCBs), the River Raisin AOC (high PCBs), and Grand Traverse Bay (high PCDD/DFs but low PCBs). Breeding adults declined in areas with high PCBs: 42% decline in Caspian terns (*Hydroprogne caspia*) in Saginaw Bay and >95% decline in herring gulls (*Larus argentatus*) in the River Raisin AOC. These two populations frequently had poor chick productivity. Relative risk ratios for infertility and embryonic mortality in gull eggs were 1.9-3.4 fold higher than reference in all three contaminated areas. The phytohemagglutinin (PHA) skin response (T cell-mediated immunity) was suppressed 51-56% in gull chicks in all three contaminated areas, and 48-50% in terns and 33% in black-crowned night herons (*Nycticorax nycticorax*) in Saginaw Bay. The second study was an NRDA preassessment investigating immune responses and reproductive success of herring gulls and black-crowned night herons in New York Harbor, where waterbird populations exposed to PCDD/DFs and PCBs had experienced significant long-term declines. Reproductive success of gulls was poor. The PHA skin response was suppressed 70-80% in gull and heron chicks. Strong negative correlations ($r = -0.95$ to -0.98) between the PHA response and dioxins

and PCBs in gull livers gave strong evidence that these chemicals contributed to immunosuppression. Additional immunological endpoints were assessed in both studies. These case studies illustrate the usefulness of cellular and physiological biomarkers in wildlife injury/impairment investigations.

6.03.T-02 Pathways of contaminant effects on bats: Implications for damage assessment and restoration

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Globally, insectivorous bats are vulnerable to certain aquatic-associated contaminants because of potential exposure through their diet of emergent aquatic insects. Most North American bat species are insectivores that forage near water, thus dietary pathways of aquatic contaminant exposure and effects are likely to be an important source of injury. Natural Resource Damage Assessment and Restoration (NRDAR) cases are often located within the range of bat species of conservation interest. However, few cases directly consider injuries to bats. To aid practitioners in conducting damage assessment and restoration for bats, we 1) systematically review the literature on diet-mediated effects of contaminants on bats, 2) contextualize the relative strength of these effects with other pathways of effect (maternal transfer, dermal, and inhalation) using a systematic literature review and meta-analysis and 3) develop a conceptual framework for aiding determination of diet-related injury to bats in NRDAR and similar processes. The conceptual framework incorporates potential pathways of exposure, bat species foraging traits, types of contamination, and types of injury that can be quantified to determine adverse effects to bats at contaminated sites compared to reference sites in the field during the NRDAR assessment or similar investigations following a contaminant release or oil spill. In some cases, the unique ecological and physiological characteristics of bats may make it beneficial to assess bats separately from other surrogate species like birds or shrews.

6.03.T-04 Modeling PFOS trophic magnification in freshwater food webs: Determination of injury for natural resource damage assessment (NRDA)

Jeff Morris¹, Heather Forth¹, Jamie Holmes¹, Fabrizio Bonatesta², Andrew McFadden¹, Lucas Savoy¹ and David Evers¹, (1)Biodiversity Research Institute, (2)Abt Global

Perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) are per- and polyfluoroalkyl substances (PFAS) that were listed as hazardous substances by US EPA in 2024. As recently listed hazardous substances, injury to natural resources caused by PFOS and PFOA are now compensable under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA; a.k.a. Superfund) through the Natural Resource Damage Assessment (NRDA) process. In this presentation, we will describe field studies and modeling work we have conducted to quantify trophic magnification across freshwater aquatic food webs and estimate PFOS concentrations in common loons (*Gavia immer*), a top predator at these sites, and their prey. Our model integrates PFOS concentrations in water and sediment in these systems to model PFOS bioaccumulation and trophic magnification up through the food web. The model provides a method for estimating exposure and injury to higher trophic level biota and their prey without conducting comprehensive food web studies.

6.04.P-Th Remediating Sediment Sites by Modifying Bioavailability, Degradation and Accessibility

6.04.P-Th-116 The potential of anaerobic biodegradation under sediment caps

Allison D Geiselbrecht¹, Evan Malczyk¹, Talia Mathews¹, Marzieh Mansoori² and Karl Rockne², (1)Floyd|Snider, (2)University of Illinois, Chicago

In situ capping is a widely used contaminated sediment treatment technology to mitigate risks to human health and the environment resulting from contaminated sediments. Long-term effectiveness monitoring (LTM) of

sediment caps in perpetuity is typically required by regulatory agencies based on the assumption that recalcitrant organic contaminants persist in the environment over very long time scales. Although anaerobic degradation and transformation of many organic contaminant classes have been established over a broad range of rates, cap design and associated performance monitoring do not routinely consider how biodegradation of residual contaminated sediments under the cap may impact overall assessment of post-remediation trends and remedy effectiveness. The goal of this presentation is to promote awareness of anaerobic biodegradation to inform future guidance and studies and to improve the baseline understanding of how anaerobic degradation can be considered for the design and LTM of capped sediment remedies. Research was conducted to understand the anticipated biodegradation processes and degradation time frames for persistent organic compounds, particularly polycyclic aromatic hydrocarbons (PAHs), under anaerobic conditions similar to capped sediments. Anaerobic degradation rates from the literature for common classes of environmental organic contaminants were compiled from a number of studies that reflect both in situ measurements and bench-scale studies using native sediments from capped sites. To demonstrate hypothetical reduction by anaerobic degradation of PAHs over time within capped sediments, available anaerobic degradation rates were applied to sediment concentrations from two capping sites. The long-term impact of in situ degradation under sediment caps, including the potential effectiveness of cap amendments to encourage biodegradation, is poorly documented. Advancing awareness through greater dissemination of the state-of-the-science on anaerobic biodegradation has the potential to expand the consideration of these processes for sediment cap remedy and LTM design, with the hope of encouraging additional research interest and potential pilot studies

6.04.P-Th-117 Are remediation efforts effective in the East Palestine disaster cleanup?

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The East Palestine, Ohio rail disaster and subsequent decisions to proceed with unwarranted burns of railcar contents have been shown to have contaminated areas in and nearby the derailment site. In addition, the air-borne plume of volatilized contaminants, as well as a mixture of combustion products, dispersed over the region, particularly to the east and southeast. This independent investigative team, as well as other research groups, have shown that the levels of contamination exceeded those where additional monitoring and cleanup were indicated. Cleanup efforts began soon after the incident in February 2023. However, the areas addressed were those in the immediate vicinity of the derailment and dismantlement of the affected railcars and trackbed. Only later was it acknowledged that the contamination was much more widespread and included local streams, area residences, subsurface zones, and some deposition areas from the atmospheric plume. Some of these cleanup projects continue to the present. Continuing sampling and monitoring have indicated a variable pattern of the effectiveness of the cleanup. This presentation will address several aspects of the completed and on-going remediation program - local wetlands and streams, and surface and subsurface contamination, both in the areas near the site as well as those further to the east and southeast. Some observations will be made regarding alternative control and remediation techniques and the likelihood of success for long-term environmental restoration. Concerns about continuing human health consequences will also be addressed.

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6.04.P-Th-118 From environmentally acceptable endpoints (EAE) to in-situ remediation: An overview of progress

Charlie Menzie, Exponent

The Environmentally Acceptable Endpoints (EAE) program emerged in the 1990s as a collaborative industry – government – academic initiative to develop soil and sediment clean-up levels that account for bioavailability. The initiative was prompted by laboratory and field studies related to assessing and remediating petroleum residues (e.g., the various fractions of TPH and PAHs) and synthetic organic chemicals (e.g., pesticides and

PCBs) in soils and sediments. Collectively these studies revealed that while a fraction of these compounds could be remediated through bioremediation and various extractions, a portion of the compounds appeared resistant or could be remediated only slowly. These fractions were variously referred to as “fast” and “slow”, “free” and “bound”, or “bioavailable” and “not readily bioavailable”. This led to growing awareness of “bioavailability” and a focus on how to evaluate this property, facilitate treatment of both the available and unavailable fractions, and establish EAE as clean-up criteria. Over the past two decades much progress has been made on evaluation and on the development of remedial technologies either by rendering the bound chemicals more available or by controlling bioavailability and bioaccessibility or by augmenting bioremediation. During this period there were regulatory developments and frameworks on how best to incorporate the EAE concepts into remedial and restoration decisions and to address potential negative consequences. This presentation provides the history, current status, and anticipated future of managing bioavailability, bioaccessibility, and bioremediation via treatment amendments. These technologies are shown within a spectrum of assessment and remediation/restoration tools ranging from removal to capping to natural attenuation/recovery that can be tailored to site conditions.

6.04.T Remediating Sediment Sites by Modifying Bioavailability, Degradation and Accessibility

6.04.T-01 Twenty-five year perspective on in-situ activated carbon amendment to sediment

Richard Luthy¹, Yeo-Myoung Cho¹ and Upal Ghosh², (1)Stanford University, (2)University of Maryland, Baltimore County

The addition of activated carbon (AC) in various forms to sediment is a proven technology for managing persistent organic contaminants. Using AC was an outgrowth of fundamental research that began more than twenty-five years ago with understanding of exactly where and how hydrophobic organic contaminants (HOCs) were associated with sediment particles. That early work employed complementary microscale analytic techniques to describe the character of sorption mechanisms directly at the sub-particle scale. Subsequent investigations showed that black carbon particles in sediment, e.g., char, coal, or pitch, acted as natural accumulators of HOCs. At the same time, work with contaminated sediments showed that HOCs associated with black carbon particles were more strongly sorbed and less bioavailable than such compounds associated with amorphous organic matter. It was from this work that the idea emerged that adding black carbon particles to sediment, like AC, would enhance organic compound sequestration and thereby reduce availability and risk. This presentation will recap some of this history and give examples of successful use of in-situ AC amendment in freshwater and marine settings. Recent work will be presented on the stability of a proposed engineered blended cover comprising aggregate base and AC at a site with shipping activity. In sum, twenty-five years' experience shows how site-specific mechanistic studies, bioaccumulation tests, and modeling validate the treatment efficacy of in-situ AC amendment and deployment in different settings.

6.04.T-02 Influence of activated carbon on the bioaccumulation of methylmercury from sediment by the amphipod, *Leptocheirus plumulosus*

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The benthic invertebrate, *Leptocheirus plumulosus*, was used to evaluate the potential for activated carbon (AC) amendments to reduce methylmercury (MeHg) bioavailability in Berry's Creek, NJ, sediments. The standard 28-day bioaccumulation assay protocol was modified to account for a strong initial pulse of net MeHg production in test sediments. Several laboratory experiments were conducted with various sediments and two different AC amendments. AC amendments, mixed into homogenized marsh sediments, reduced the bioavailability of sediment MeHg to *L. plumulosus*. A 5% AC amendment reduced the biota-sediment

accumulation factor ($BSAF = \text{ng MeHg/g ww tissue} / \text{ng MeHg/g dw sediment}$), which normalizes tissue concentrations to sediment concentration, by 2- to 4-fold. Addition of AC, however, often resulted in higher sediment MeHg concentrations in the homogenized marsh sediments. The AC appeared to alter the balance between MeHg production and degradation in sediment, but the impact varied with sediment chemistry. Overall, the net effect of AC amendment was moderate reduction in amphipod MeHg concentrations in some cases, increasing concentrations or no effect in other cases. AC amendments significantly decreased total mercury (Hg) and MeHg in porewater in all cases, which could decrease efflux from contaminated sediments. We also compiled the available literature on amphipod MeHg bioaccumulation. While sediment MeHg is a significant positive predictor of amphipod MeHg uptake, MeHg bioavailability (BSAF) generally declines with increasing concentrations of sediment MeHg and sediment organic carbon. BSAF values for Berry's Creek sediments were generally in the lower range of literature values. The mechanism(s) of AC impact on net Hg methylation need to be determined. The complexity of the physical and biogeochemical drivers of net methylation will require site-specific testing of definite application methods prior to potential use of AC in remediation. **Disclaimer/Disclosure:** This research was supported by The Dow Chemical Company. The Smithsonian Institution provided support for interns and postdoctoral scientists.

6.04.T-03 Blended cover with activated carbon for in-situ stabilization of DDx/dieldrin in sediment

Richard Luthy and Yeo-Myoung Cho, Stanford University

In-situ activated carbon (AC) sediment amendment is known to immobilize organic contaminant flux from sediments, thereby reducing contaminant bioavailability and associated risks. To address concerns of field-specific scour conditions and hydrodynamic forces, Stanford conducted a case study for an AC-blended cover at the United Heckathorn Superfund Site in Richmond, CA. We designed experiments to assess the stability of a blended cover under site-specific fluid shear induced by flume-generated currents based on prior estimates of maximum vessel scour and bed shear stress. The team conducted mesocosm laboratory studies using site-collected sediment with passive samplers to test water quality and blended cover material options. The experiment tested a blended cover with AC and coarse materials designed to withstand hydrodynamic conditions and AC stability in a current flume. The cover comprised medium-size gravel ($D_{50} = 15 \text{ mm}$) with 4% granular AC by weight. Flume erosion studies showed minimal AC loss (1-2% of total AC) under shear forces of 9-31 Pa, which was equivalent to or exceeded the estimated worst-case erosional conditions in the channel induced by a hypothetical, stationary tugboat propelling at high power thrust. Mesocosm studies showed rapid reductions in freely dissolved (C_{free}) DDx and dieldrin in the blended cover layers and surface water with C_{free} DDx reduced by over 98%. The combination of both stability and performance testing demonstrates that an engineered blended cover-AC design would be a feasible remedial option at the site, and that this testing approach can be applied to evaluate in-situ treatment in other locations.

6.04.T-04 Lessons learned from in-situ activated carbon amendment applications to treat dioxin/furan contamination in the Scanlon and Thomson Reservoirs, St. Louis river area of concern

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The Minnesota Pollution Control Agency (MPCA) partnered with the US Environmental Protection Agency (USEPA) Great Lakes National Program Office (GLNPO) on two Great Lakes Legacy Act (GLLA) Projects to remediate dioxin/furan contaminated sediments in the Scanlon and Thomson Reservoirs. Due to the geologic and hydrodynamic constraints of the reservoirs, remedial capping and dredging and removal were not viable remedial alternatives. The selected remedy for the sites was application of a pelletized form of powdered activated carbon (PAC) amendment to minimize uptake of the contaminants from the sediment to benthic invertebrates. Approximately 238 tons of pelletized PAC were placed over a 13.7-acre area at the Scanlon Reservoir. Upon completion, the Thomson Reservoir project is projected to place 18,600 tons of pelletized PAC over approximately 66 acres. Pelletized activated carbon placement at this scale is a new and novel way of mitigating the bioavailability of contaminants, leading to design and implementation unknowns. Strategies for

dust mitigation during transfer and placement of the amendment were developed during full-scale placement at both sites. Quality assurance and quality control procedures to verify PAC placement thicknesses were independently developed for each site and modified during placement to achieve the best possible data. Accessing and placing pellets in shallow and variable water depths were also overcome with varying degrees of success. Public outreach and messaging were important to notify the public of the objectionable nature of the dissolved pellets prior to mixing with the underlying sediments. Finally, robust plans were developed to monitor the activated carbon and in-situ porewater concentrations post-placement to track remedy effectiveness.

6.04.T-05 In-situ sequestration and degradation of PCBs in sediments with bioamended activated carbon

Kevin R Sowers and **Upal Ghosh**, *University of Maryland, Baltimore County*

Polychlorinated biphenyls (PCBs) are a widespread contaminant in sediments in the U.S. and a frequent cause of fish consumption advisories. While PCB biodegradation was first observed in the field nearly four decades ago, engineering for field-scale remedies has been elusive. Our recent lab-scale and field studies have demonstrated the effectiveness of activated carbon (AC) amended with PCB-degrading microorganisms for reducing the inventory of legacy PCBs in sediments while also reducing bioavailability to the food chain. We describe in situ treatment of PCB of several impacted sediment sites treated with bioamended AC. The innovative aspect of the technology is the application of large numbers of PCB-transforming microorganisms to circumvent the threshold PCB concentration for growth. Anaerobic organohalide respiring bacteria and aerobic PCB oxidizing bacteria are grown in production-scale bioreactors and applied to sediments using a pelleted AC agglomerate (SediMite™) as a delivery system. The bioamended AC serves not only as a solid substrate for the delivery of the microorganisms but also adsorbs and concentrates hydrophobic PCBs in close proximity to the biofilm of PCB-transforming bacteria. Based on the optimal concentration of cells and carbon loading rates for each site, we conducted field applications of bioamended AC at sites in the U.S. The effect of treatments was monitored by measuring total PCB concentrations in sediment cores and in porewater using passive samplers. Methods were successfully developed for production-level scale-up of the microorganisms without residual POPs and the development of inoculation systems to deploy active PCB-transforming microorganisms for full-scale treatment of PCB impacted sediments. Deployment methods for bioamendments include deployment into sediment with AC pellets with a modified venturi air mover, conveyor belt and by manual broadcasting. Methodology, challenges associated with deployment and effect of treatment on sediment and porewater concentrations of PCBs will be discussed. The results demonstrate the successful treatment of PCB impacted sediments using a combination of in-situ treatment with both anaerobic dechlorinating and aerobic degrading microbes. These field studies show the effectiveness and engineering scalability of bioremediation as an in situ strategy to help address the widespread need to reduce contamination of the aquatic food web from exposure to sediment-bound PCBs. **Disclaimer/Disclosure:** This work was supported by the National Institutes of Environmental Health and Safety Small Business Innovation Research Program (R43ES032365 and R44ES032365), and U.S. Department of Defense Environmental Security Technology Certification Program (ER-201215). The authors are co-inventors of patents related to the technology described in this abstract for which they are entitled to receive royalties. In addition, U.G. and K.R.S. are partners in a startup company (RemBac Environmental) that has licensed technologies related to the bioamendment; U.G. is a partner in a startup company (Sediment Solutions) that has licensed SediMite™.

6.04.T-06 Evaluation of surface sediment amendments in mercury contaminated sediment – How biogeochemistry plays a role

Steve Dent¹, **Paul Ho**¹, **Jada Damond**¹, **Jennifer Crawford**² and **Chris Eckley**², (1)CDM Smith, (2)EPA Region 10

The Black Butte Mercury Mine Superfund site in Lane County, Oregon, has caused mercury contamination in the downstream Cottage Grove Reservoir (CGR). CGR is operated as a flood control reservoir, so portions of the reservoir experience periods of flooding and drying throughout the year. While the permanently inundated

sediments in the low pool region of the reservoir have the highest concentrations of Hg-contaminated sediment, elevated Hg methylation has been observed in the seasonally inundated sediments and the wetlands that line the inlet of CGR. Due to the extent of Hg contamination and the nature of the flood-control reservoir, in-situ amendments were considered as potential treatment options. To address this, EPA Region 10 and CDM Smith conducted a series of bench-scale treatability studies to identify the optimal surface sediment amendment for maintaining low MeHg and THg porewater concentrations and MeHg sediment concentrations, limiting its release into surface water and subsequent uptake by biota. Amendments were chosen based on studies demonstrating their ability to sequester mercury, including engineered materials, activated carbon, and biochar. In these bench-scale studies, the performance of these amendments was evaluated as they were applied to several biogeochemical conditions mimicking those at the site. In an anaerobic sediment microcosm experiment, amendments were applied to sediments collected from various locations within CGR, which had distinct characteristics affecting Hg fate and transport. In a subsequent bench-scale mesocosm study, amendments were applied to seasonally inundated sediments simulating the wetting and drying cycles that CGR undergoes. Amendment effectiveness varied substantially between sediment types for both mercury and geochemical parameters, underscoring the need for site-specific evaluation before selecting a remedy. Mesocosm experiments exhibited more uniform performance among selected amendments, with their effectiveness with MeHg in solid phase increasing over successive wetting and drying cycles. MeHg concentrations in porewater remained below detection across all amendments within the mesocosm tests. This study provides insights into the physical and biogeochemical factors influencing amendment effectiveness, informing potential future pilot scale studies at the site and strategies for mitigating mercury contamination in aquatic environments at other sediment sites. **Disclaimer/Disclosure:** This study was funded by EPA, conducted at EPA facilities, and all samples were analyzed by EPA or under contract to EPA. The findings and conclusions in this presentation have not been formally disseminated by the U.S. EPA and should not be construed to represent any agency determination or policy. Any mention of trade names, products, or services does not imply an endorsement by the U.S. Government or the U.S. Environmental Protection Agency. The EPA does not endorse any commercial products, services, or enterprises.

6.05.P-Tu General: Engineering, Remediation and Restoration

6.05.P-Tu-112 Recycling and material recovery of low dimensional materials incorporated perovskite solar cells: A life cycle perspective

Santiago Londono and Ilke Celik, Portland State University

Low-dimensional (LD) materials such as: 0D (e.g., graphene quantum dots), 1D (e.g., nanowires and nanoribbons), 2D (e.g., graphene), are increasingly integrated into perovskite solar cells (PSCs) to improve both power conversion efficiency and operational stability, given its technology maturity their environmental implications remain insufficiently understood. This aims to do a life cycle assessment of alternative integration pathways emphasizing the use of greener solvents and scalable manufacturing and recycling methods for PSCs based on LD materials. Specifically, we assess the environmental impacts of dispersing reduced graphene oxide (rGO), molybdenum disulfide (MoS₂), and graphene quantum dots (GQDs) into three layers of a PSC including the electron transport layer, absorber, and hole transport layer using alternative solvents such as ethanol and water-based dispersions. The LCA follows ISO:14040 and 14044 guidelines, applies a cradle to end of use system boundary, and one kilowatt hr of generated energy as functional unit. Impact assessment is conducted under TRACI 2.1 method, with life cycle inventories derived from published experimental studies and modeled in GaBi using databases from Ecoinvent. While results are not completed, preliminary findings indicate that substituting conventional solvents like dimethylformamide and N-methyl-2-pyrrolidone with less hazardous alternatives may reduce environmental impact metrics across LD-PSC integrations. These findings are expected to support more environmentally conscious choices as technology advances toward commercial scale.

6.05.P-Tu-113 PFAS-free future: Advancing water treatment with supercritical oxidation

Elham Akbari, Gangadhar Andaluri and Md Saiful Islam, Temple University

Per- and polyfluoroalkyl substances (PFAS) are recalcitrant environmental pollutants, resisting conventional treatment due to their robust carbon-fluorine bonds. Supercritical water oxidation (SCWO), leveraging water's unique reactivity above its critical point (374°C, 22.1 MPa), presents a promising strategy for their complete mineralization. Under supercritical conditions, water generates reactive free radicals, enabling the rapid cleavage of PFAS molecular structures. This study evaluates SCWO's performance in degrading a suite of PFAS, including perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorobutanoic acid (PFBA), across a range of conditions (400–600°C, 23–30 MPa, 1–180 min residence time). Analytical results, obtained via liquid chromatography-mass spectrometry (LC-MS) and ion chromatography, reveal destruction efficiencies exceeding 99% for carboxylic PFAS, with negligible formation of fluorinated intermediates. Current efforts extend to emerging regulated compounds, such as perfluorohexanesulfonic acid (PFHxS) and perfluorobutanesulfonic acid (PFBS), aligning with shifting regulatory frameworks. Reaction pathways and fluorine fate are under investigation to clarify degradation mechanisms. The process demonstrates pronounced sensitivity to temperature and oxidant concentration, underscoring avenues for optimization. These outcomes position SCWO as a sustainable, scalable alternative to incineration and landfilling, offering a transformative approach to remediating PFAS-contaminated water. Ongoing research targets energy efficiency and field-scale deployment, advancing SCWO's practical utility.

6.05.P-Tu-114 Surface water quality for a proposed two-stage ditch program, Upper Cache River Watershed, Arkansas

Tanvir Hasan Kajol¹, Amelia Atwell² and Jennifer Bouldin¹, (1)Arkansas State University, (2)Wofford College

Agricultural runoff degrades water quality and causes eutrophication in the Gulf of Mexico (GOM), with the Upper Cache River Watershed (UCRW) in Arkansas contributing nutrients to the lower Mississippi River. The Arkansas Department of Environmental Quality has identified streams in the UCRW as nutrient runoff sources. Two-stage ditches (TSDs) are being constructed in channelized agricultural streams to mitigate this issue and improve water quality. TSDs are a unique floodplain restoration management strategy that play a role in treating agricultural runoff, reducing nutrient, sediment exports, reduce turbidity, and total suspended solids (TSS) from watersheds on a much larger scale than conventional trapezoid-shaped ditches, and develop better habitat for improved water quality. The Nature Conservancy is installing TSDs in the UCRW (Stratton Ditch, Greene County, AR) to be monitored prior, during, and after construction to determine the efficacy of the TSD system in reducing nutrients (total nitrogen, TN; total phosphorus, TP; Nitrate, Nitrite, Orthophosphate), TSS, Turbidity. The UCRW's East Slough (EASL) and Big Ditch Creek (BCDI), two sub-watersheds that were previously monitored, have total TN, TP, and turbidity levels that exceeded state standards. The previous three years sampling period revealed that at EASL, the site had a mean turbidity of 231 ± 22.3 NTU during primary flow events, exceeding the 75 NTU limit 82% and in storm flow events, the mean of 665 ± 122 NTU, exceeded the 250 NTU limit 59% of the time during the three years sampling period. Additionally, TP concentrations in BCDI were more than four times greater than the state standard, and TN values were much greater than in any other site sampled for the UCRW. In addition to measuring water quality data, monitoring sediment toxicity will be used to identify if sediment-bound contaminants are present prior to construction. Total lead and dissolved lead will also be monitored to determine the extent to which toxic levels are present. The goal of installing TSD systems in UCRW's channelized Stratton Ditch will help to understand the effectiveness of the TSD system on overall water quality improvement for future implementation on a larger scale, and it could be a solution to reducing nutrient and sediment loads from the lower Mississippi River Basin Watersheds, which are primarily responsible for nutrient loads entering GOM. **Disclaimer/Disclosure:** Cache River Watershed Monitoring for Two-stage Ditches, Project- GHP-22-100

6.05.P-Tu-115 Prediction of monthly rainfall erosivity factor (R Factor) for soil erosion using quadratic regression models under climate change scenarios in South Korea

Minseok Park, Yonghwee Seo, Jinsol Hong, Kijong Cho and Seunghun Hyun, Korea University, South Korea

Rainfall erosivity (R-factor) refers to the potential of rainfall to cause soil erosion. Due to the requirement of high temporal resolution data, the conventional EI30 method is limited to apply for predicting R factor under future climate change scenario. In addition, intense and erratic rainfall patterns under climate change make R-factor prediction difficult. This study aimed to 1) develop a quadratic regression model (QRM) to estimate the monthly R factor using daily rainfall data in South Korea and 2) apply the model to predict R factor under three climate scenarios (SSP1-2.6, SSP2-4.5, and SSP5-8.5) across the country using IDW method. Hrly rainfall data (1973-2007) collected at 58 weather stations and future daily rainfall data (2031–2100) simulated by 3 climate scenarios were utilized in this study. For model development, a subset data of 1973-2002 (n=262,800) was used and two key predictors (day10 and rain10) were extracted for building the QRM. Another subset data of 2003-2007 (n= 38,325) was used for validation of R-factor predicted by the QRM. As a result, the predicted monthly R-factors were statistically well correlated (mostly, $R^2 > 0.71$) with result of conventional EI30 method, representing the reliability of the QRM. Moreover, result of spatial distribution across the country (2031-2100) simulated by IDW method revealed that R factor will be substantially higher in the central-western inland and southwestern coastal regions of the country. The QRM is a simple and reliable method for estimating monthly R factor. Predicting future R-factor enables us to identify vulnerable areas and months and aids in designing proactive management strategies to mitigate soil erosion risk under climate change. **Disclaimer/Disclosure:** This study was supported by the Korea Environment Industry & Technology Institute (KEITI) through Climate Change R&D Project for New Climate Regime (RS-2022-KE002294)

6.05.P-Tu-116 Apples to oranges? Testing whether an equation used to estimate concentrations of aquatic microorganisms can be applied to calculate abundances of suspended microplastics

Anne L Gilewski, J. Evan Ward, Sandra Shumway and Bridget Holohan, University of Connecticut

A major challenge in marine microplastics (MP) research is the heterogeneous nature of plastic particles and their distribution and abundance in the water column. Two recently published papers enumerated environmental MP in seawater by filtering water samples, counting a subset of MP on each filter, and extrapolating total MP abundance using an equation originally developed for the “epifluorescence enumeration of microorganisms”. Microplastic particles and bacterial cells have different densities and morphology, leading to questions regarding whether they can be counted similarly. To assess the validity of this approach, a laboratory study was conducted that reproduced the methodology with known concentrations of MP. Using 6 µm and 45 µm fluorescent polystyrene microspheres (Fluoresbrite®, Polysciences, Warrington, PA 18976), two concentrations were evaluated: 30 particles and 1000 particles L⁻¹. Following the authors’ methodology, comparisons were made between concentrations obtained from counting selected regions of interests (ROI) and applying the microorganism calculation, and full-filter counting. Several technical issues arose during the study including significant clumping of MP at the 1000 particles L⁻¹ concentration, and long (> 48 h) filtration time when using the suggested AnodiscTM/nitrocellulose filter combination even after the 100-µm pre-filtration step. Technical issues aside, results demonstrated that MP concentrations based on counting ROI and microorganism calculation were several orders of magnitude greater than concentrations based on whole filter counts. Although these studies attempted to estimate MP of smaller size in the field (~1-12 µm and ~1-43 µm, respectively), the method should be applicable to any MP size. Based on the current laboratory study, estimations of environmental MP concentrations by counting ROI and extrapolating with the microorganism calculation yield greatly overestimated values and, therefore, should not be used in further studies where the accuracy of environmental MP concentrations is a priority.

6.05.P-Tu-117 Sustainable water treatment for underserved areas: Biochar vs. activated carbon in the gift of water filter

Jeantel Cheramy, Andrew L Rainey, Andrew R. Zimmerman and Maurelli Thomas, University of Florida

Access to clean drinking water remains a fundamental human right, yet many communities continue to face serious threats from contamination by heavy metals such as lead (Pb), arsenic (As), copper (Cu), iron (Fe), and cadmium (Cd). Industrial discharge, agricultural runoff, and improper waste disposal contribute significantly to the presence of these toxic elements in water supplies. Once introduced, these metals persist in the environment, accumulate in the human body, and cause long-term health issues, including neurological damage, kidney dysfunction, developmental delays, and cancer. This study investigates whether the Gift of Water (GOW) filtration system, a low-cost, point-of-use water treatment system originally designed for microbial removal, can also effectively reduce heavy metal concentration. The study aims to identify affordable filtration media that can improve water safety in underserved regions. To evaluate this, we compared activated carbon (AC) and biochar (BC) as filtration materials. We spiked tap water with Pb, As, Cu, Fe, and F at 130% of the U.S. EPA's Maximum Contaminant Levels (MCLs). We then filtered the water through the GOW system using either AC or BC and analyzed the samples using Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). Our results showed no significant difference between AC and BC in removing Fe, Cu, and Pb. However, BC removed arsenic more effectively ($p = 0.0193$). Both filtration media reduced all tested metals by over 90% after filtration, but BC demonstrated comparable or superior performance at a lower cost. These findings position biochar as a sustainable, cost-effective filtration alternative for decentralized water treatment. Integrating biochar into systems like the GOW filter can help underserved communities reduce heavy metal exposure and improve access to safe drinking water.

6.05.P-Tu-118 Synthesis and evaluation of zirconium-based metal-organic frameworks (MOFs) adsorption efficiency for remediation of PFAS-impacted groundwater

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Per- and polyfluoroalkyl substances (PFAS) pose significant environmental challenges due to their persistence in ecosystems and potential adverse effects on human health and wildlife. Contaminated groundwater resources are of particular concern as they serve as vital drinking water sources. This study focuses on synthesizing and assessing zirconium-based MOFs as efficient adsorbents for the remediation of PFAS-impacted groundwater. In this study, we proposed to synthesize fluorinated ligands with suitable frameworks for new MOFs that can bind and remove PFAS. We have also chosen to explore the potential of the commercially synthesized zirconium-based MOFs (MOF-66, MOF-67, and MOF-74), which are known for their high surface area and chemical stability. Characterization of the synthesized MOFs was performed using X-ray diffraction (XRD), scanning electron microscopy (SEM), and Brunauer-Emmett-Teller (BET) surface area analysis to confirm their structural integrity and porosity. The adsorption efficiency of the MOFs was assessed through a series of batch experiments using model PFAS, including PFOS, PFOA, 5:3 FTCA, 6:2 FTS, PFHxA, PFHxS, PFBS, and GenX, to investigate their interactions with the MOF materials. Quantification of PFAS concentrations was performed using Agilent liquid chromatography-tandem mass spectrometry (LC-MS/MS). We explored initial contact time (4-30 hr), initial PFAS concentration (5ppm-5ppb), and pH (3-10) to elucidate the kinetics and isotherm models governing the adsorption processes. The results from this study will demonstrate whether zirconium-based MOFs exhibit remarkable adsorption capacities for different PFAS classes and their potential as effective remediation technologies. The study will not only elucidate the adsorption kinetics and mechanisms involved but also provide insights into the applicability of MOFs in groundwater remediation.

6.05.P-Tu-119 Assessing strategies for antibiotic removal from wastewater using quantitative mass spectrometry

Kerry Jacobs, Erin Trathen, Matthew Falkner, Tala Navab-Daneshmand, Gerrad Jones and Manuel Garcia-Jaramillo, Oregon State University

Antibiotic resistance poses a major threat to human health as the abundance of antibiotic-resistant bacteria

increases globally. Wastewater poses a unique opportunity for confluence of antibiotics in the urine and the bacteria from human waste and other biotic sources. As a result, wastewater treatment plants (WWTPs) aim to remove antibiotics from wastewater to reduce the opportunity for creation of antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARBs), also known as “superbugs”. Existing wastewater treatment methods remove some antibiotics, but many are still found at high enough concentrations in effluent to trigger the creation of ARGs. Identifying the type of wastewater treatment processes that remove antibiotics most efficiently can inform other WWTPs on which processes to include that will address the issue of antibiotic prevalence in their effluent. In this study, influent, intermediate effluent, final effluent, and solids were collected from 40 WWTPs across the country, extracted using solid phase extraction, and analyzed using a targeted approach on a high-resolution mass spectrometer. Thirteen common antibiotics before and after a variety of wastewater treatment processes were quantified, and additional antibiotics annotated using open-access mass spectral libraries. By comparing the concentrations or relative abundance of each antibiotic before and after the surveyed treatment processes, the efficiency of these treatment methods can be tested and communicated to WWTPs globally.

6.05.P-Tu-120 Implications of USEPA January 2025 inorganic arsenic toxicity value update on site screening and remediation

Chrissy Peterson, Dana McCue, Mayble Abraham and Kailey Brady, EHS Support LLC

Arsenic is naturally occurring and ubiquitous in the environment; however, arsenic is also used in manufacturing and processing. Because of this widespread natural and anthropogenic occurrence, arsenic is frequently identified as a constituent of concern for contaminated sites. Risk assessments of contaminated sites involve evaluation of chemical concentrations through screening and risk characterization activity. Human health risk-based screening levels (RBSLs) are developed using toxicological values and are used for comparison to Site concentrations. Toxicological values are also used in risk characterization which identifies potential unacceptable levels of risk to human receptors that might require land use restrictions or remediation efforts. United States Environmental Protection Agency released an update to the toxicological assessment of inorganic arsenic (CAS Number 7440-38-2) in January 2025. New developments in epidemiological research resulted in revision to the non-cancer and cancer oral toxicity values. The revised toxicological values are several orders magnitude lower than previously published toxicity values used for risk characterization. Due to the toxicological value update, RBSLs are decreased and quantified cancer risks and noncancer hazards increased at the same order of magnitude. This presentation will discuss implications of the revised inorganic toxicity values on site remediation, the increased importance of developing site-specific background concentrations, and a case study evaluating remediation alternatives based on RBSLs and site-specific background values.

6.05.P-Tu-121 Evaluating sediment basins for pyrethroid and *Hyaella azteca* toxicity removal in agricultural runoff

Berkley N. Anderson, Marie Garcia and Thomas Michael Young, University of California, Davis

Agricultural runoff containing sediment-bound pyrethroid insecticides can be transported to nearby waterbodies and adversely affect receiving ecosystems. Sediment basins can be used as a best management practice (BMP) to remove such hydrophobic particle-bound contaminants by allowing particles to settle. The purpose of this study was to evaluate the efficacy of sediment basins for pyrethroid and *Hyaella azteca* (*H. azteca*) toxicity removal using large scale settling column experiments. Agricultural runoff samples were collected from four locations in Monterey County, CA. Large scale settling column experiments were conducted to assess pyrethroid removal after 0-, 6-, and 24-hr settling timepoints. *H. azteca* survival at each timepoint was measured using 96-hr toxicity tests and three toxicity methods were employed to assess different exposure pathways and pyrethroid phases inducing toxicity. At field relevant holding times/basin depths pyrethroid removal ranged from 74.0% to 98.8% and significant increases in *H. azteca* 96-hr survival were observed. The results of this study support sediment basins as a promising solution to reduce pyrethroid concentrations and their associated

toxicity in agricultural runoff. The prospects, challenges, and potential next steps in the application of sediment basins for pyrethroid removal will be presented.

6.05.P-Tu-122 Source tracing phosphorus laden sediments in the hyper-eutrophic Upper Klamath and Agency Lakes

Corey De la Cruz, Daphne Siyuan Guo and Gerrad Jones, Oregon State University

The Upper Klamath and Agency lakes (UKL) located in Southern Oregon suffer from frequent hyper-eutrophic conditions driven by excessive phosphorus, which impairs water quality threatening both human and fish populations. Excessive phosphorus in the lake promotes large-scale blooms of *Aphanizomenon flos-aquae* and microcystin hepatotoxin producing *Microcystis aeruginosa* cyanobacteria. Severely reduced water quality has increased mortality rates of endangered juvenile Shortnose (*Chasmistes brevirostris*) and Lost River (*Deltistes luxatus*) suckers along with threatening human beneficial use of the lake waters. Over the past 20 years federal and state agencies, along with local stakeholder groups, have implemented various management strategies to achieve the proposed 40% reduction target for external phosphorus loading set by the Oregon Department of Environmental Quality (ODEQ). However, excess phosphorus concentrations remain a persistent issue in UKL and only 10% total phosphorus loading reduction has been successfully achieved to date. Phosphorus has a high affinity to clay, and therefore identifying the dominant sources of phosphorus laden sediments is essential for understanding and managing its transport to Upper Klamath Lake. We will utilize a combination of multi-dimensional analysis techniques, including machine learning algorithms, with elemental mass spectrometry fingerprints of sediments in the UKL and its tributaries to forensically trace phosphorus sources. This source identification will support more targeted and effective strategies to reduce sediment-bound phosphorus loads and improve water quality in the lake.

6.06.P-Th Late-Breaking Science: Engineering, Remediation and Restoration

6.06.P-Th-071 Development of a lead background concentration at the westside lead Superfund site

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Lead is a toxic element that can be prevalent in the environment due to its historical use in paints and leaded gasoline, its release from industrial processes (e.g., smelting, waste incineration), and its natural occurrence in geological formations. In 2024, the U.S. Environmental Protection Agency (EPA) reduced the Regional Screening Level for lead in residential soil from 400 parts per million (ppm) to 200 ppm. The lower screening level may exceed anthropogenic background concentrations of lead in urban soils. U.S. EPA Region 4, Research Triangle Institute (RTI) International, and Geosyntec Consultants conducted an urban background study to evaluate lead concentrations near the Westside Lead Superfund Site in Atlanta, Georgia. The study was conducted by collecting 30 incremental samples using the incremental sampling methodology (ISM). Samples were collected from randomly selected locations within a 5-mile by 5-mile grid, homogenized in the field, and analyzed using 1) a handheld X-ray fluorescence (XRF) device and 2) in an analytical laboratory using the U.S. EPA Method 6010D. Lead was detected in 100% of samples. XRF concentrations ranged from 29.1 to 436.3 ppm (geometric mean: 80.9 ppm), and analytical laboratory concentrations ranged from 15.0 to 300.0 ppm (geometric mean: 51.3 ppm). The average background lead concentrations in Atlanta's urban soils were less than the updated lead Regional Screening Level. XRF results were highly correlated with laboratory results ($R^2 = 0.99$) but were systematically higher. This discrepancy may be due to differences between analytical instruments, as XRF measures total metals, while laboratory methods measure acid-digestible fractions. Statistical analysis demonstrated that lead datasets followed lognormal distributions. Removing outliers reduced variability and lowered estimated background levels. The 95-95 Upper Tolerance Limit (UTL) for background lead was 252.8 ppm, based on laboratory data, decreasing to 204.9 ppm after excluding one outlier. For XRF data, the 95-95 UTL was 346.5 ppm, decreasing to 280.4 ppm after removal of the outlier. These are both

greater than the new Regional Screening Level (200 ppm). These findings highlight the importance of incorporating background studies when establishing cleanup goals, as naturally occurring or diffuse anthropogenic inputs may have contributed to widespread urban impacts that are unrelated to releases impacting a site.

6.06.P-Th-072 Evaluating selenium bioaccumulation and the efficacy of phytoremediation in an industrial wetland

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Selenium is a natural, bioaccumulative element that is essential for biological life. When present in excess, however, selenium has the potential to pose risk to fish and birds. Additionally, some species of selenium (i.e. selenomethionine) are more bioaccumulative than others. Since selenium bioaccumulates, one potential solution for excess selenium removal is phytoremediation (i.e. using plant life to remove pollutants). We conducted a pilot-scale investigation of selenium phytoextraction in an industrial wetland by periodically harvesting and removing Phragmites, which was already established in the area. In addition to assessing selenium removal, we also studied preferred uptake pathways by comparing water-to-plant and soil-to-plant bioaccumulation for selenium and its species. The wetland was divided into four areas which varied in Phragmites coverage and density. We found that Phragmites could effectively remove selenium, with preferential removal of more bioaccumulative selenium forms. However, the amount of selenium that could be removed through Phragmites harvesting varied substantially among study plots, with greatest effectiveness in areas with tall, dense vegetation and higher aqueous selenium concentrations. Selenium concentrations in Phragmites were more closely linked to concentrations in water than to concentrations in sediment, suggesting that sediments are not the primary source of selenium to Phragmites and the food web in this system. However, a more detailed understanding of mass balance would be needed to predict the extent to which a scaled-up phytoremediation effort would mitigate selenium bioaccumulation. Considering that Phragmites is an invasive species, its periodic harvest could potentially have additional benefits such as reducing seed dispersal from the area. Taken together, our findings support phytoextraction via available plant life as a potentially useful remedy component for selenium removal in dense Phragmites wetlands.

6.06.P-Th-073 Supplemental contaminant source and creek sediment remediation at a previously remediated Superfund site, Pennsylvania, U.S.A.

William J Reese III and Nick Rashid, AECOM

The Whitmoyer Laboratories Superfund Site (Myerstown, Pennsylvania) is a former veterinary pharmaceutical manufacturing facility. The on-site area covers approximately 22 acres and is traversed by Tulpehocken Creek and a portion of the Union Canal. The principal contaminant of concern is arsenic. In 2002, the Site was remediated, which included excavation and off-site disposal of soil impacted with high concentrations of arsenic, consolidation (under the cap) of soils with lower arsenic impacts, construction of a 2-foot thick permeable soil cap to prevent direct contact with residual subsurface arsenic-impacted soils, institutional controls (deed restrictions), and hydraulic control and treatment of impacted groundwater. Routine post-remedy sediment monitoring revealed an area below the canal-creek confluence with elevated arsenic levels in the surficial creek sediment. It is speculated that this area of elevated arsenic in sediment was either overlooked during the original remedial investigations, due to spatial scale of sediment data collected, or reflects newly contaminated sediments due to potential uncontrolled source(s) of arsenic loading to the creek in this specific area. Supplemental sediment investigations were performed between 2017 and 2019 to better understand the horizontal and vertical extent of the impacted sediments. Numerous contemporaneous investigations revealed that overburden shallow groundwater with elevated arsenic concentrations has the potential to seasonally discharge to the creek from the right (southern) bank at this specific area of interest, a former soil hot spot area, as overburden groundwater was not fully controlled by the bedrock pumping wells of the on-site groundwater recovery system. To address this newly identified source of arsenic to Tulpehocken Creek, an overburden

groundwater interceptor trench system was constructed in 2023. Additionally, remedial alternatives are being evaluated to remove this area of contaminated sediments from the creek, cap the underlying impacted soils, and reconstruct the creek bed to mitigate the potential for erosion and recontamination of the new cap. This presentation will focus on the means and methods used to locate and delineate the area of contaminated creek sediment and determine the supplemental remediation required, including the overburden groundwater interceptor trench system as well as the pending streambed remedial work, at this previously remediated Superfund site.

6.06.P-Th-074 Efficient and sustainable removal of perfluorocarboxylic acids in water using UV-activated nZVI-N-biochar

Angely Irizarry Chico, Sivasankar Annamalai and Sangchul Hwang, Texas State University

Per- and polyfluoroalkyl substances (PFAS) have become a major global environmental challenge due to their extensive use, environmental persistence, bioaccumulative potential, and associated risks to human and ecological health. Addressing PFAS contamination requires not only effective removal but also sustainable remediation technologies to prevent secondary pollution. Over the past decade, a wide range of PFAS destruction strategies have been explored, including bioremediation, sonochemical treatment, high-temperature incineration, smoldering, mechanochemical degradation, electron beam irradiation, gamma irradiation, chemical reduction, and advanced oxidation processes. Despite these efforts, many existing approaches face limitations related to high energy demands, incomplete mineralization, or the generation of harmful byproducts. In this study, an environmentally friendly and cost-effective technology was developed for remediating perfluorocarboxylic acids (PFCAs), a major subclass of PFAS that includes compounds such as perfluorooctanoic acid (PFOA), perfluoroheptanoic acid (PFHpA), perfluoropentanoic acid (PFPeA), and perfluorobutanoic acid (PFBA). To achieve this, water hyacinth biomass was employed as a sustainable precursor to synthesize a core-shell structured, nitrogen-doped biochar loaded with nanoscale zero-valent iron (nZVI-N-WHBC800). The synthesis involved a Mannich reaction followed by high-temperature carbon reduction, resulting in a multifunctional material with strong adsorption capacity and catalytic activity. The nZVI-N-WHBC800 composite was tested as both an adsorbent and a catalyst in the UV-assisted degradation of PFCAs. Experiments were conducted using an initial concentration of approximately 800 µg/L of mixed PFCAs under various treatment conditions. The results indicated that the removal percentage of PFCAs was calculated to be <2%, 13%, and 76% for the control, nZVI-N-WHBC sorption, and UV/nZVI-N-WHBC800 reduction reactions, respectively. These findings demonstrate that the nZVI-N-WHBC800/UV system is an efficient, low-cost, and environmentally sustainable technology for PFCA remediation.

6.06.P-Th-075 Nanosized manganese dioxide catalysts for fenton-like oxidation of dichloroethanes and 1,4-dioxane

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This study investigates a modification to conventional Fenton-based in situ chemical oxidation (ISCO) for groundwater remediation, targeting chlorinated solvent co-contaminants that persist under standard treatment. The degradation kinetics of 1,4-dioxane, 1,2-dichloroethane (1,2-DCA), and 1,1-dichloroethane (1,1-DCA) were evaluated using nanosized manganese dioxide (nMnO₂) as a replacement catalyst for Fe(II) in hydrogen peroxide (H₂O₂) reactions. Unlike Fe(II), which is limited by narrow pH requirements and solubility constraints, nMnO₂ catalyzes hydroxyl radical production over a broader pH range while remaining stable in suspension. Stirred batch reactors were utilized to study the kinetics of 1,4-dioxane and DCA degradation under various suspension conditions. Batch experiments demonstrated that low doses of nMnO₂ (~0.02 g/L), Beta- or alpha- phase variants, high H₂O₂ concentrations (>100 mM), and near-neutral pH (5–10) promoted effective 1,4-dioxane degradation. Under these conditions, 100 ppb 1,4-dioxane was completely removed within 1.5 days, though re-spiking of H₂O₂ was sometimes required. Glycolate and formate were identified as partial oxidation products, accounting for ~10% of carbon balance. Common groundwater constituents such as

bicarbonate, natural organic matter, and sediments did not inhibit degradation. While phosphate slowed reactions by sorption to nMnO₂, it extends H₂O₂ persistence. Both DCAs showed limited degradation, so more work is being done to understand the mechanisms of their degradation. Flow-through column experiments with beta-nMnO₂-coated sand and H₂O₂ achieved complete 1,4-dioxane removal to below detection limits within ~2 hr. The short time in columns may be due to rapid and abundant reactive oxygen species generated by the high density of nMnO₂ in small volume pore water. However, 1,2-DCA exhibited limited degradation under the tested conditions. These findings suggest that nMnO₂ offers several advantages over Fe(II)-driven Fenton chemistry, including catalytic activity across a wider pH range, resistance to dissolution or precipitation, and potential for placement in subsurface barriers. While the nMnO₂-H₂O₂ system effectively mineralizes 1,4-dioxane at environmentally relevant concentrations, further experimental work is needed to confirm applicability to the DCAs. Further work will also focus on kinetic modelling and flow-through column experiments, specifically with particle transport and dispersion.

7.01.P-Mo Indigenous Knowledge and Chemicals Management

7.01.P-Mo-92 Game-based learning for tribal youth: Understanding toxics risks through first foods

Nadine French, *Negonnekodoqua Blair and Mason Murphy, Confederated Tribes of the Umatilla Indian Reservation*

Communicating complex environmental science to young audiences is essential for cultivating the next generation of Tribal environmental stewards. The Confederated Tribes of the Umatilla Indian Reservation (CTUIR) are developing an educational game focused on bioindicators and toxics in aquatic food webs to engage middle and high school students. This interactive, modular game introduces key concepts such as food web dynamics, ecological indicators, and the direct and indirect impacts of toxics on culturally significant species, including salmon, lamprey, and freshwater mussels. The game design process is grounded in collaboration with Tribal educators, natural resource staff, and cultural knowledge holders to ensure alignment with CTUIR values and learning goals. Gameplay modes support both collaborative and competitive learning and are adaptable to classrooms, after-school programs, and community events. Planned evaluation will gather feedback from student participants and educators to assess engagement, comprehension of key concepts, and cultural relevance. By bridging technical science and culturally grounded knowledge, this effort supports environmental education, youth empowerment, citizen science, and community engagement in CTUIR's toxics reduction and river restoration initiatives.

Disclaimer/Disclosure: Negonne Blair is submitting the abstract on behalf of Nadine French, lead author, undergraduate at the University of Montana- Western. Student email: nadinefrench@ctuir.org Nadine's work is supported by the US EPA grant 44-02J37501-0

7.01.P-Mo-099 Using CTUIR cultural literature to guide toxics risk prioritization in the Umatilla River subbasin

Ben Todd¹, **Nadine French²**, *Negonnekodoqua Blair² and Mason Murphy², (1)Whitman College and the Confederated Tribes of the Umatilla Indian Reservation, (2)Confederated Tribes of the Umatilla Indian Reservation*

Mainstream chemical risk assessments rarely reflect the values, lifeways, and ecological relationships of Tribal communities to First Foods. To address this gap, the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) are developing a Toxics Strategic Plan grounded in First Foods principles, culturally defined health, and place-based stewardship. This project synthesizes CTUIR cultural literature - including the Umatilla River Vision, Uplands Vision, oral histories, and natural resources management documents - to construct a values-based framework for evaluating environmental risk. The framework identifies key priorities such as ecological integrity, ceremonial use, and intergenerational responsibility. We apply this framework to assess the relevance and limitations of existing scientific data and environmental regulatory practices in the Umatilla River subbasin. This approach highlights critical data gaps and establishes a path forward for toxics reduction actions and the

development of a CTUIR Toxics Inventory that reflects Tribal priorities and informs future monitoring, risk assessment, and policy decisions grounded in Tribal knowledge, sovereignty, and environmental management. **Disclaimer/Disclosure:** This work is supported by the Whitman College Internship Grant and the USEPA grant 44-02J37501-0

7.02.A.T Inspiring Next Generation of Young Scientists - High School Students Share Their Research

7.02.A.T-01 San Diego Bay water quality risk assessment: Persistent organic pollutants analysis in a hands-on research project

Miguel F Vasquez¹, Damian Shea², Claire Wang¹ and Goran Bozinovic^{1,3}, (1)Boz Life Science Research and Teaching Institute, (2)Statera Environmental Inc., (3)University of California, San Diego

Water quality chemistry provides pollution assessment and an indicator for the test environment's general ecological risk and trends. Pollution comes from many sources and chemical pollution is not restricted to current or recent use chemical infiltration with the prevalence of persistent pollutants. Notably, persistent organic pollutants (POPs) such as pesticides and plasticizers from human sources and activity can make their way through waterways, contaminating the environment from source to destination. The persistence of pollutants varies on the environmental conditions and the current form of these persistent pollutants, whether as metabolites, by-products, or reacted species. Mitigation of a large-scale problem also requires educating and engaging the surrounding community and young students to inform on status, reduce current risk, and continue future monitoring efforts. San Diego Bay is a busy, shallow water bay next to the second largest city in California, shipbuilding industries, naval bases, and lies just north of the Tijuana River Estuary outlet into the Pacific Ocean. This combination exposes the bay to many types of pollutants, chemical and biological. Using novel composite integrated passive samplers (CIPs) designed to capture polar and non-polar organic pollutants, local high school students and researchers deployed these samplers across the bay to assess current use and legacy chemicals. Additionally, students and researchers collected biological samples from bivalves and marine mammals from the bay to compare bioaccumulation data of organic pollutants with the specificity and results from the CIPs data at each site. Participating students learned not only the experimental side of environmental toxicology but also science communication and presenting data to the public through a complete educational program designed to provide students with real-world research alongside science professionals.

7.02.A.T-02 High school students investigate sediment toxicity in the Tijuana River Estuary using fish embryo morphology and microbiome sequencing

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Pollution in the Tijuana River Estuary (TRE) jeopardizes both the abundant wildlife and surrounding human communities. In a collaborative education and research initiative, high school students work alongside researchers to investigate the biological impacts of pollutants in the TRE by examining the effects of estuarine sediment on Japanese medaka (*Oryzias latipes*) embryos. Students participated in all stages of the scientific process: designing exposure experiments, collecting morphological and developmental data, and analyzing their findings. Sediment collected near the U.S.-Mexico border was elutriated in 15 ppt saltwater and used to expose medaka embryos to a range of concentrations under controlled laboratory conditions. Students monitored survival, heart rate, developmental staging, and morphological endpoints at two critical timepoints during heart development: three days post-fertilization (dpf) - when the sinus venosus, atrium, ventricle, and bulbus arteriosus differentiate, and seven dpf - when the pericardial sac forms, marking the final stage of heart development. Students also analyzed data from 16S rRNA sequencing of sediment samples to explore how microbiome composition may be associated with pollution. Through this project, students not only gain theoretical and practical knowledge of experimental design, embryology, microscopy, environmental toxicology, microbiology, and data analysis, but also develop a personal understanding of the environmental

challenges in their communities. As inhabitants of San Diego, a region directly impacted by the pollution of the Tijuana River Estuary, they are able to comprehend the gravity of ongoing transboundary pollution issues and see firsthand how science can be used as a tool to investigate complex environmental problems with local relevance. By contributing to real-world investigations of pollution in the Tijuana River Estuary, students contributed meaningful data to environmental monitoring efforts. This program inspires the next generation of young scientists by immersing precollege students with little research experience in every step of the scientific process while fostering community involvement in environmental protection.

7.02.A.T-03 Organic pollutants and gut metagenomes of mesopelagic fish: A search for toxicant bioindicators in the deep ocean

Claire Willaredt^{1,2}, **Zuying Feng**^{1,2}, **Damian Shea**³ and **Goran Bozinovic**^{1,2}, (1)Boz Life Science Research and Teaching Institute, (2)Portland State University, (3)Staterra Environmental Inc.

While humans are vastly unfamiliar with the deep ocean, their industrial activities generating and depositing persistent organic pollutants in the ocean have already adversely affected marine ecology and biodiversity. Using high-performance gel permeation chromatography and gas chromatography–mass spectrometry, community college and university undergraduate students from Portland area detected a wide range of persistent organic pollutants analyzing livers of fish living hundreds of meters deep near the Oregon coast. Besides chemical contaminants ‘derivatives, students identified 12 organochlorine pesticides and PCBs which had been widely phased out since 2004 internationally. While the production of these legacy toxicants is restricted, they persist in marine environment because of their characteristic biotic and abiotic degradation resistance. Expectedly, liver toxicant concentrations were higher in lanternfish (*Stenobrachius leucopsarus* and *Lampanyctus tenuiformis*), likely due to their vertical migration and exposure to greater toxicant concentration near the surface. This data will complement a more comprehensive assessment of several more deep-sea fish families and correlate liver body burdens to intestinal microbial composition and expression patterns of genes regulating critical enzymes implicated in metabolic pathways and detoxification mechanisms.

7.02.A.T-04 Interactive effects of dietary perturbation and thermal stress

Benjamin Deviney¹, **Jenna Nicole Jedlicki**^{1,2,3} and **Goran Bozinovic**^{1,2}, (1)University of California, San Diego, (2)Boz Life Science Research and Teaching Institute, (3)San Diego State University

The forefront of scientific research is constantly addressing unanswered questions which require people to carefully design and execute experiments to infer biological and environmental relevance. To better prepare the next generation of researchers, we involve undergraduates directly in our research, catalyzing their scientific interest and critical thinking skills. Climate change is transforming the face of our planet, increasing temperatures and the severity of weather events. During droughts, food availability may be reduced, altering diets. We characterized the interaction of thermal and dietary stressors by exposing fruit flies to elevated temperatures and an unbalanced diet and measured lifespan and locomotor ability to identify physiological outcomes. To better understand metabolic shifts governed by altered gene expression and microbial diversity alterations during heat and dietary stress, we correlated whole-body 16s rRNA microbial sequencing data with female and male fly head gene expression quantified with 3'-end shallow RNA sequencing. By involving undergraduates in research, firsthand, a seed of curiosity is planted. Instead of performing an experiment where the answer is already known, they are required to come up with and test hypotheses. Undergraduates learn to think critically about confounding factors when learning experimental design. This active-learner approach fosters long-term scientific curiosity, maturing into inquisitive research, healthy skepticism, and responsible environmental stewardship.

7.02.A.T-05 Ecological risk assessment research immersion program improves students' science literacy and STEM aptitude

Maysoon Lehmeidi Dong¹, **Zuying Feng**², **Miguel F Vasquez**^{1,2} and **Goran Bozinovic**^{1,2}, (1)University of California, San Diego, (2)Boz Life Science Research and Teaching Institute

The Tijuana River Estuary (TRE), the largest coastal wetland in Southern California, has long been a focal point of transboundary pollution concerns between the United States and Mexico. To address this issue through community engagement and secondary education, the Boz Life Science Research and Teaching Institute and the University of California Division of Extended Studies launched research immersion programs for pre-college students to conduct comprehensive environmental health assessments. High school students collected sediment and water samples from four locations during both dry and wet seasons to conduct sea urchin embryotoxicity testing. The chemical composition of these samples was analyzed using both qualitative non-target and quantitative targeted methods via GC-MS and LC-MS/MS. Additionally, novel Composite Integrative Passive Samplers (CIPS) were deployed to measure time-weighted average concentrations of polar and non-polar contaminants. 16S rRNA sequencing was employed to explore correlations between microbial diversity and chemical pollution. Throughout the program, students engaged in the full scientific process—interpreting literature, formulating hypotheses, designing and executing experiments, and applying biostatistics and bioinformatics to interpret results and assess biological significance. The program culminated in a symposium where students presented their findings to scientists, educators, policymakers, and industry professionals. Beyond identifying public health risks and informing policy, the overarching goal of both programs is to build a foundation for long-term international collaboration and student exchange, empowering the next generation of STEM leaders and environmental stewards.

7.02.A.T-06 A pre-college research experience: Quantifying chronic pollution effects on dolphins from San Diego Bay via chemical profiling, gene expression and microbial sequencing

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Pre-college students engaged in applied molecular biology course investigating the impact of long-term exposures to environmental pollutants on bottlenose dolphins (*Tursiops truncatus*) housed by the U.S. Navy Marine Mammal Program in San Diego Bay. This unique dolphin population, living in netted ocean enclosures closely monitored with consistent diet and healthcare, provides a model for studying chronic chemical exposure under relatively controlled conditions. Students participated in the analysis of environmental contaminant profiles collected using Composite Integrative Passive Samplers (CIPS) and chemical body burden data from mussels collected at the same sites. In the lab, students extracted RNA from archived dolphin liver samples, comparing gene expression profiles between younger and older dolphins to quantify long-term pollutant exposure effects at the molecular level. Liver and blubber samples were also analyzed for body burdens of persistent organic pollutants, including PCBs, PAHs, pesticides, pharmaceuticals, and plasticizers. Students processed intestinal tissue biopsies for gut microbiome analysis using 16S rRNA sequencing, correlating liver body burdens to gut microbiome composition and expression patterns of genes regulating critical enzymes implicated in metabolic pathways and detoxification mechanisms. Throughout this experience, students experience every step of the scientific process, from interpreting primary literature, designing and performing experiments, to conducting data analysis, gaining insight to molecular biology laboratory techniques, environmental chemistry, and microbiology. Moreover, they get an opportunity to interact and network with science professionals from academic, government, and industry settings to better understand the different career paths relevant to their research immersion experience. This program allowed students to gain conceptual understanding, technical skills, and environmental awareness by conducting real-world investigations of pollution effects on marine mammals' health.

7.02.B.T Inspiring Next Generation of Young Scientists - High School Students Share Their Research

7.02.B.T-01 Exploring social effects on behavior and microbiome of fruit flies by high school students

Jenna Nicole Jedlicki^{1,2,3} and Goran Bozinovic^{1,3}, (1)Boz Life Science Research and Teaching Institute, (2)San Diego State University, (3)University of California, San Diego

Engaging high school students with research project-based curriculum promotes development of critical thinking skills, scientific literacy, and how to analyze and interpret data. Students developed a hypothesis and designed an experiment testing the sex-specific impact of social environment on *Drosophila melanogaster*, or the fruit fly. Two separate populations of 12 flies were housed in a chamber, with a mesh barrier preventing direct contact. Three scenarios were compared: 1) single-sex: each population consisting of the same sex 2) non-mating: separated female and male populations, and 3) mating: mixed sex populations. Flies were collected at three timepoints: 3 days (sexual maturation), 7 days (early adulthood), and 14 days (middle adulthood). DNA was extracted from the bodies of each group and sequenced using 16S rDNA sequencing to determine bacterial identity and abundance. From each group, two males were subjected to an aggression assay at the same three timepoints. Students collected data and used bioinformatic tools such as R to analyze and visualize data. Their findings include decreases in aggression in mating flies, and differences in bacterial composition among groups at days 7 and 14. By engaging in all parts of research, students developed a better understanding of the scientific method and learned to design and execute an experiment in its entirety. They will also develop critical communication skills through presenting their work to an audience of peers and science professionals.

7.02.B.T-02 Applied biostatistics and bioinformatics: Data science literacy improves STEM aptitude and environmental science research experience among pre-college students

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Biostatistics and Bioinformatics—the application of computational and analytical methods to biological problems—is based on collection, management, analysis, and interpretation of data in life sciences. The 11-week applied data research immersion introduces pre-college and college students with no prior computational or analytics experience to principles and applications of advances in sequencing technologies, genomics, transcriptomics, and structural informatics. Most analyses are performed in “R” programming language. Students’ experiences are structured on critical assessment of primary scientific literature, principles of experimental design, and utility of published peer-reviewed data sets emphasizing analytical applications in genomics and proteomics to infer biological, sequencing, and gene expression data relevance. This initial experience is followed by another 11-week session of applying learned skills to authentic research data-rich projects, with topics ranging from public health and epidemiology, developmental toxicogenomic, comprehensive non-targeted and targeted chemical assessment of persistent organic pollutants, to evolutionary comparative genomic analysis and regenerative molecular biology. We hypothesized that the same content can be delivered to pre-college, undergraduate, and graduate students and that prior coding or analytic experience is not necessary for students to succeed. With the sample size of over 700 students and analysis based on pre- and -post-course assessments, exams, confidence surveys, and ability to perform, interpret, and communicate results from multivariate statistics, principal component analysis (PCA), and RNA Sequencing, our data strongly suggest that biostatistics and bioinformatics should be introduced to STEM students as soon as possible, preferably during the high school formative academic experience. Such engagement builds confidence, academic resilience, and should positively correlate with pursuit of and retention in STEM Majors.

7.02.B.T-03 Effects of tourist season on sea water quality at the Croatian UNESCO heritage island of Hvar: Student STEM research immersion program promotes environmental stewardship and community outreach

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Croatian island of Hvar, a UNESCO Heritage site, is one of the most popular tourist destinations in Europe, characterized by an extensive coastline. Hence, the environmental impact of tourism on sea water quality is critical to assess. Composite Integrative Passive Samplers (CIPS) were deployed at 16 sites on the island by a team of four researchers and six local high school students. The team also collected bivalves and echinoderms from each site for bioconcentration and 16s rRNA microbial sequencing analysis. Chemical classes found both in CIPS and mussels included legacy chemicals like PCBs and pesticides (i.e. DDT), pharmaceuticals, stimulants, and steroid hormones. Local high school students, having no prior applied science experience, acquired research skills, developing community environmental awareness and presenting data publicly. Moreover, they are an important part of our analytical team, learning now to process, manage, and analyze data in R. Their experience, besides presenting some of this work at the recent SETAC Europe 2025 meeting in Vienna, will be to author their work in a reputable peer-reviewed environmental science journal.

Disclaimer/Disclosure: Milicic Foundation, UC San Diego Division of Extended Studies

7.02.B.T-04 Environmental research as a tool to promote STEM and data science literacy among pre-college students

Zuying Feng¹, **Maysoon Lehmeidi Dong²**, **Miguel F Vasquez¹** and **Goran Bozinovic^{1,2}**, (1)Boz Life Science Research and Teaching Institute, (2)University of California, San Diego

Engaging pre-college students in life science research traditionally done by graduate students, P.I.s and science professionals is critical for promoting and improving science literacy, environmental stewardship, and building competent leadership. For the past 6 years, Boz Life Science Research Institute and UCSD Division of Extended Studies have offered research immersion programs to high school students, most of whom had no practical science experience, based on comprehensive ecological risk assessment of the Tijuana River Estuary (TRE). We use an 11-weeks program model to engage high school students from various socioeconomic and academic backgrounds in all aspects of research experience: learning how to critically assess primary science literature, devise testable hypothesis and experiment design, execute statistically viable experiments, generate / manage / and analyze the data, and formally communicate their results. As the largest coastal wetland in Southern California, Tijuana River Estuary has long been a focal point of transboundary pollution concerns between the United States and Mexico. High school students help collect sediment and water samples from four locations based on proximity to the border, Pacific Ocean, and urban areas during both dry and rainy weather events to conduct sea urchin embryotoxicity testing, microbial sequencing, and both non-targeted and targeted chemical profiling. Moreover, novel Composite Integrative Passive Samplers (CIPS) were utilized to quantify time-weighted average concentrations of persistent organic pollutants (POPs) of concern in estuarine waters. The program culminated in a science symposium with students' presentations to professional science audiences from academia, industry, and government. Besides quantifying and mitigating ecological public health risks of local and internationally relevant pressing environmental issues, the program's goal is a long-term empowerment of next generation STEM and community leaders focused on environmental and human health protection.

7.02.B.T-05 Empowering environmental health literacy in Colonias: A community science approach

Luis Adrian Gomez, **Lindsay Sansom** and **Garett Sansom**, *Texas A&M University*

Environmental science education must be both inclusive and action-oriented, particularly in historically marginalized communities like the South Texas colonias. Colonias, home to over 500,000 residents in Texas alone, face persistent challenges in water quality, infrastructure, and environmental health literacy. To address these disparities, we developed the South Texas Community Science Program (STCSP) as part of the Juntos para un Mejor Mañana (Together for a Better Tomorrow) initiative - an innovative, hands-on program that empowers residents through environmental health science and advocacy. Between 2023 and 2024, the program engaged over 80 students and community members from colonia neighborhoods, offering intensive instruction in water and soil sampling, risk assessment, and environmental policy frameworks. Each participant earned a

certificate of completion, formalizing their role as community scientists and environmental health stewards. Central to this effort were culturally grounded pedagogies, incorporating trusted promotoras as instructors and integrating continuous feedback from local community advisory boards to ensure relevance and cultural competence. This presentation will outline the successes and challenges encountered in implementing the STCSP, offering practical strategies for other educators seeking to advance environmental literacy in underserved communities. Key program outcomes include increased technical knowledge, strengthened community voice, and heightened civic engagement. Notably, many students reported an increased desire, and early successes, in pursuing higher education and careers in STEM fields. Attendees will also hear directly from participating students through video interviews that share their reflections on how the program expanded their environmental awareness and inspired future advocacy and academic pathways. By merging rigorous environmental science with community empowerment, the STCSP provides a replicable model for inclusive teaching and environmental justice. Attendees will leave with actionable insights and tools to design and implement similar initiatives, fostering the next generation of environmental leaders in communities most affected by environmental health disparities. **Disclaimer/Disclosure:** This project was supported by Methodist Healthcare Ministries of South Texas, Inc. and the Gulf Research Program of the National Academies of Sciences, Engineering, and Medicine (NASEM GRP).

7.03.P-Tu Navigating the Misinformation Maze: The Scientist's Mission to Preserve Scientific Integrity

7.03.P-Tu-123 Polymer & plastic additives in global commerce - combining producer knowledge, cheminformatics, database management & modeling tools to bring context & clarity in a challenging environment

Craig Warren Davis¹, Mike DeLee² and John Norman³, (1)Exponent, (2)Dow Chemical Company, (3)American Chemistry Council

Plastic products are ubiquitous in modern life. This is due part to the fact that polymers and plastics are incredibly versatile – with performance characteristics that can be achieved using various catalysts, additives, copolymers, and processing aids. While this makes plastic an ideal functional material it also introduces additional chemistries into the value chain, which may remain, to some degree, in the finished products. This has given rise to increased public concern over the availability of regulatory, health & environmental data for these materials as well as questions over the rigor with which different products are evaluated across different regions of the world. Key questions from stakeholders include, (1) how many of these materials are actively in commerce across the globe, (2) how these materials are used and in what quantities, (3) are these materials subject to rigorous chemical registration and management frameworks, and (4) are there sufficient health & environmental data available to support the continued safe use of these materials in the global market. There are challenges to addressing these questions, some are scientific in nature (i.e., data poor substances, limited model domains), some are logistic (multiple or conflicting identifiers, complex substances), and some are institutional (access to regulatory evaluations/data, legal frameworks). All these challenges further complicate effective risk communication between technical and non-technical stakeholders, leading to confusion, distrust, and skepticism. To address these challenges, ICCA have developed a public, open access database of regulatory, health and environmental information for chemicals previously identified in UNEP's "Chemicals in Plastic" database as well as those further identified as "actively in commerce" via global industry survey and available public product data. This includes data on use, technical function, regional availability, and polymer type(s). Data from the US EPA's CompTox dashboard are integrated to aid with accurate substance identification and the curation of health & environmental hazard data. Future versions of the database aim to include information on regulatory hazard and risk assessments as well as experimental and modeled physical-chemical, fate, and hazard data, where available, to aid in the rapid risk-based prioritization of these substances, particularly in developing regulatory regions.

7.03.T Navigating the Misinformation Maze: The Scientist's Mission to Preserve Scientific Integrity

7.03.T-01 Who controls the microphone? Recapturing the public trust

William L. Goodfellow¹, Patrick D. Guiney² and Timothy J Canfield³, (1)Exponent, (2)ECOTOX-Guiney Consulting, LLC, (3)U.S. Environmental Protection Agency

In recent years, the scientific community has faced a growing challenge: the erosion of public confidence in scientific statements and findings. This current situation is compounded by the increasing influence of untrained individuals who leverage social media 215s to disseminate their interpretations of scientific information. These “influencers” often prioritize sensationalism and brevity, leading to an environment where short sound bites and attention-grabbing headlines overshadow more deliberate, evidence-based discourse. The result is a public that is often misinformed or skeptical about scientific truths, which poses significant risks to informed decision-making and policy development. To address this issue and reclaim the “authoritative voice” in the public discourse, scientists must innovate in their communication strategies to help level the playing field on information dissemination. Recapturing the microphone from untrained influencers requires a multifaceted approach that leverages technology and creativity. Scientists must adapt to the evolving media landscape, embracing innovative communication tools that resonate with the public while upholding the principles of scientific rigor. One promising approach is the enhancement of risk communication, ensuring that complex scientific data is presented in a clear, concise, engaging, and relatable messages. Using storytelling techniques is one way to do this. Other potential ways to accomplish this end goal could involve the strategic use of front-end bullet points in the form of cover sheets for scientific papers, providing a snapshot of key findings and implications. These bullets can be hyperlinked to detailed supportive data within the paper, allowing readers to delve deeper into the research if they choose where the scientific foundation for the information claims can be provided. Such a format would not only cater to the brevity demanded by modern media consumption of the public; but also maintains the integrity and depth of scientific information required to ensure confidence that the information provided is accurate and sound. Additionally, the use of infographics and animated visuals can transform static data into engaging narratives that capture the attention of the public consumer; but also, would foster easy understanding of the topic at hand. Several other approaches for counter-acting influencers will be discussed.

7.03.T-02 Credibility, incentives, synthetic propaganda and publishing: Why toxicology needs a reformation!

Christopher J. Borgert, Applied Pharmacology & Toxicology Inc.

Life sciences (LS) generally and toxicology specifically face a credibility gap. ‘Research’ increasingly seeks to reduce biology to mathematic modeling aimed at the production of descriptive algorithms rather than to better understand how the physical world operates through hypothesis generation and severe testing by falsification. This leads to dichotomous thinking and monolithic paradigms (e.g., follow “the” science), which arguably stifles discovery and promotes censorship of whatever is not deemed to be “the” science. Misplaced incentives may be the root cause, as asserted by the newly appointed Director of NIH, Dr. Jay Battacharya, a leader who intends to reform those incentives. Mis-incentives are especially germane to regulatory science, where the need for understanding is often subjugated to policy-making. The need to make decisions and to create a predictable regulatory environment are anathema to the discovery process, but neither can regulations follow the tides of scientific investigation. Profound changes may be needed, particularly in this era of algorithms-over-understanding. Prominent examples of these clashes include BPA, glyphosate, PFAS, and legacy chemicals that continue to fuel a lucrative economy of injury litigation. Adding to the complexity is the weaponization of science enabled by technological advances in AI. Not only can AI be used to satisfy the myriads of mis-incentives to which scientists are subjected, but as exposed by Dr. Bernhard Sabel, unscrupulous individuals and foreign governments use AI to flood the literature with AI-generated “science,” not only for personal profit but for interference in global markets to gain strategic economic advantages. Set within the context of an outdated and arguably counterproductive scientific publishing apparatus with similar mis-incentives, toxicology needs a systemic reformation. Reforms include de-coupling investigative research in toxicology from the regulatory process; prioritization of replication, falsification and translation in investigative research above

novel discovery; prioritization of safety assessment (translation to species of concern) over the current process of hazard-identification-by-speculation, and the development of tools to forensically identify and expunge research reports that lack transparency and authenticity.

7.03.T-03 Beyond the first conclusion: Why science demands re-evaluation in light of new data

Ellen M. Mihaich, Environmental and Regulatory Resources

If misplaced incentives are a significant cause of the integrity crisis in science, nowhere can that cause be seen more clearly than in the tension between regulation and scientific investigation. Here, purposes are not merely conflated; they collide head-on. While the tension is understandable, something must give if toxicology is to regain credibility among the public. Incentives for scientists in academia include securing governmental funding for research and generating as many highly-cited publications as possible. Since scientific publishing long ago de-prioritized falsification, replication, and rigorous testing in favor of new discoveries, the peer-reviewed toxicology literature is highly skewed towards unreplicated, novel reports of chemical effects. Regulatory agencies, compelled by statute to use the best available science but also compelled by public opinion to appear “protective,” must often place less emphasis on replicability. Although highly reliable by design, the cost of guideline toxicology studies and the tendency for regulatory agencies to consider only the “positive” data or the lowest reported toxic dose, industry has little incentive to repeat studies or to conduct research to clarify the relevance of results obtained in laboratory test species. This creates a situation where a robust attempt to understand the relevance and transferability of new toxicological findings is rare, and even when done rigorously, decision-makers and the public are typically unable or unwilling to accommodate new understanding. Several examples will be presented that illustrate the reluctance to revisit decisions made from a regulatory perspective that essentially result in a case of mis-information by not taking into consideration the best available science.

7.03.T-04 PFAS and fluorinated chemicals: An information disconnect in the legislative process

Christopher Rendall-Jackson, Farella Braun + Martel LLP

I plan to present on various state legislative efforts that perpetuate a misunderstanding about the health risks posed by fluorinated chemicals, and opportunities for scientists to counteract such misunderstandings. More specifically, I’ll discuss legislative findings and restrictions regarding PFAS in consumer products that are overbroad, and subsequent legislative actions to narrow such restrictions, that may perpetuate the misunderstanding that health risks posed by certain PFAS apply to a much broader group of fluorinated chemicals. I’ll conclude by discussing opportunities for scientists to work to correct such misunderstandings. Certain state legislation misattributes the health risks posed by certain PFAS to a much broader group of fluorinated chemicals. Where legislative findings are focused on risks from certain types of PFAS, but attribute those risks to a much broader group of fluorinated chemicals, the resulting legislative restrictions apply to a much broader group of chemicals than necessary to address the identified risk. For example, some legislation broadly defines PFAS as a class of fluorinated organic chemicals containing at least one fully fluorinated carbon atom, and sometimes indicate that detection of total organic fluorine indicates the presence of PFAS, but the legislative findings about the risks created by PFAS do not necessarily apply to all of the chemicals encompassed by such definitions. Those broad legislative restrictions may create a misimpression about the health risks of that broader group of restricted fluorinated chemicals. Subsequent legislative actions to narrow such restrictions may then perpetuate this misunderstanding. Those subsequent actions generally do not narrow the broad definitions of PFAS and instead narrow the categories of products targeted. By not recognizing that the risks from certain PFAS are being misattributed to a much broader class of fluorinated chemicals, and instead suggesting that the risk from those fluorinated chemicals is best controlled by focusing on how they are used in certain products, these subsequent legislative actions may perpetuate the misimpression being created about that broader group of fluorinated chemicals. I’ll conclude by discussing various opportunities scientists have to counteract such misimpressions, including by participating in the legislative and rulemaking processes and in litigation matters.

7.03.T-05 Errors in data and analyses persist in spite of transparency measures

Andrew Deines, Exponent

You may have heard of the “replication crisis”, but what about the reproducibility crisis? The reproducibility crisis acknowledges a pervasive failure across scientific disciplines for different scientists to arrive at the same outcomes, when using the same data and analytical methods reported in a study. This reproduction failure doesn’t arise from the natural operation of the scientific method with new data and results overturing old, but rather, it reflects misinformation: errors, omissions, bias, or even maleficence in analysis and reporting. I will present a vignette from a recent work where data analysis and review identified multiple errors, resulting in the reporting of erroneous and misleading results. Peer review is the first and perhaps best line of defense against misinformation. Take for example, SETAC’s own journal -- Environmental Toxicology and Chemistry--which has a relatively robust policy on data transparency. Yet, while “the Journal strongly encourages authors to make all data and software code on which the conclusions of the paper rely available to readers” and while all data and must be made available to reviewers, if not the general public, the peer review process has no requirement that reviewers assess either the accuracy of data or that the software code correctly implements the data processing or analysis described in text. The policy is in fact ambiguous whether the analysis code must be made available to peer reviewers. The availability of data and software code is overall improving transparency – and perhaps confidence - in results by opening the “black box” of data analysis and making the replication crisis more visible. At the same time, the arrival of AI means we are living through a paradigm shift in the way that data are accessed and analyzed. AI provides powerful analytical tools; some of these tools might lessen the replication crisis, such as by assisting in software code development, while other AI tools might make it worse--by putting data analyses deeper into a “black cloud”.

7.04.P-Tu Risk Communication—the Essence of Science Solutions

7.04.P-Tu-124 Consequences of incident-specific factors for risk communication: A comparison of two recent case-studies involving a refined liquid petroleum product and solid materials from a built asset

Norman Forsberg, Emily Morrison, Nancy Bonnevie and Adam Ayers, Arcadis Inc., USA

Effective risk communication following an accidental spill or release (i.e., incident response) is an information dependent process that must adapt over time in response to public concern as interactions between environmental risk assessment scientists, managers, policy makers and the interested public evolve. As a result, risk communication in incident response situations evolves in a manner that is directly related to public interest, and the rate and extent to which relevant and reliable information can be gathered, organized, analyzed, and interpreted consistently among stakeholders. Event-specific factors (e.g., public interest, regulatory familiarity with the released material, the physical/chemical nature of the released material, understanding of the physical and toxicological hazards of the spilled/released materials, environment in which the event occurred, and relevant receptors and exposure pathways) can lead to distinct risk communication pathways and life-cycles. Additionally, the availability (or lack thereof) of relevant and accepted regulatory frameworks with which to base assessments and align stakeholder’s expectations is critically important. Two recent case-studies (both highly generalizable) will be used to explore this topic. The first case-study involves a spill of refined liquid petroleum product into a river system, where regulatory stakeholders were familiar with the chemical, physical, and toxicological hazard characteristics of the material and had in place an existing regulatory framework that supported a quantitative assessment of risks to human health and the environment. The second case-study involves a release of the manufactured parts from a built asset into the aqueous environment, with the released materials generally regarded as stable to the environment and the built asset handled as non-hazardous waste the end of its lifecycle (i.e., similar to non-consumable items like building materials or furniture). The case-studies will be compared as a means of identifying the relative importance and potential consequences of event-specific factors on the risk assessment process and risk communication efforts. Findings will also be discussed within the context of other types of environmental disasters that are relevant to this session (such as hurricanes and floods).

7.04.P-Tu-126 Development of a database for identification, hazard assessment and risk assessment support for plastic additives in commerce

Kara Franke¹, Julie Panko¹, Bjoern Hidding², Mike DeLee³, Craig Warren Davis⁴ and John Norman⁵,
(1)ToxStrategies, (2)BASF SE, Germany, (3)Dow, (4)Exponent, (5)American Chemistry Council

Plastics play an essential role in numerous areas of modern life due to their versatility, with characteristics that can be optimized for use in packaging, textiles, electronics, construction materials, and more. However, there is rising global concern about the safety of chemicals used in the manufacturing of plastics (e.g., functional additives) and their potential to be released in the environment. More than 13,000 chemicals associated with plastic materials were identified by the United Nations Environmental Programme (UNEP) in their 2023 report and were screened for hazards and potential risks. However, the approaches used to compile this data were limited to information in the public domain and the data is spread out across multiple publications, making it difficult to interpret and understand plastic additives and the available health and safety information. In response, the user-friendly ICCA Plastic Additives Database was developed to build on this work and make risk related information on uses, hazards, and risk assessments more accessible to facilitate risk communication more equitably to stakeholders and regulators of varying levels of technical expertise. Additions and updates to the database have focused on chemicals known to be used as plastic additives in global commerce using supplemental sources such as US EPA CompTox Chemicals Dashboard, Chemical Abstracts Service (CAS), collaboration with SpecialChem, and a survey of additive manufacturers and producers. The database contains the identification, use and function of plastic additives that are currently confirmed in commerce or that have been in commerce for the past 10 years; physical chemical properties of the chemicals that inform their fate and transport in the environment; hazard classifications of the chemicals according to harmonized determinations; and lists of existing chemical management laws regulating specific additives; and includes an overview page for each substance with quick reference data visualizations for uses, functions, and regions of use; and supporting pages that present detailed data in a manner that is accessible to regulatory agencies and risk assessors around the globe and with varying levels of risk assessment expertise. Additional functionality related to hazard and risk assessment is planned, including the curation of existing hazard and risk assessments for available substances, to aid in screening level risk-based prioritization of these substances. **Disclaimer/Disclosure:** Database development was funded by the International Council of Chemical Associations.

7.04.P-Tu-127 Can there be justice for all?

Tamara R. House-Knight and Francis Ramacciotti, GHD

The evolving landscape of chemical regulations in the United States (U.S.) presents significant challenges to not only businesses, but communities that are located near highly industrialized areas. Regulations enacted by the U.S. Environmental Protection Agency (USEPA) and other federal agencies create standards, criteria, guidelines that apply to the entire country and are intended to create consistency that is protective of human health. Individual states may take the lead in establishing state standards or defer to Federal regulations. The rollback or non-enforcement of federal environmental regulations poses significant threats to environmental justice, which could disproportionately impact marginalized and vulnerable communities. Without robust federal oversight, low-income and communities of color may face increased exposure to environmental hazards such as air and water pollution. These groups often lack the political power and resources to challenge harmful practices or demand remediation. Additionally, the weakening of regulatory frameworks undermines long-standing efforts to address systemic inequities in environmental protection, widening the gap between affluent and disadvantaged areas. As federal enactment and enforcement of chemical regulations become increasingly uncertain, in addition to the elimination of the federal Office of Environmental Justice and External Civil Rights, what happens with engagement and support with communities who may have environmental concerns. This presentation will examine how risk communication and community engagement can be effective tools for communities in addressing the proposed regulatory actions such as reconsideration of regulations on power

plants, reconsideration on regulations on oil and gas industry, and elimination of environmental justice program grants.

7.04.P-Tu-128 Empowering communities: Colorado's FREE PFAS testing and assistance program (TAP) for private well users

Meghan Williams and Erin Looney, Colorado Department of Public Health and Environment

In February 2024, the Toxicology and Environmental Epidemiology Office at the Colorado Department of Public Health and Environment launched the PFAS Testing and Assistance Program (PFAS TAP). The goals of PFAS TAP are to: 1) expand our understanding of PFAS contamination in Colorado's groundwater; 2) provide data-informed recommendations to limit PFAS exposure; 3) use education and outreach to reduce exposure and encourage positive behavior change in private well users; 4) effectively reach lower-income households with real assistance to protect health; and 5) collaborate with county partners to promote well water testing and education in targeted communities. The program offers an affordable and effective solution for private well users by providing free PFAS testing and free water filters to eligible households. Anyone in Colorado with a private well can sign up for free PFAS testing, and eligible participants can get free filters that meet NSF standards for contaminant reduction. In addition, PFAS TAP conducts targeted outreach in priority areas to focus resources where they are most needed. In this presentation, we will share data gathered thus far on the magnitude and extent of PFAS contamination in private wells and report on lessons learned.

7.04.P-Tu-129 Using beer to engage the public on scientific topics

Sam Nutile¹, Lynne Beaty¹, Adam M. Simpson¹ and Sara Stalhman², (1)Penn State Behrend, (2)Pennsylvania Sea Grant

The introduction and spread of non-native species are one of the largest threats to the integrity of aquatic ecosystems. Non-native animals and plants compete with native species for resources, harbor and introduce new diseases and parasites, alter the flow of contaminants throughout the food web, and decrease the recreational and economic value of aquatic habitats. While the initial introductions of non-native species are generally the result of broader global transport systems, the spread of established species is often facilitated by public users of wild areas. As such, educating the public on the existence of non-native species and providing tools by which to identify and prevent the spread of such species can be a highly effective mechanism to limit the distribution of these invaders. Through partnership with a local brewery, we have launched a beer draft series, Lagers for Lakes, that has highlighted the existence of 8 non-native species in waters of the Great Lakes over the past four years. The release of each beer has been accompanied by a launch party that has provided information to attendees on the species of interest, as well as general knowledge of methods to limit the spread of non-native species. With the inclusion of carnival style games, raffles, snail racing, and information stations, we aim to reach a diverse audience of varying demographics. To date, we have engaged with over 200 individuals at these launch events and received continued engagement through passive information that available upon the purchase of the themed draft. This presentation will share the successes and challenges associated with this type of outreach event and offer the potential for collaboration on similar efforts in other areas focused on the same or similar scientific topics. By combining scientific outreach with products of local businesses, our efforts reach those most likely to make an impact in the battle against non-native species.

7.04.P-Tu-130 Translating bioavailability science into action—A success story of collaborations, technology development and regulatory innovation in the USA

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While the science behind pollutant bioavailability was maturing within the scientific community 25 years ago, there was little impact on hydrophobic pollutant sediment risk assessment, which was based on total concentrations. Sediment remediation practice largely relied on disruptive sediment removal that often failed to achieve risk reduction goals. We learned from careful observations of nature that black carbon particles natively

present in sediments sequestered hydrophobic pollutants rendering them less bioavailable. This observation provided powerful insights that needed to be translated into practice for both risk assessment and for designing remedies. Translation of bioavailability science into practice had to progress on three fronts: 1) testing and development of methodologies to alter hydrophobic pollutant bioavailability in sediments through amendment of strong sorbents, 2) development of reliable metrics of measurement of bioavailability changes in sediments, and 3) regulatory engagement and innovation to enable acceptance of new metrics of assessing risk posed by hydrophobic pollutants in sediments and consideration of new approaches for in-situ management of polluted sediments through bioavailability manipulation. This presentation will provide an overview of the developments in the science of pollutant bioavailability in the last two decades with a focus on the following aspects: 1) development of the novel sediment remediation technology of activated carbon amendments into sediments to reduce bioavailability that has now become a mainstream approach in the US, 2) development and standardization of passive sampling technologies to measure freely dissolved concentrations as a key metric for pollutant bioavailability, and 3) regulatory innovation through active collaboration and dialog among researchers, regulators, and practitioners that enabled creation of an environment where new approaches based on the latest science could be tested and flourish. As global attention moves to address newer environmental challenges, the lessons learned from past successes in science translation can guide more effective designs and sustainable solutions. **Disclaimer/Disclosure:** The authors thank multiple past and ongoing funding sources including the DOD SERDP program, NIEHS Superfund Research Program, Alcoa, Dow Chemical Company, and the USEPA.

7.04.P-Tu-131 Building and expanding an interactive CEC network

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An interactive network has been developed, as part of a larger CEC initiative, to foster connections and collaboration among individuals and organizations working on contaminants of emerging concern (CECs). Initially launched as a pilot effort, this network integrates insights from Advisory Committee meetings, gap analyses, legal scans, and research initiatives to map key players and activities within the CEC landscape. This network is designed to support research, engagement, and programming, and brings together a variety of contact information and meaningful relationships to enhance communication and resource sharing in this field. The network is currently being expanded into the Great Lakes region and is being refined through user feedback and beta testing to ensure broader applicability and impact. This evolving 215 aims to support a growing community of CEC end users by facilitating partnerships, informing policy, and driving community-engaged collaboration. A final network framework will serve as an ever-changing tool, and will be widely distributed to help achieve goals and priorities that extend beyond the southeast.

7.04.P-Tu-132 Dear Dearbhla, nature's agony aunt: Sentient species and their role in environmental science and conservation

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Dear Deabhla... much like an agony aunt gives life advice, we can turn to indicator species, and 'model' organisms for environmental advice. What will happen if temperatures rise in freshwater ponds? How might microplastics change algae populations in rivers? *Daphnia*, or waterfleas, have been recognised as sentient species in freshwater environments for a long time, and are widely used as a proxy, or model, species to understand how environmental challenges might affect ecosystems. Dearbhla, the *Daphnia*, takes a novel approach to science communication by taking the model organism, bringing them out of the laboratory and anthropomorphising them, quite literally in the form of a *Daphnia* costume. This allows us to highlight the scientific important and development of *Daphnia*, or water fleas, as an amazing model organism that are used globally as indicators of good environmental health. They have been historically used for freshwater toxicity

testing of chemicals, and more recently nanomaterials and microplastics. For context, *Daphnia* are transparent organism and are keystone species in freshwater environments because they link the primary producers (algae) with higher trophic levels in the food web (fish and other insects etc). Under healthy/normal conditions they will reproduce genetically identical daughter offspring and will only produce male *Daphnia* when stressed, which is reflected in Dearbhla having a removable brood pouch. A range of methods that have been developed to monitor environmental health such as the use of fluorescent particles and dyes, video capture methods to explore how their movement changes and a range of molecular methods to determine their cellular response to pollutants. Dearbhla can bring all of this research to the public in an engaging and visual way and supports some of the hands-on activities. Some of these events have included science festivals, exhibitions and open day events where Dearbhla has attended, and acted as an ‘agony aunt’ to answer the public questions on environments issues. We have found this to be really engaging due to the visual nature, the opportunity to take photos and the novel element- many people haven’t seen a *Daphnia* so having a human sized one draws some interest! Dearbhla creates a novel lens through which to engage different audiences with new environmental science and ecotoxicological approaches, which allowing two-way communication between model organisms and the public.

7.04.T Risk Communication—the Essence of Science Solutions

7.04.T-01 Communicating risk as a State Environmental Agency: Strategies, challenges and lessons learned

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Risk communication is a crucial component of managing sites with environmental contamination. Yet state environmental agencies may not have the mechanisms to support the amount of consistent and proactive communication that is needed to adequately inform the communities impacted by their work. The Oregon Department of Environmental Quality Environmental Cleanup Program’s current regulatory structure does not require consistent risk communication or proactive community engagement in decision making about Cleanup sites. Furthermore, there is no mechanism for engaging the public holistically about environmental risks from different sources in their community. The lack of regulatory requirements and financial resources to support proactive engagement creates inconsistency in the program’s ability to practice effective risk communication. A lack of effective risk communication can exacerbate public health outcomes associated with environmental contamination and disproportionately impact poorer, more vulnerable communities. To address these policy and funding gaps, the Cleanup Program has determined that strategic planning to guide the future of the program is critical. Strategic planning is an important step towards systematically supporting and normalizing more proactive and effective risk communication across the program moving forward. In this presentation, we will share strategies, challenges, and lessons learned through examples of engaging community members in conversations about programmatic policy-making and cleanup site decision-making. We will highlight strategies that have been effective, such as collaborating with partner agencies like the Oregon Health Authority and local community organizations, as well as participating in events already planned in the impacted community. We will also reflect on lessons learned, such as the feedback loop between the level of engagement coming from a concerned community and the resources that the Cleanup Program allocates to communicate about risks with that community. These examples provide successful strategies as well as lessons learned to help other agencies, scientists, leaders, and policy makers plan for future risk communication approaches in a variety of environmental situations.

7.04.T-02 Community-engaged investigation of environmental exposure from 2023 train derailment at East Palestine, OH

Oindrila Ghosh, Carla A. Ng, James Fabisiak, Julianne Beier and Maureen Lichtveld, University of Pittsburgh

The Norfolk Southern train derailment in East Palestine, OH in February of 2023 resulted in a significant spill of semivolatile and volatile organic compounds (SVOC/ VOCs) including vinyl chloride (VC), impacting a community of 5000 people and the surrounding environment. VC leaked into the environment, potentially impacting indoor air and drinking water. Emergency crews conducted a controlled burn of the spill, further impacting the environment, and may have used fluorochemical-containing firefighting foam. The University of Pittsburgh is conducting an environmental health study as a collaborative effort between the Schools of Engineering, Medicine and Public Health by deploying a community-engaged approach to collect, bank and analyze (a) environmental-, (b) biospecimen- and (c) psychosocial stress data to monitor impacts of exposures to chemicals spilled during the disaster. In this presentation, we share highlights from the environmental sampling campaign along with lessons learned on effective risk communication. This study adopts a community-based participatory approach, with oversight and guidance provided by a Community Advisory Board (CAB). A group of Community-based Researchers (CbRs) from EP along with a group of Pitt students were trained to collect environmental samples. As part of the sampling campaign, the CbR and student teams worked together to collect samples from 100 homes located within an 8-mile radius of the derailment site. Three key activities comprised one environmental sampling event: i. Measuring VC levels in household basement air using colorimetric gas detection tubes in real time ii. Collecting grab samples of indoor air, drinking water and garden soil samples from individual households to analyze presence of VOC/SVOCs and PFAS iii. Installation and retrieval of Polyurethane foam (PUF) outdoor passive air samplers to monitor VOCs/SVOCs and PFAS over a period of 2-4 weeks. Given the time-sensitive nature of grab air sampling, pairing CbRs with students proved most effective. Allowing CbRs to take ownership of team dynamics helped align the work with scientific goals while maintaining community trust. We also learned the importance of clearly communicating the study's scope during trainings and meetings—dialogue without instilling fear. Ensuring that participants and CbRs understood the connections between the environmental, biomedical, and psychosocial components of the study encouraged more active and engaged participation. **Disclaimer/Disclosure:** The "East Palestine (EP) Community-Engaged Environmental Exposure, Health Data, and Biospecimen Bank" is one of six time-sensitive R21 projects funded by the NIH to investigate the health and environmental effects resulting from the accident that is currently conducted at University of Pittsburgh.

7.04.T-03 The cart & the horse: Communicating and aligning on assessment scope & problem formulation decisions in a resource-constrained environment—A case study of ecological risk assessment of 1,3-butadiene

Craig Warren Davis¹ and Neeraja Erraguntla², (1)Exponent, (2)American Chemistry Council

Regulatory evaluations of chemicals often include public stakeholder engagement at multiple points throughout the process. Problem formulation and scope definition at the onset of an evaluation serves to define the boundaries of the assessment and may include decisions on (1) the inclusion or exclusion of exposure pathways, receptors, or stages of the value chain (i.e., waste stages) as well as (2) the approach to evaluating risk to certain compartments or receptors based on previous knowledge of chemical properties and/or use and emissions scenarios. Specifically, the decision to conduct a quantitative vs. a qualitative risk assessment for a compartment or receptor(s) is often based on the concept of a de minimis potential for exposure, hazard, or both. In the case of difficult to test substances (i.e., gasses) or substances with limited exposure and/or toxicological data, this initial assessment may be qualitative or semi-quantitative, leading to uncertainty, particularly when communicating the basis and rationale for these decisions and their respective outcome(s) to stakeholders with diverse backgrounds and expertise. Recently, the US EPA has conducted a risk evaluation of 1,3-Butadiene in which they have performed qualitative assessments of risk to the environment and associated ecological receptors (including wildlife). While this assessment is based on limited detection in surface water and the physical-chemical properties of the substance, this decision has been questioned by stakeholders during the recent Science Advisory Committee on Chemicals (SACC) public forum, with some stakeholders requesting re-evaluation using a formal quantitative risk evaluation framework. Without direct evidence of hazard and/or enhanced exposure, it is doubtful that further evaluation will yield an alternate outcome and represents a

significant resource re-allocation by EPA when substantive comments and questions remain to be addressed in other elements of the substance evaluation (i.e., occupational and general population assessments). This work seeks to (1) discuss the approach used by EPA and its limitations /challenges as well as (2) evaluate opportunities where enhanced risk communication and transparency may have improved stakeholder understating and alignment using this case study chemical.

7.04.T-04 A reel partnership: How engaging the community leads to better science and reduces exposure of refugee and immigrant fishers to toxics

Jenee A. Colton¹, Jennifer Alina Lanksbury², Richard Jack² and Chelsea Mitchell², (1)Dept. of Natural Resources and Parks, King County, (2)King County

Immigrants and refugees, particularly those with low-income and limited English proficiency, are often disproportionately impacted by environmental contaminants. Though they may have some knowledge about toxics, fishers often want more guidance on safer choices, not just which fish to avoid. In 2021, King County partnered with the Refugee Federation Service Center (RFSC) to explore contaminants in fish targeted by south King County immigrant and refugee communities. Our King County team manages a long-term monitoring program that measures toxics in marine and freshwater fish. We lacked fish consumption exposure information for communities who rely on local fishing for sustenance and as a cultural tradition. To help fill this gap we engaged with the RFSC, a non-governmental organization who represents several southeast Asian and Latino fishing communities, to understand the locations and species fished, and parts eaten by local fishers. We developed a fisher survey in seven languages that the RFSC implemented to explore the fishing habits of these communities. Among other new information, the survey revealed popular fishing areas and species not currently tracked by our monitoring program. We used this information to design a fish sampling pilot project to 1) inform safer choices by the immigrant and refugee fishers and 2) fill some data gaps in our monitoring program. Community fishers from the RFSC collected the fish in 2023 and samples were analyzed for a range of organic contaminants (e.g., PCBs, PBDEs, and PFAS) and mercury. We relied on RFSC input to select the most effective communication strategies for our results to reach community members. We worked with the state to establish fish eating recommendations targeting these communities and delivered this information via multiple approaches to maximize the impact. Our local public health agency and the RFSC assisted us in risk communication messaging which we relayed in nine languages. Through this project we started a long-term, mutually beneficial relationship with the RFSC that has vastly improved the utility of our science. Having specific exposure data means local communities we can go beyond generalized exposure assumptions that were not protective of the most vulnerable fishing populations in King County. **Disclaimer/Disclosure:** Funding for this project was provided to the RFSC from King County's Waterworks grant program.

7.04.T-05 Community-academic partnership to advance PCB science and remediation in the Portland Harbor

Alexis Slade¹, Shannon Watkins¹, Andres Martinez¹, Martine Mathieu², Cassie Cohen³ and Keri Hornbuckle⁴, (1)University of Iowa, (2)North Carolina State University, (3)Portland Harbor Community Coalition, (4)Civil And Environmental Engineering, University of Iowa

The community-academic collaboration between the Portland Harbor Community Coalition (PHCC) and the Iowa Superfund Research Program (ISRP) aims to interweave community, scientific, and regulatory knowledge to address PHCC concerns about PCBs. PHCC works for robust, equitable cleanup of the ten-mile Portland Harbor Superfund site in Portland, Oregon. Together, PHCC and the ISRP conducted a 3-part study of airborne Polychlorinated Biphenyls (PCBs) in the surrounding community. We draw on the principles of Community Based Participatory Research. For example, we build on community resources by leveraging PHCC's deep local knowledge of the site, cleanup activities, and regulatory parties to develop hypotheses, design data collection methods, and interpret findings. We seek co-learning; for example, ISRP investigators are building comfort with new approaches to sharing scientific findings (e.g., sharing in community before peer review). Moreover,

PHCC and the ISRP have collaborated to make sense of and disseminate findings. After interpreting findings together, we shared with community members who helped collect data, PHCC coalition members, a local indigenous community group, and finally local and state regulators. We are working to have research findings inform remediation and to address new emerging research questions. Partnerships between impacted communities and academic research centers can inform practice by combining vital community and scientific knowledge.

7.04.T-06 The confluence of environmental health and doctor clinics

Andrea Kingcade and Kristy Richardson, Colorado Department of Public Health and Environment

Environmental regulations, intended to protect public health, typically require information about the potential hazards and impacts of exceeding a standard. However, the regulators then refer individuals to their healthcare providers to discuss concerns about health impacts of exposures. Yet, community members tell us their healthcare providers are often unaware of health effects associated with, or exposure sources of, environmental contaminants. Even with a legacy contaminant, like lead, that is widespread in Colorado, less than 25% of children who should get tested were tested in 2024. Healthcare providers' lack of awareness about environmental health issues presents a particular challenge in disproportionately-impacted communities that lack equitable healthcare access, are more vulnerable to exposures and health impacts, and have less agency to advocate for themselves and their communities regarding environmental contamination and health concerns. This talk will highlight the Colorado Department of Public Health and Environment's collaborations with healthcare providers to address this important need. We'll present the bridges we are making for public health intervention and the key lessons learned from this partnership.

7.05.P-Mo Science Translation in Regulatory Programs and Impacts to Local Water Quality Improvement Efforts

7.05.P-Mo-113 Science translation of passive sampling measurements requires modeling interpretation and active regulatory engagement

Upal Ghosh¹, Nathalie Lombard¹ and Dev Murali², (1)University of Maryland, Baltimore County, (2)DC Department of Energy and Environment

Regulatory programs managing large rivers polluted with legacy pollutants like polychlorinated biphenyls (PCBs) have faced major challenges in accurately quantifying sources and developing approaches for remediation. Remedial investigations of sites contaminated with PCBs have traditionally focused on mapping sediment contamination to develop site conceptual models and select remedy options. This sediment-focused approach often leads to an incomplete understanding of the impacts of ongoing inputs to the water column and over-estimation of potential effectiveness of sediment remediation. A study by the National Research Council found that disruptive sediment removal at Superfund sites often failed to achieve risk reduction goals. Recent scientific research has demonstrated the importance of freely dissolved concentrations of hydrophobic and bioaccumulative chemicals to assess risk and determine key sources that need to be controlled. However, pollutants like PCBs are sparingly soluble making it difficult to measure directly. The development of equilibrium passive sampling methods has accelerated over the last decade and enabled measurements of ultra-low concentrations of bioaccumulative chemicals in the aquatic environment. Yet, use in the regulatory context has been limited due to the lack of precedent and solid demonstrations tied closely to regulatory needs. Here we demonstrate a unique collaboration between academia, Federal agency, and local regulatory agencies to perform measurements of surface water, sediment porewater, and gas-phase PCB concentrations using polyethylene passive samplers to provide the first accurate mass balance of freely dissolved PCBs in the Anacostia River in Washington DC. This mass balance enabled identification of a major tributary and contaminated sediment hot-spots as the sources having the most negative impact on the water body, while also demonstrating that the atmosphere served as a sink for PCBs from the surface water. By coupling the mass balance model with a food

web model, we were able to connect the media concentrations to bioaccumulation in fish and compare management options. We demonstrate that sediment cleanup alone will not be able to achieve target reductions of PCBs in fish and dissolved concentrations from the problem tributary needs to be controlled. The framework presented here for passive sampling and interpretation can be used to provide robust and sustainable solutions for other river systems. **Disclaimer/Disclosure:** We acknowledge support for this work from the DC Department of Energy and Environment and the Maryland Department of Environment.

7.05.P-Mo-114 Sensitivity analysis of surface water quality criteria derivation: A case study of ecological criteria for PFOS by three countries

Betsy Ruffle¹, Belinda Goldsworthy², Bryant Gagliardi³, Christine Archer¹, Craig Warren Davis⁴, Paul G. Koster van Groos⁴ and Anita Thapalia⁴, (1)AECOM, (2)EnRiskS, Australia, (3)AECOM, Australia, (4)Exxon Mobil

Nationally adopted ecological surface water quality criteria (SWQC) for perfluorooctane sulfonic acid (PFOS) vary several orders of magnitude between different jurisdictions. The variability between SWQC emphasizes the importance of understanding how criteria are derived and the assumptions, ecotoxicity data and methodologies underpinning the values. We present a systematic investigation across three jurisdictions, Australia, Canada, and the USA, to characterize and assess the underlying drivers of the differences between their chronic aquatic life SWQC for PFOS. The investigation focused on three variables: 1) selection of ecotoxicology studies, specifically the methods for evaluating study quality and exclusion criteria; 2) data reduction, or the methods for selecting a single toxicity value for a species or genus; and 3) SWQC derivation, or the processes for analyzing the reduced dataset and deriving a final criterion. The studies selected by each jurisdiction and rationales for excluding certain studies were compared. Each country's derivation methodology was applied to the other country's final data set to assess differences in data reduction and SWQC derivation approaches, including evaluating differences in species sensitivity distribution software and model fits. The underlying dataset is the primary variable determining differences in SWQC outcomes across jurisdictions. Decisions regarding study suitability differed considerably with only a limited number of studies used by all three jurisdictions in chronic criteria derivation. Differences in data processing steps and application of uncertainty factors also have a significant impact. Greater transparency and consistency in processes, including data selection criteria, would be useful and may help to reduce differences. The results of this study can inform policy decisions related to updating future SWQC values and derivation methodologies.

7.05.P-Mo-115 Translating water quality datasets in State regulatory programs and impacts to local water quality improvement efforts

Isabella Nelson¹, Katie Murray² and Kim Anderson¹, (1)Oregon State University, (2)Oregonians for Food and Shelter

Regulatory programs impacting water quality involve the application and translation of complex toxicological science, and communication with non-scientific audiences, in order to potentiate positive changes on the ground. Oregon is home to a unique regulatory program, the Pesticide Stewardship Partnership (PSP), that aims to protect water quality through partnerships with industry and voluntary stewardship. The PSP operates through a limited water quality monitoring program that samples stream reaches adjacent to a number of land uses, including urban, agricultural and forestry. Analysis of grab samples for pesticides identifies compounds of concern and these data are reported back to stakeholder groups by state agencies. Stakeholders include university extension and local soil and water conservation districts, some of whom have obtained funding from the PSP program to build local partnerships, implement targeted education, and effect change. Historically, the PSP program has achieved notable successes by focusing on effective Best Management Practices that are targeted to relevant locations and pesticide uses in a given watershed. In recent years, problems with data analysis and interpretation are impacting the program's ability to target and address issues effectively, and this has limited overall programmatic outcomes. Accurate application and translation of rigorously conducted science by regulatory bodies is essential to ensuring not only the success of programs on the ground, but also

lasting water quality improvements. This presentation highlights specific challenges related to science application from Oregon's Pesticide Stewardship Partnership Program and makes recommendations for improving outcomes based on a re-analysis of 10-year water monitoring data sets from two distinct watershed basins. All of these data are publicly available, and we have employed rigorous processes and peer review to meet program goals and outcomes.

7.05.P-Mo-116 Assessing environmental monitoring data quality and reliability in TSCA risk evaluations using 4-tert-octylphenol as a case study

Jennifer Bare, Chloe Louise Fender, Cynthia Torres and Julie Panko, ToxStrategies

Environmental and human health risks are determined for existing chemicals undergoing an EPA TSCA risk evaluation. One component of this risk assessment is evaluating environmental concentrations with via monitoring datasets, gray and peer-reviewed literature, and modeling. Here, we spatially and temporally assessed data available in EPA's Water Quality Portal (WQP) for use in TSCA risk evaluations using 4-tert-octylphenol (4-tOP) as a case study. Data from 2012 through 2022 (the last year of available data) were evaluated based on the Draft TSCA Systematic Review Protocol and prior risk evaluations. The groundwater, surface water, and sediment datasets in WQP were moderate to large in size (i.e., >600 samples) but were highly censored, with detection frequencies less than 10%. The soil dataset was very limited (approximately a dozen samples), but it had the highest detection frequency, approximately 33%. Across all media, two of the maximum values were equal to the detection limit for the sample. Overall, the highly censored datasets limit fate and transport analyses based on 4-tOP chemical properties and meaningful spatial or temporal comparisons. As with most monitoring databases, complete details for a quality and reliability assessment may not be readily available or may require thorough review of database parameters, particularly for a database such as the WQP, which combines several reporting organizations. Although each TSCA existing chemical risk evaluation is meant to be fit-for-purpose and rely on a weight-of-evidence approach that includes more than WQP data, a standard approach is needed for determining environmental concentrations, particularly when deciding whether to (1) perform a qualitative or quantitative assessment or (2) rely on measured or facility-specific modeled data. Finally, when characterizing environmental concentrations, a standard approach is needed for handling non-detects in highly censored datasets, because the handling method may impact EPA's determination of unreasonable risk.

7.06.P-We The Growing Interface Between Science and Policy: Challenges and Strategies for Making Progress

7.06.P-We-121 From risk to resilience: Identifying needs for addressing contaminants of emerging concern in Hawai'i

Lauryn Rachel Hansen and Eileen Nalley, University of Hawai'i, Manoa

Hawaii's ecosystems and communities face increasing threats from contaminants of emerging concern (CECs), including pesticides, pharmaceuticals, and microplastics. CECs encompass a broad range of chemicals that are not routinely monitored, pose a real or suspected threat to human and ecosystem health, and are poorly understood. These pollutants may jeopardize native species, contaminate local waterways and food systems, and, in turn, disrupt community well-being and island stewardship efforts. Addressing CECs is thus more than an issue of environmental protection; it is a matter of environmental justice, ensuring that those most impacted have a voice in the management and restoration of Hawaii's natural resources. Recognizing this, Hawaii Sea Grant is collaborating with state and federal agencies, researchers, and community organizations to develop a comprehensive needs assessment and monitoring guide for CECs in Hawaii and the U.S.-Affiliated Pacific Islands. This guide will identify existing CEC efforts, highlight opportunities for expanding monitoring and management, and emphasize the importance of protecting local resources. By integrating scientific expertise, regional insights, and models from other regional and international frameworks, this effort aims to guide best practices for prioritizing contaminants, evaluate monitoring gaps, and inform management decisions. A

proactive, interdisciplinary framework can empower decision-makers to implement science-based policies that safeguard native biodiversity, protect ecosystem services, and promote food and water security. Additionally, it fosters collaboration that bridges policy and research to strengthen Hawaii's resilience against emerging pollution threats and work towards equitable access to clean water and healthy environments.

7.06.P-We-122 A communication strategy to limit the use of PFAS, flame retardants and other organohalogen chemicals of concern

Arlene Blum, Rebecca Fuoco and Anna Soehl, Green Science Policy Institute

Since 2010, the Green Science Policy Institute has developed a joint strategy with academic and NGO collaborators to more effectively use our peer-reviewed scientific papers to protect human and environmental health. We design policy-relevant research projects and use our communication strategy to bring our research results to government and business decision makers. This strategy has contributed to our papers having high impact scores, multiple citations, and thousands of downloads. Wide communication of our papers has contributed to changes in policy, manufacturing, and purchasing for reduced use of organohalogen chemical classes of concern: flame retardants, PFAS and antimicrobials. Examples include: (1) Our 2011 and 2012 studies finding flame retardants in juvenile products and furniture contributed to the 2013 update of California's furniture flammability standard and the federal 2021 US COVID-19 Regulatory Relief and Work from Home Safety Act. These updates increased fire safety, reduced the need for flame retardants in these products, and contributed to healthier homes across North America. (2) Our 2010 San Antonio Statement, 2015 Madrid Statement, and 2017 Florence Statement documented the scientific consensus on the need to reduce the use of organohalogen flame retardants, PFAS and antimicrobials. These statements provide a scientific basis for manufacturers and retailers to remove harmful organohalogens from the products they manufacture and sell. (3) Our peer-reviewed papers from 2016 to 2022 finding PFAS in US drinking water, food packaging, carpeting, cosmetics, and textiles contributed to remediation and policy change, decreasing harm from PFAS. Our communications strategy, which will be discussed in more detail: •Select a research topic to support policy in the public interest. Collaborate with expert authors from multiple institutions. Once a manuscript is accepted, work with the journal to select an embargo release date several weeks in the future for maximum impact. Compose a press release in accessible language with a "hook" to attract attention. Query journalists and then share the embargoed press release and paper. Educate journalists on the problem, research results, and policy recommendations and establish relationships. Utilize paper to protect health and the environment from harmful chemicals. We encourage scientists to consider such a communications strategy to increase the reach and impact of their publications.

7.06.T The Growing Interface Between Science and Policy: Challenges and Strategies for Making Progress

7.06.T-01 It's time for science-based recommendations to be used to inform action on plastic chemicals in the global treaty

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The unparalleled rise in global plastic production has resulted in widespread pollution and exposure of humans and wildlife to thousands of hazardous chemicals. Many of the chemicals used across the plastics life cycle meet criteria for persistence, bioaccumulation, mobility, and toxicity, characteristics that are used globally to designate chemicals of concern. The majority of these compounds remain unregulated under existing multilateral environmental agreements, warranting further action. In response, the United Nations Environment Assembly (UNEA) has mandated the development of an international legally binding instrument to end plastic pollution, for which negotiations began in 2022 and continue with the next meeting set for August 2025 in Geneva. Although current treaty negotiations have begun addressing a short list of plastic chemicals, significant gaps remain. These include a comprehensive perspective on the diversity of plastic chemicals and their related mixture effects, insufficient regulatory mechanisms, lack of chemical transparency and industrial accountability, and reliance on ineffective recycling strategies that reintroduce toxic substances into consumer products and the environment. While regulatory gaps persist, the presence of harmful chemicals in plastics contributes to major public health burdens, with high annual costs. Vulnerable populations—including children, reproductive-aged individuals, and vulnerable communities—face heightened risks. We therefore recommend six critical components for the treaty to effectively reduce the impacts of plastic chemicals: (1) reduction and simplification of polymers and chemicals, (2) safer and sustainable design of plastic chemicals, (3) incentives for change, (4) holistic (full-life-cycle) approaches for alternatives, (5) just and equitable interventions, (6) centering human rights. Group-based regulation should replace individual chemical approaches to prevent regrettable substitutions. Lastly, binding, global obligations, rather than fragmented or voluntary measures, are vital for chemical safety, circularity, and accountability across the plastics life cycle. A strong, effective treaty is a critical opportunity to achieve a safer, more sustainable future for humans and environmental health.

7.06.T-02 On the inside: Elements of corporate culture that contribute to manufacturing doubt in public health

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Examples of corporations manufacturing doubt to protect profits while obscuring public health have been identified across many industries. These investigations focus primarily on how corporations manufacture doubt to influence external audiences. There are fewer explorations of how companies control information and manufacture doubt internally, since internal corporate communications are tightly controlled and active or former employees may face retribution for speaking about their experiences. Using the case of one company's role in the global contamination of per- and polyfluoroalkyl substances (PFAS), our work explores strategies of manufacturing doubt and narrative manipulation inside the corporation. Understanding how corporate leaders and executives advance internal priorities by controlling scientists and scientific communications provides insights into the practices, controls, and culture that may shape decision making and external messaging pertaining to public health crises. We rely on two principal data sources for our analysis: internal 3M documents (principally those made public via the State of Minnesota vs. 3M Company lawsuit), and first-hand accounts of scientists working within 3M during a five-year period (1997-2002) spanning 3M's decision to publicly disclose the presence of 3M fluorochemicals widespread in the blood of the population and in the global food web. We find that hierarchical position, access to information (current and historical), functional isolationism, and intimidation may be employed to control what data are collected, how data are shared, and the interpretation of the data. While our analysis focuses primarily on the manifestation of these tactics on individual employees, the resulting internal ignorance can magnify across workgroups, departments, or regions, ultimately shaping a consolidated corporate viewpoint. There are few analyses of tactics employed within a corporation to control the production and dissemination of knowledge. Using a current public health crisis, our discussion introduces

specific accounts useful in developing an understanding of corporate culture that may compromise public and environmental health.

7.06.T-03 Questionable safety vs. known harms: Flame retardants in lithium-ion battery enclosures

Anna Soehl¹, Arlene Blum¹, Miriam L. Diamond², Ariana Spentzos¹, Anna Shalin³ and Jolie Miller³, (1)Green Science Policy Institute, (2)School of the Environment, University of Toronto, Canada, (3)University of Toronto, Canada

Rechargeable batteries are an important tool for achieving net-zero carbon emission goals. By 2030, the market value of lithium-ion batteries is forecasted to surpass \$400 billion (predicted ca. 25% growth). However, as the current technologies pose a risk of highly energetic fires, safety standards are being hastily adopted, often resulting in the unnecessary use of harmful flame retardants. While safety standards do not directly mandate the use of flame retardants, adding them to plastics remains a cost-effective method to meet common combustion tests. For example, plastics that meet UL94 V-0 ratings often contain 10-20% flame retardants by weight but are rarely disclosed. Such ratings were developed to prevent the spread of fire from small ignition sources.

However, the current requirements for fire-resistant battery enclosures are not justified—there are no publicly available data demonstrating that flame-retarded plastic enclosure can slow or stop highly energetic thermal runaway fires. The unnecessary use of flame retardants in battery enclosures inside many products (earbuds, e-bikes, electric vehicles, etc.), is concerning since these chemicals can: • harm workers and nearby communities during production, migrate out of plastics during use into air, dust, water, our food supply, animals, and people, contaminate waste and recycling streams, increase smoke, soot, and toxic gases such as dioxins and furans during combustion—the main causes of fire deaths and likely contributing to firefighter cancer. This talk will alert participants to the need to research and communicate with decision makers in business and government the identity of flame retardants in electronic enclosures, their environmental fate, adverse health effects, and the importance of evidence-based safety standards as well as investing in safer battery technologies. Commonly used flame retardants come with serious public and ecological health hazards and should only be used when their benefits have been demonstrated and outweigh known hazards.

7.06.T-04 Textile perspective is critical to drive solutions to microfiber pollution in California

Krystle Wood¹,Carolynn Box¹ and Kyla Kelly², (1)Materevolve, (2)California Ocean Protection Council

Microplastics, including microfibers, are ubiquitous and continue to be persistent and extremely challenging, if not impossible, to effectively remove once in the aquatic environment. The California Statewide Microplastics Strategy calls for workshops to identify sector-specific recommendations and immediate actions that could be enacted to reduce microplastic pollution in California. For the Textile Sector, Materevolve, in collaboration with the California Ocean Protection Council, convened a range of stakeholders, including textile designers, textile manufacturers and chemists, apparel brand representatives, ocean researchers, toxicologists, and others to identify short term recommendations, long-term policy needs, barriers, and research priorities to reduce microfiber pollution. Specifically, the workshop drove collaborative discussions between industry, scientists, and policymakers on four key focal areas to better design textile systems to prevent and mitigate the prevalence, toxicity, and persistence of microfiber pollution from source to sink. These four focal areas included material and chemistry innovation, textile design and manufacturing, product use and care, and end of life considerations. As a direct result, a report entitled California Statewide Microplastics Strategy Workshop: Textile Sector was developed and is available as a public resource. This report identifies key actions the State of California could take to advance solutions to microfiber pollution, such as identifying best practices to reduce fiber release during textile design and manufacturing and generating incentives for innovators who are looking to re-design current textile systems. This workshop broadened the discussion to new and important stakeholders from the textile sector and demonstrated that collaboration and connection between diverse, cross-sector stakeholders is critical to making progress and advancing science and policy solutions to microfiber pollution.

7.06.T-05 The global PFAS science panel: Informing decision-makers about hazards and risks of per- and polyfluoroalkyl substances (PFAS)

Jamie DeWitt, Oregon State University

The Global PFAS Science Panel (GPSP) was founded in 2018 by two of the organizers of the meeting that resulted in the Zurich Statement on future actions on per- and polyfluoroalkyl substances (PFAS). Although not global in membership, including members from the European Union and the United States, the GPSP attempts to be global in its reach. The GPSP is comprised of regulatory and academic scientists committed to the GPSP mission of fostering development of high-quality scientific research, stewarding information exchange, and coordinating advancement of science and policy to address PFAS as a global concern. The panel works to facilitate international collaboration among scientists, policymakers, and other partners and attempts to identify research priorities as well as collect and synthesize existing information with respect to PFAS, including persistence, mobility, bioaccumulation, exposure, biomonitoring, and toxicity data. The panel also works to establish a system to differentiate between essential and non-essential uses of PFAS and work toward the global phase-out of non-essential uses of PFAS. The GPSP also coordinates scientific inputs for policy processes and communicates with scientists, policymakers, and partners throughout the scientific community. The GPSP outlined the "essential use concept" for PFAS that has been implemented by several states within the United States and is a guideline for addressing PFAS in the European Union. This concept asserts that if a PFAS use is not necessary for the health, safety, and functioning of society and if a more sustainable alternative is available, that use is not considered essential. The GPSP also has worked to outline the true societal costs of PFAS uses and define the P-sufficient approach for PFAS that asserts that persistence is sufficient to phase-out PFAS in products and processes. The GPSP builds relationships between scientists and policymakers through scientific publications and targeted meetings that bring together scientists and policymakers working together to fill information gaps on PFAS. **Disclaimer/Disclosure:** JCD declares that she receives state and federal funding to study the immunotoxicity of PFAS and serves as a plaintiff's expert witness in cases involving PFAS.

7.06.T-06 The six classes concept and a strategy towards a healthier world (science + communication + decision makers = positive change)

Arlene Blum, Rebecca Fuoco and Anna Soehl, Green Science Policy Institute

This talk will demonstrate how rigorous scientific research communicated to decision makers and strategic policy work can reduce the use of harmful organohalogen chemicals in products worldwide. Rather than addressing the tens of thousands of chemicals in everyday products one at a time, the Six Classes concept, developed by the Green Science Policy Institute, educates decision makers in government and business about six chemical families containing many of the harmful substances in consumer products. This approach can prevent a cycle of "regrettable substitution," whereby a phased out harmful chemical is replaced with a closely related "chemical cousin" which is likely to cause similar harm. This talk will focus on success stories from two organohalogen classes—PFAS and flame retardants. Organohalogens are not found in mammalian biochemistry and not recognized by efflux transporters which protect our cells. In the 1970s, Arlene Blum and Bruce Ames found that organohalogen flame retardants commonly used in children's pajamas were mutagens and likely carcinogens. Furthermore, their carcinogenic metabolites were found in the urine of a child wearing flame retarded pajamas for one night. Through their scientific publications, media outreach, and policy engagement, the use of these chemicals in children's sleepwear was stopped soon after the publication of their research findings—one of the earliest examples of successful science-based chemical regulatory change in the U.S. However, TDCPP or Chlorinated Tris (Tris(1,3-dichloro-2-propyl) phosphate), continued to be used in furniture, vehicles, and other products after being removed from children's pajamas. A coalition of scientists, trade associations, manufacturers, and firefighters were then able to change flammability standards so that flame retardants were no longer needed in furniture and children's products—leading to reduced harmful exposures and healthier homes. Ongoing success stories are provided by PFAS, for which the class concept is especially useful. Research papers on PFAS in US drinking water, fast-food packaging, cosmetics, and school uniforms, widely communicated and shared with decision makers in government and business, have contributed to the

reduced use of PFAS in these products. This talk should encourage scientists to design policy relevant research and share their findings with decision-makers with the objective of reducing harmful chemical exposures.

7.06.T-07 From assessment to action: Building a southeastern contaminants of emerging concern program

Brooke Saari¹, *Katy Smith², Lola Renauer¹ and Catherine Janasie³, (1)South Carolina Sea Grant Consortium, (2)University of Georgia, Marine Extension and Georgia Sea Grant, (3)National Sea Grant Law Center, University of Mississippi School of Law*

Contaminants of emerging concern (CECs) are chemicals increasingly detected in the environment that may pose risks to both ecosystems and human health. Despite their potential impacts, the distribution and effects of CECs remain poorly understood, and many are not yet regulated under current environmental laws. To address this gap, a comprehensive needs assessment was conducted to guide an interdisciplinary approach for investigating CEC drivers and identifying actionable solutions. Through a multi-faceted needs assessment effort, which included a literature review, a targeted policy scan, and the formation of an Advisory Council (AC), research gaps were identified and project priorities refined. These priorities informed the launch of a competitive research program, which has now funded six projects across three states (FL, GA, and SC). The program uses a collaborative study group model that brings together faculty, students, and outreach professionals to enhance awareness of CECs and broaden end user engagement in the research process. In addition to the research program, the initiative has expanded its impact through the hiring of a graduate student, a law student, and a summer undergraduate intern; the development of engagement and outreach initiatives; and the creation of an interactive regional network to foster ongoing collaboration and knowledge exchange. These early efforts have focused on identifying and addressing CEC-related needs in the southeastern U.S., intending to create scalable tools and strategies that can be applied more broadly. The findings from the funded projects will serve as a foundation for continued networking, education, and community-engaged research. This presentation will highlight the methodology used to identify regional needs, the role of end user engagement in shaping research priorities, and the broader implications for managing CECs in diverse environmental contexts.

7.06.V The Growing Interface Between Science and Policy: Challenges and Strategies for Making Progress

7.06.V-01 A potential approach to addressing microplastics—California's safer consumer products program

Dicle Yardimci, *Topher Buck and Anne Doherty, California Department of Toxic Substances Control*

Microplastics (MPs) are small pieces of plastic, generally considered to have a maximum dimension of 5 millimeters. MPs are persistent, mobile, and ubiquitous in the environment. They have been detected in every ecosystem and in all media, including water, air, soil, household dust, drinking water, and foods and beverages that humans consume. Exposure to MPs may cause physical stress and cellular, tissue, and organ damage; disruption of the endocrine and immune systems; and have negative impacts on mobility, reproduction, and development. MPs have been detected in human blood, lungs, placentas, testes, breast milk, and stool. Wildlife can mistake MPs for food, which can impair wildlife survival and reproduction. California's Department of Toxic Substances Control (DTSC) is concerned about the adverse impacts of exposures to MPs on the general public, pregnant people, children, workers, wildlife, and the environment. In 2023, we proposed to add MPs to our Candidate Chemicals List. If we succeed in doing so via the rulemaking process, we will gain the authority to regulate consumer products that contain or have the potential to generate MPs. Additionally, we are in the early stages of evaluating consumer products that may contain or generate MPs during their life cycles for potential regulation, anticipating the Candidate Chemical Listing is successful. This talk will provide an overview of DTSC's Safer Consumer Products Program and the basis of our proposal to add microplastics to the Candidate Chemicals List. It will also highlight what a potential microplastic regulation could look like

under the DTSC program. In the past, we have successfully promoted safer chemistries for nail products, detergents, tires, paints, carpets, and children's products via rulemaking.

7.07.P-We General: Policy, Management and Communication

7.07.P-We-128 Identifying and prioritizing sites in Oregon with suspected per- and polyfluoroalkyl substance contamination

Brian G Church, Daniel Hafley, Sarah Van Glubt, Franziska Landes, Amanda Wozab, Julie Harvey and Ratnanjali Adhar, Oregon Department of Environmental Quality

The Oregon Department of Environmental Quality (ODEQ) initiated rulemaking in 2024 to list several per- and polyfluoroalkyl substances (PFAS) as hazardous substances in the state, allowing for state-led environmental cleanup of PFAS at contaminated sites. In preparation, ODEQ's Environmental Cleanup program created a framework to identify suspect PFAS sites and prioritize initial actions that take into account several factors including but not limited to agency resources, local demographics and environmental justice indicators, natural resource vulnerability and known impacts, and a "worst first" approach for targeting industries and associated industrial properties. Accordingly, the industry tiering and site ranking framework balances different agency priorities and attempts to set realistic goals. Roughly 100 suspect sites have been identified and ranked across Oregon thus far, and this list is expected to grow as more data become available. This presentation explores the framework, its goals, initial outcomes, and next steps.

7.07.P-We-129 Public engagement on contaminants of emerging concern in carrots and kale irrigated with recycled and river water

Richard Jack¹, Edward P. Kolodziej², Kristina Westbrook¹, Erika Kinno¹, Hannah McDonough,³ Douglas Collins⁴ and Hatch Jason³, (1)King County, (2)University of Washington, Tacoma, (3)Washington Water Trust, (4)Washington State University, Puyallup

King County, in Washington State, has a strong commitment to environmental protection, restoration, and preservation of our shared water and land resources. Together with our Tribal partners, cities, and regional non-profits, we have invested millions of dollars in water quality improvements, riparian habitat, restoring functional floodplains, as well as preserving water rights and farmland. As our climate shifts to hotter and drier summers, with reduced snowpack to feed our rivers and streams, having sufficient cool, clean stream flows to support salmon recovery has become more and more of a limiting factor. Building trust and two-way communication with stakeholders is critical to fostering collaborative informed science-based decisions. Changing perspectives, attitudes, and behaviors to allow keystone species like salmon to recover in our region requires dedicated communication and outreach efforts above and beyond good science. This project, a collaboration between local government, a conservation non-profit, and two state universities, studied contaminants of emerging concern (CECs) in membrane bioreactor-derived recycled water and Sammamish River water. We also analyzed soils, carrots, and kale irrigated by both waters. Our goal was to inform farmers and consumers about the risks and benefits of using recycled water instead of river water irrigation. We wanted to inform them to encourage shifts in farmer and consumer perceptions of recycled water irrigation and ultimately leave more surface water available for fish. This project produced detailed estimates of CEC risk to consumers and agronomic benefits to farmers for produce grown on both recycled water and river water over two growing/irrigation seasons. We invested in a variety of fact sheets and graphical interpretations of these risk assessment findings. This poster will highlight these risk and benefit communication tools and techniques. Personal face-to-face outreach, with consumers and producers at farmer's markets and via outreach groups, allowed our non-profit partners to communicate how switching to recycled water irrigated crops can provide more abundant, safe, local food. Before and after surveys helped us understand the increased acceptance of recycled water irrigation. We anticipate an increased willingness of farmers to switch to recycled water leaving

cooler water in the river for salmon in the future. **Disclaimer/Disclosure:** This work was funded in part by King Wastewater Treatment Division and the Bullitt Foundation.

7.07.P-We-130 Enhancing accessibility in watershed sustainability assessment: A user-friendly framework

Megan Dodge, Jose Aguilar Escamilla, Caitlyn Arango, Jasmine Cleveland, Stephen Mensah, Perry Wilson and Stacey L Harper, Oregon State University

Effective watershed management requires tools that integrate sustainability metrics in a way that is both accessible and broadly applicable. However, existing frameworks and case studies are often overly complex, limited in scope, and lack a user-friendly interface, making them difficult for non-experts and other interested parties to use effectively. To address this gap, we developed a new sustainability-focused watershed assessment framework designed for clarity, versatility, and ease of use. Our approach involves reviewing existing models, identifying key sustainability indicators, and designing a streamlined interface that simplifies data input and interpretation. The resulting framework allows users to assess watershed sustainability across environmental, social, and economic areas with minimal technical expertise. We hypothesize that the tool will be valuable for policymakers, educators, and students. Future work will involve usability testing with these target groups to evaluate its effectiveness and gather feedback for iterative refinement of the user interface. This work provides a practical and scalable solution for watershed sustainability assessment, enhancing accessibility of analysis and supporting public awareness of issues within watersheds.

7.08.P-Th Late-Breaking Science: Policy, Management and Communication

7.08.P-Th-076 Mentoring and skills development training in One Health (MSDT-One Health): An innovative national mentoring and training program in One Health and toxicology

Wilson Kiiza Rumbeiha¹, Laura Van Winkle¹ and La Cole Blackshire², (1)University of California, Davis, (2)Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis

The NIH NIGMS has awarded a new 5-yr mentoring and skills development training grant in One Health (OH) to a consortium of institutions led by the University of California, Davis. Collaborating institutions include SETAC, Meharry Medical College, and Iowa State University. One Health research is an integrated approach aimed at optimizing the health of people, animals, and the ecosystem in their shared environment.

Environmental toxicants are environmental stressors and they negatively impacting the health of people, animals and the environment. Currently, OH research is facing a major challenge: the lack of engagement of toxicologists and/or people with toxicology knowledge in OH transdisciplinary research. This is partly due to inadequate training of toxicologists in skills essential for successful transdisciplinary OH research and/or a lack of awareness of opportunities in this field as OH is not offered as a major at most undergraduate institutions. To address this gap, the MSDT-One Health national training program has been developed with three specific aims (SA). SA1: Develop and deliver a yr long mentoring program for STEM undergraduate students. SA2: Develop and deliver a yr long professional development training and mentoring program for early career scientists (Post-docs, Fellows, Project Scientists, Assistant Professors) to increase their awareness of the opportunities in OH research and to increase knowledge and their ability to obtain funding necessary to conduct OH research, including community engaged toxicology research. SA3: Develop case studies and e-modules at the intersection of OH and toxicology and make them widely available. Expected outcomes include: a) a total of ~100 undergraduate students mentored in OH with a toxicology emphasis; b) 5-10 early career scientists securing intramural seed funding and 2-3 early career scientists securing extramural funding to conduct OH research with community engagement components; c) development of highly interactive case studies in one environmental health toxicology (5) and learning modules on OH (3) to be widely available for training undergraduates and early career researchers. Ultimately, this research education program in OH and toxicology will strengthen the biomedical research enterprise by adding to the number of scientists with the necessary knowledge to engage in transdisciplinary OH research. Supported by NIGMS R25GM160671

7.08.P-Th-077 Online toxicology module and case study learning resources for mentoring and skills development training in One Health (MSDT-One Health)

La Cole Blackshire, Wilson Kiiza Rumbeiha and Laura Van Winkle, University of California, Davis

The MSDT-One Health program seeks to increase the number of biomedical scientists with a foundation in toxicology to engage in transdisciplinary One Health (OH). Currently there is an unmet need in the biomedical research workforce: a lack of toxicologists and/or a lack of biomedical scientists with knowledge and skills to comfortably engage in transdisciplinary OH research. Increasing the number of scientists with a toxicology background in transdisciplinary research will improve human and animal health by generating new knowledge on how environmental toxicants impact human and animal health. The new MSDT-One Health program is targeting STEM undergraduates and early career researchers to increase their toxicology and OH knowledge and research skills. This will be partly achieved by requiring mentees without toxicology training or background to take training in toxicology by completing toxicology case studies and e-modules developed through prior funding from the NIGMS (1R25 GM 113989, R25 GM 139200). These resources are freely accessible 24/7 online to anyone with hand held devices or computers connected to the internet and the resources are maintained and housed at the University of California Davis. Examples of e-modules include Principles of Toxicology, Pathophysiology, Neurotoxicology, Developmental toxicology, Reproducibility and Rigor and Environmental Toxicology. Examples of case studies include studies of Lead, Vaping, Ethanol, Arsenic and Aflatoxins. These case studies and e-modules are especially beneficial to junior biomedical research scientists who lack prior training in toxicology and assist with professional development by providing a framework and guided further training in toxicology. New modules and case studies will also be developed and then added through the newly funded NIH NIGMS mentoring and skills development training grant (1R25GM160671) to strengthen skills in transdisciplinary One Health with a special focus on key skills needed for toxicology research.

8.01.T Real World Pacific Northwest: What It Takes to Build Solutions for Endangered Species and Agriculture

8.01.T-02 Agricultural commodity group: An introduction to cropping systems in Western Washington

Kara Rowe, KR Creative Strategies

The Western Washington Agricultural Association serves agricultural interests across western Washington with a majority of membership located in the highly productive Skagit, Snohomish, and Whatcom Counties. This talk will provide an overview of the cropping systems in these regions.

8.01.T-03 Oregon farmer: Environmental stewardship success over the last 30 years

Jason Sandahl, Ag Aligned Global

The concept of environmental stewardship has existed in agriculture for decades. This talk will highlight how farmers have complied with regulatory pressure across various sectors including pest management, sales/marketing and labor.

8.01.T-04 Oregon crop consultant: Pest management decision making in Willamette Valley Oregon

Michelle Armstrong, Wilbur-Ellis

Drawing from experience in the Oregon Hazelnut Commission and as a crop consultant, this talk will provide an overview of the complex world of farmer decision making in Willamette Valley, and the role of crop consultants in this increasingly complex environment.

8.01.T-05 ESA-species expert: Threatened and endangered salmon in the Willamette Valley

Anne Mullan, National Marine Fisheries Service

Land use has a large influence on the habitat for two main ESA-listed salmonid species in the Upper

Willamette. This talk will provide an introduction to the species, their status, current threats, and recent habitat conservation efforts.

8.01.T-06 ESA-species expert: Threatened and endangered terrestrial species in the Willamette Valley

Kelsey King, *U.S. Fish and Wildlife Service*

Drawing from experience in environmental toxicology and ESA-species recovery, this talk will provide an overview of ESA-listed terrestrial species in the Willamette Valley and concerns related to environmental contaminants.

8.01.T-07 Conservation specialist: ESA-species and farm-level conservation planning

Giulio Ferruzzi, *U.S. Department of Agriculture, Agricultural Research Service*

Ecological Sciences leads the technology for NRCS in environmental compliance technologies to ensure science-based conservation for our Nation's resources. This talk will provide an overview of conservation science in Washington.

8.01.T-08 Conservation specialist: Incentivizing conservation for ESA-listed species in Washington and Oregon

Kevin Scribner, *Salmon Safe*

Salmon-Safe works with West Coast farmers, developers, and other environmentally innovative landowners to reduce watershed impacts through rigorous third-party verified certification. This talk will explore the concept of incentivizing conservation.

8.02.P-Mo Unlocking Insights From Multivariate Environmental Data

8.02.P-Mo-003 Four decades of PFAS in aqueous film forming foams (AFFF): Temporal and manufacturer-specific trends

Christian Rude¹, **Jiaju Fu**², **Boris Droz**¹, **Jennifer Field**¹ and **Gerrad Jones**¹, (1)*Oregon State University*, (2)*Oregon State University, China*

The firefighter's most effective tool for fighting large petroleum and polar solvent-fed fires is the aqueous film forming foam (AFFFs). AFFF formulations contain per- and polyfluoroalkyl substances (PFAS) to achieve low surface tensions which allow them to spread across fuel surfaces extinguishing fires and preventing reignition. While effective, many PFAS contained in these foams are recalcitrant. PFAS-containing AFFFs have been used at airports, military bases, and some chemical manufacturing facilities, resulting in groundwater contamination. People are exposed to contaminated groundwater when plumes intersect private or public wells or local surface waters. Research into PFAS toxicity is ongoing and exposure to PFAS is strongly correlated with immune dysregulation, liver disease, and some types of cancer. Thorough knowledge of AFFF PFAS composition is needed to account for PFAS in soil and water, and to predict their fate, transport and hazard in the environment. To this end we acquired high resolution mass spectrometry data (HRMS) for a collection of 215 AFFFs produced by 10 different manufacturers from 1974 to 2018. Samples were analyzed with a SCIEX ExionLC UPLC system coupled with an AB SCIEX 7600 mass quadrupole time of flight spectrometer (QTOF) equipped with an electrospray ionization (ESI) source using SWATH data-independent acquisition in the negative ionization mode. PFAS quantification was accomplished across three AFFF dilutions, while nontarget analysis was carried out with 1000X dilutions to capture less abundant suspect and nontarget PFAS components. Quantification of PFAS was accomplished in SCIEX OS; nontarget analysis was performed in R using patRoom. Target, suspect, and nontarget HRMS data clearly distinguished between fluorotelomer and electrochemical fluorination manufacturing of AFFFs. PFAS concentrations were highest in 3M foams, which also contained characteristic branching of polyfluorinated tails typical of electrochemical fluorination. Clear differences in chemical signatures differentiated AFFF made using fluorotelomer-based PFAS including Ansul, National Foam, and Solberg manufacturers, while Chub National Foam and Chemguard were similar. Continuing work

includes positive-mode acquisition and volatile analysis by gas chromatography QTOF mass spectrometry with the goal of creating chemical fingerprints to aid in source attribution and informing appropriate chemical and toxicological analysis at contaminated sites.

8.02.T Unlocking Insights From Multivariate Environmental Data

8.02.T-01 Unveiling rubber additives and emerging transformation products in complex matrices with multiple suspect and nontargeted screening strategies

Wei Wang, Melissa Gonzalez and Edward P. Kolodziej, Interdisciplinary Arts and Sciences, University of Washington, Tacoma

Synthetic consumer products, while often beneficial and useful, contribute to the dispersal of complex chemical mixtures and their transformation products (TPs) into the natural environment. A growing concern is the release of numerous chemical additives and TPs from rubber products to the ambient environment. Rubber products, including automobile tires, hoses, tapes, cables, electrical wires, contain complex additive formulations (e.g., antioxidants, vulcanizing agents, plasticizers) that are essential for the structural integrity and durability. However, constant environmental exposure and abrasive wear can facilitate the reaction and migration of these chemicals and their TPs, some of which, like N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine quinone (6PPD-Q), exhibit significant toxicity and pose ecological risks to sensitive habitats. Despite their extensive use, a critical knowledge gap persists regarding the specific compositions, occurrence, and fate of these additives and TPs among various environmental compartments. This study describes the application of high-resolution mass spectrometry (HRMS)-based suspect and nontargeted screening methods for identifying and elucidating the structures of multiple emerging rubber additives and TPs. Multiple annotation strategies, including: (1) bottom-up fragmentation pattern-based screening, (2) case-control differential analysis, and (3) in-house library-based suspect screening, were developed. These strategies were applied to various complex matrices, spanning environmental and biological samples. The data revealed extensive biotic and abiotic transformations of rubber additives, leading to the identification and structural elucidation of a large array of TPs (confidence level ≥ 3). Complementary analysis confirmed the ubiquitous occurrence of these chemicals and their TPs across various environmental compartments. These findings illustrate significant applications of screening strategies in the identification of novel contaminants from consumer products. Furthermore, they highlight strategies for comprehensive characterization of rubber additives and related TPs to aid a more complete risk assessment of emerging rubber-derived environmental contaminants.

8.02.T-02 Residual mapping applied to multivariate PCB source apportionment models: A case study from the Portland Harbor Superfund site

Glenn W Johnson¹, James Peale² and Sara Moore³, (1)GeoChem Metrix, (2)Geosyntec Consultants, Inc., (3)Formation Environmental

Multivariate mixing models (receptor models) are widely used for source apportionment of polychlorinated biphenyls (PCBs). However, a challenge arises when two or more contributing sources have highly similar, near colinear source profiles. To address this, we borrow a tool from linear regression: analysis of residuals. We present results of linear mixing model as applied to three PCB sediment datasets from the Portland Harbor Superfund Site (PHSS) all analyzed by USEPA Method 1668A. We present a five end-member constrained least squares mixing model applied to each of the three data sets. Consistent with previously published PHSS receptor models (Megson, et al., 2023; Rodenburg, et al., 2015) the end-members in our model match commercial PCB products that accounted for the vast majority of Monsanto sales: Aroclors 1242, 1248, 1254 and 1260. For the residual analysis, we calculated measured vs. model predicted levels for all variables in the model. We present results using two graphical formats (1) measured vs. predicted congener profile comparisons, and (2) residual maps showing excess measured vs. predicted levels. For this presentation, we focus primarily on PCB-15 residuals. In all three datasets, we observe high residual PCB-15 levels (i.e., high excess measured PCB-15) in one area of the site where the samples are otherwise consistent with predominant

Aroclor 1242 and 1248 contributions. Other areas of PHSS have high contributions from these two Aroclors, but samples in those areas do not exhibit high residual PCB-15 to the same degree. Recent research suggests that this congener profile may reflect a polychlorinated terphenyl (PCT) product: Aroclor 5432, marketed in the early 1970s as a replacement for PCB-containing hydraulic fluids. PCT Aroclor contained up to 5 percent PCBs as an unintended byproduct. The congener profile of the PCB fraction of Aroclor 5432 is similar to Aroclor 1242, but with excess PCB-15.

8.02.T-03 Chemical signatures of biosolids-amended soil: Risks and remediation potential

Marie Olland, Tala Navab-Daneshmand, Gerrad Jones, Manuel Garcia-Jaramillo and Lewis Semprini, Oregon State University

As global population grows, so does waste generation, with nearly half of all solid waste being land applied, concerns over contaminant exposure are increasing. While biosolids land application offer great sustainability benefits, current regulations focus on pathogens and a limited number of metals, ignoring the wide range of organic compounds that pose potential environmental and human health risks. We hypothesize that the combination of different remediation strategies will reduce biosolids introduced contaminant abundances, providing insights to inform safer biosolids management practices. This study investigates the impact of biosolids amendments on soil chemical composition and assesses the remediation efficacy of biochar and grass growth over the course of nine weeks. Samples included soil pots with combinations of untreated soil, biosolids-amended soil, biochar-amended soil, and both biosolids and biochar-amended soil, all with and without grass growth. Ultra-high performance liquid chromatography coupled with high-resolution time-of-flight mass spectrometry enabled detailed chemical characterization. Novel workflows employing non-target and suspect screening, ation potential of biochar. In these samples, highlighted compounds associated with microbial metabolites and accumulaand multivariate statistical and machine learning techniques were used to analyze chemical profiles across samples. Our results show over a 2-log increase of pharmaceuticals such as miconazole, and metoprolol with known environmental toxicity in biosolids-amended soils. Furthermore, biosolids and biochar treated samples showed a divergence in temporal chemical profile trends, demonstrating the promising remedition of hydrophobic compounds pointing to biotransformation and sorption as dominant pathways for biochar remediation. While grass contributed less to altering chemical profiles, this study highlights the need to address chemical risks associated with biosolids land application and demonstrates potential remediation strategies to mitigate them. Our findings contribute to a more holistic understanding of waste-derived contaminants in the environment and supports the development of sustainable data-driven waste management practices.

8.02.T-04 How much data is enough? an information-theoretic look at redundancy in chemical datasets

Mahmoud Fakhry, Rithika Ganesna and Gerrad Jones, Oregon State University

Environmental forensics increasingly leverages high-dimensional analytical techniques which can generate hundreds to thousands of chemical variables per sample. While these data-rich approaches promise improved forensic resolution, they also increase analytical complexity, redundancy, and cost. This raises a key question: how much of the data is actually useful? To address these issues, we developed an information-theoretic framework to evaluate and prioritize datasets based on their internal redundancy and informational value. We applied this framework to a dataset of 203 aqueous film-forming foam (AFFF) formulations to assess the relative information content of the two NMR datasets. To quantify informational value, we computed normalized Shannon entropy (ranging from 0 to 1, where 1 indicates maximal information content and 0 indicates none) and pairwise mutual information (MI) (also scaled 0 to 1, with 1 indicating total redundancy and 0 indicating fully independent variables). Dimensionality was first reduced via non-metric multidimensional scaling (NMDS), followed by hierarchical and k-means clustering to extract grouping structures for analysis. Across most clustering configurations, the ^{19}F NMR data showed substantially higher entropy values (0.591–0.791) and lower redundancy (MI = 0.19) compared to the ^1H NMR data, which exhibited lower entropy (0.380–0.462) and high redundancy (MI = 0.97). These results indicate that ^{19}F data contain more unique and

non-redundant features, making them more potentially more informative for AFFF classification and source tracking. This generalizable approach offers a quantitative method to evaluate and prioritize high-dimensional analytical datasets when instrument time or resources are constrained. While demonstrated here using NMR data in the context of AFFF forensics, the framework can be readily applied to other data types, such as high-resolution mass spectrometry (HRMS), to support informed decisions across diverse environmental applications.

8.02.T-05 Strategies for nontarget identification of transformation products of gas-surface multiphase chemistry in indoor dust

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Humans increasingly spend most of their time in indoor spaces that are highly contaminated with xenobiotics from building materials, personal care products, furniture, appliances, cleaning products and biogenic emissions. Many of these contaminants are primarily found in the condensed phase, where they have long lifetimes and are prone to slow gas-surface multiphase chemistry. Previous work has shown that products of this chemistry can be observed in indoor dust, however, the thousands of chemicals that have been observed in dust and tens of thousands of resulting theoretical products present a significant analytical challenge. As a result, it is unknown how widespread these transformation products are or how much of a human health risk they pose. To identify the prevalence of the transformation products of gas-surface multiphase chemistry, we exposed four genuine indoor dust samples to ozone and hydroxyl radicals to collect a time series. We then analyzed these dust samples over time using gas chromatography and liquid chromatography with multiple separation chemistries coupled to high-resolution mass spectrometry for comprehensive and complimentary sample characterization. Initial suspect screening shows the presence of products previously observed in literature, but the scale of this dataset and presence of many unknown products makes suspect screening and library matching impossible for large-scale analysis of product formation. Instead, by utilizing modern automated analysis tools for this large dataset, we can identify features that exhibit product-like or precursor-like kinetic behavior, assign molecular formulas to these features, and connect precursors to products using exact mass differences. This molecular network allows large-scale identification of likely products of gas-surface multiphase chemistry, the transformation processes that result in the most common products and increase confidence in the overall prevalence of transformation chemistry in the indoor environment.

8.02.T-06 Ectotherm risk assessment of chronic heat exposure and nutritional bottlenecking

Benjamin Deviney and *Goran Bozinovic, University of California, San Diego*

Climate change forces organisms to contend with extreme temperatures coupled with more frequent and longer-lasting extreme weather events. Food sources may be scarce during droughts, limiting dietary options. Predicting survival and both short and long-term adaptive responses to sudden environmental stressors requires an understanding of the complex interactions among critical variables governing phenotypic outcomes. Ectotherms, such as insects, have temperature-sensitive metabolic rates, and respond to thermal shifts altering behavior and physiology. Widespread, chronic heat exposures, however, limit escape from heat stress, increasing mortality rates. Moreover, dietary limitations can result in unbalanced nutrient intake with dire metabolic consequences. Chronic exposures to elevated temperatures and protein-supplemented diets allow for an investigation of the effects of combined stressors. We exposed female and male *Drosophila melanogaster* to elevated temperature and a high-protein diet to quantify the effects of individual and cumulative stress. While higher protein intake lowers locomotion scores (ART-ANOVA, $p < 0.001$), elevated temperature consistently decreases score in males (ART-ANOVA, $p < 0.001$) but not in females. Over a 20-day exposure, female fly lifespan significantly decreased on the high-protein diet (log-rank test, $p < 0.001$), whereas male lifespan was unaffected. We hypothesize that the combination of heat and high protein intake significantly alters metabolism and perturbs microbiota. To better understand the adaptive mechanisms and relationship between the altered

microbial diversity and metabolic gene regulation during heat and dietary stress, we correlated whole-body 16S rRNA microbial sequencing data with female and male fly head gene expression quantified via 3'-end shallow RNA sequencing. This study helps identify adaptive mechanisms to heat and dietary stress and metabolic sex-specific biomarkers of exposure in lab-reared fruit flies. Our data serves as a reference to future studies implicating natural, genetically variable natural populations of fruit flies.

8.03.P-Th Where the Alternatives Meet the Road: Finding Substitutes for 6PPD in Vehicle Tires

8.03.P-Th-120 Turning the Corner on Hazardous Tire Compounds: A Management Framework for Tire Additive Pollution

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Motor vehicles on the world's 36 million kilometers of roads maintain traction through tires and are a major form of transport across most of the world. Tires are complex technical mixtures consisting of ~40 – 50% natural and synthetic rubber, 28% carbon black, 15% steel, and 16-17% fillers and additives. Therefore, emissions of tire particles represent a substantial source of chemicals to the environment, with ~185,000 tonnes of tire additives and fillers being emitted in the U.S. to the environment annually. Together, the large emissions and known human and environmental toxicity of tire additives mean that tire additives represent a global pollution challenge. The implications of tire additive emissions were recently exemplified by the finding that 6PPD-quinone (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone) was acutely toxic to coho salmon (*Oncorhynchus kisutch*) and other salmonids. Further research has shown widespread human exposures, with 6PPD-quinone detected at frequencies of ~60-100% in urine, cerebrospinal fluid, and serum samples. This global pollution challenge demands a commensurate policy response, which current policies do not provide. This presentation will introduce a policy approach created by a group of environmental chemistry, toxicology, and environmental engineering researchers to manage tire additives. We propose five principles for managing tire additives: prioritizing nonhazardous alternatives, ensuring comprehensive regulation, transparency in tire compositions, characterizing hazard, and international harmonization. Following these principles, we outline a Management Framework for Tire Additive Pollution (MF-TAP) that recommends a phased regulatory approach, data transparency, independent expert panels, and internationally coordinated governance to drive the development and use of alternative, nonhazardous compounds. Under this approach, we recommend a phased replacement of 6PPD, with an initial rapid phase-out in favour of a drop-in substitute, followed by a thorough review and, if warranted, phase-out of those drop-in substitutes to avoid a regrettable substitution. Strong provisions in the Global Treaty to End Plastic Pollution around chemical additives of concern could be an excellent way to implement the MF-TAP. Managing tire additives according to the MF-TAP will allow us to better address this global pollution challenge and reduce the impacts of hazardous tire additives on humans and ecosystems.

8.03.P-Th-121 Collaborative innovation to replace 6PPD in tires: Advancing safer alternatives for aquatic health and tire safety

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N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is a critical antidegradant used in tires to prevent premature failure and ensure passenger safety. However, its transformation product, 6PPD-quinone, has been

identified as extremely acutely toxic to coho salmon and other salmonids, posing an urgent environmental health threat in urban waterways. This presentation provides an overview of a collaborative innovation forum that was convened to support multi-sectoral dialog and engagement (including tire manufacturers, chemical suppliers, regulators, academic researchers, and NGOs) to identify strategies to accelerate the development and adoption of safer, effective alternatives to 6PPD. Forum discussions revealed a shared urgency to replace 6PPD, while acknowledging the technical complexity of substitution due to 6PPD's multifunctionality, ubiquity across tire components, and critical role in tire safety. Participants explored the dual imperative of maintaining tire performance and reducing human and ecological toxicity. Key outcomes of the forum included: - A call for defining minimum toxicological criteria for safer alternatives, including transformation products. - Support for tiered testing strategies to expedite "fail fast" screening and reduce innovation costs. - Recommendations for rational chemical design to reduce transformation to toxic quinones while preserving performance. - Recognition of gaps in toxicological and performance standards that impede innovation and regulatory clarity. - Broad interest in pre-competitive collaborations, shared data 215s, and joint testing programs. The forum catalyzed momentum toward establishing a shared roadmap for innovation, investment, and potential voluntary commitments to phase out 6PPD. Participants emphasized the need for multidisciplinary R&D funding, involving material scientists, toxicologists, and environmental engineers alike. While acknowledging the challenges, interested parties expressed commitment to advancing safer solutions that protect aquatic life and public health without compromising tire performance. This case illustrates the power of collaborative innovation to address urgent chemical challenges in a complex, high-stakes application. It offers a scalable model for cross-sector engagement in developing safer, sustainable alternatives across the value chain.

8.03.P-Th-122 Are in vivo and in vitro approaches aligned in evaluating the toxicity of PPDs and their quinones in coho salmon?

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Acute mortality events in urban-dwelling coho salmon (*Oncorhynchus kisutch*) have been attributed to 6PPD-quinone (6PPD-Q), an oxidation product of the tire-derived antioxidant N-(1,3-dimethylbutyl)-N'-phenyl-1,4-phenylenediamine (6PPD), drawing increasing attention to the ecological risks of PPD-class antioxidants. While in vivo tests in fish are valuable tools for evaluating ecological effects of chemicals, they require substantial resources and are limited in scalability due to ethical considerations related to animal welfare. In contrast, in vitro approaches are expected to enable rapid and cost-effective screening, but the extent to which in vitro results correspond to organism-level responses remains insufficiently explored. In this study, we conducted both in vivo tests using farmed coho salmon in Japan and in vitro tests using fish-derived cells to evaluate the toxicity of 6PPD, 7PPD, 8PPD, and their respective quinone derivatives. In the in vitro assays, only 6PPD-Q exhibited measurable effect, whereas the other compounds did not show observable effects within the tested concentration ranges. In the in vivo tests, 6PPD-Q induced adverse effects consistent with previously reported toxicity thresholds. Results for the other PPDs and quinones, as well as comparisons between in vivo and in vitro outcomes, will be presented in detail during the meeting. Our findings suggest that in vitro tests can reliably reflect certain aspects of in vivo toxicity, supporting their potential role as effective and complementary tools for initial screening and risk assessment of tire-derived chemical contaminants.

8.03.T Where the Alternatives Meet the Road: Finding Substitutes for 6PPD in Vehicle Tires

8.03.T-01 Defining safer: A path to restricting to use of 6PPD in tires

Stephanie Gill and Craig Manahan, Washington State Department of Ecology

The Washington State Department of Ecology started assessing chemicals in products to find safer alternatives in 2019 with the inception of the Safer Products for Washington Program. When 6PPD-quinone was identified in 2020 by the University of Washington and Washington State University as the second most toxic chemical to

aquatic life and the direct cause of coho salmon mortality in the Puget Sound, the Washington State Legislature prioritized finding alternatives to 6PPD and identified 6PPD in tires as a priority for the Safer Products program. Because of the extreme toxicity of 6PPD-quinone, the established Safer Products hazard criteria were modified to support additional data needs to identify antiozonants that are safer than 6PPD and the transformation product 6PPD-quinone. We will present on the strategic plan defined in 2021 to develop a robust and scientifically supported alternative assessment for the emerging contaminants 6PPD and 6PPD-quinone, including identifying data needs and developing the path to a defensible hazard criterion. We will review the results of the Ecology-funded toxicological research and rubber testing and identify our priority data needs to allow for a Safer Products program regulatory recommendation to restrict the use of 6PPD in tires. The presentation will include strategies to avoid regrettable substitutions while prioritizing the critical need to eliminate 6PPD and 6PPD-quinone from the environment and a look ahead on what to expect over the next couple of years as we hunt for a safer alternative to 6PPD.

8.03.T-02 Manufacturers perspectives on efforts to replace 6PPD in motor vehicle tires

Tracey J. Norberg, *U.S. Tire Manufacturers Association*

The tire manufacturing industry is committed to finding an alternative to N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) that is safer for coho salmon and other affected fish species while ensuring that the performance and safety of tires is maintained for the life of the tire. In conducting the California Department of Toxic Substances Control Safer Consumer Products Alternatives Analysis (AA), we have encountered a number of challenges that will be the focus of this presentation. Thirty-six tire manufacturers from around the world have opted to conduct the AA jointly as a consortium. Having successfully obtained approval for our Stage 1 AA, the Consortium is now carrying out Stage 2. Working as a consortium has allowed us to pool resources and knowledge, yet working with such a large group has imposed some timing and communication constraints, which will be discussed. We have developed anonymized surveys as a way of harnessing the extensive research experience of our members while protecting their intellectual property. A high-level summary of the survey approach will be presented. While not required by the AA process, our group has also funded basic research to address, at a preliminary level, both the toxicity and in-rubber performance of potential alternatives. Some of the challenges in interpreting and using these findings will be presented. The AA is also being conducted simultaneously as some other independent tire research efforts, some of which may touch on the same issues as the AA at least peripherally, so new research could inform or impact the outcome of the AA. This AA is not being conducted in isolation from a regulatory standpoint, and there has been a need to coordinate and share information with non-California regulators at state, federal and international levels. The presentation will also discuss concerns about the potential for “free riders” to not join our consortium or conduct an AA themselves and simply wait for someone else to identify potential solutions. As the California AA program is currently structured, this disincentivizes companies from responding to a priority product listing in a timely manner and jeopardizes the success of this program. The presentation will conclude with a discussion of some of the steps that manufacturers would need to take in order to implement any identified alternative.

8.03.T-03 Challenges and new approaches in conducting alternatives assessment for 6PPD in motor vehicle tires

Thomas Lewandowski, *Jiaru Zhang, Tatiana Manidis and Thomas Dugan, Gradient*

The Priority Product motor vehicle tires containing the antidegradant N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is currently undergoing alternatives analysis (AA) in accordance with California's Safer Consumer Products (SCP) program. On behalf of the U.S. Tire Manufacturers Association (USTMA) 6PPD AA Consortium, Gradient co-authored the preliminary Stage 1 AA report, which identified seven possible alternatives to 6PPD that merit further evaluation. We are now proceeding to Stage 2, the first priority product to do so under the SCP program. The complicated chemistry, performance, and safety requirements of tires pose significant challenges in finding alternatives to 6PPD. Similarly, the lack of knowledge about the nature of the toxicological mechanism(s) by which ozonated 6PPD may produce reported effects in aquatic

environments, as observed in recent laboratory studies, introduces uncertainty into the identification of possible alternatives. This presentation will summarize some of the novel aspects of the tire AA, including the need to prescreen over 70 possible alternatives, the need to incorporate toxicity to certain salmonids (i.e., non-standard test species) into the hazard evaluation process, heavy emphasis on the hazards of transformation products (i.e., 6PPD quinone and potential ozonation products of possible alternatives), and the need to infer likely performance at the product level from material level screening tests, typically carried out under different study designs. We will also discuss some of the expected challenges in carrying out the Stage 2 AA, including quantification of economic impacts, filling in data gaps on hazards using read across methods, and conducting the AA under regulatory time constraints while research on possible alternatives continues. We will also discuss some of the important considerations in reaching a final AA decision, including the need to select alternatives that are long term solutions and can meet chemical registration requirements to allow for timely implementation. **Disclaimer/Disclosure:** Gradient, the authors' employer, has received funding from the US Tire Manufacturers Association for the work described.

8.03.T-04 Driving change: California's approach to safer alternatives to 6PPD in tires

Kelly A. Grant, *Thomas A Bruton and Xiaoying Zhou, California Department of Toxic Substances Control*
A foundational feature of California's Safer Consumer Products Program (SCP) is that product manufacturers perform Alternatives Analyses (AAs) for regulated products. The guiding principle is that product manufacturers have the best technical knowledge to evaluate performance and technical feasibility of alternatives. However, AA is a complex discipline. The quality 6PPD AAs varied widely, with some of the AAs lacking key elements. Just four of the 19 AAs submitted are currently compliant; but, because USTMA's Consortium AA is among those, more than 90% of the California tire market is compliant. Our evaluation of the AAs provided valuable lessons learned about the SCP AA process that can strengthen our program and may benefit other regulatory AA programs. Among the challenges for the 6PPD AA is the need to evaluate hazards of the transformation products, as well as the parent chemical; regulated entities' unfamiliarity with AA concepts and processes; and the rigors of the SCP AA requirements. We will discuss these learnings, as well as the results of the 6PPD AA, a short summary of which is provided below. In March 2024, 75 tire manufacturers submitted Preliminary AAs, screening a handful of approaches with the potential to replace 6PPD or reduce exposure to it. Manufacturers focused their efforts on replacing 6PPD with other anti-degradants. Collectively, the industry proposed 20 different chemical alternatives. Some of the proposed chemicals are commercially available, while some others seem to be pre-patent. Several of the alternatives are also members of the PPD class and may share some of the hazard traits of 6PPD. Evidence suggests that the acute toxicity of 6PPD-quinone to coho salmon is unique among the PPD-quinones. Unfortunately, other hazard traits of the PPD-quinones are minimally characterized. In the next step, manufacturers will prepare Final AA reports that provide detailed analyses of the hazards, exposures, and lifecycle impacts of the alternatives. These Final AA reports are due starting in August 2026.

8.03.T-05 CCPD: A promising safer alternative to 6PPD-Comparative aquatic toxicity and environmental fate assessment

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6PPD and its ozonation transformation product 6PPD-quinone (6PPD-Q) are significant aquatic toxicants, with 6PPD-Q causing acute mortality in coho salmon (*Oncorhynchus kisutch*) via stormwater runoff. In response, regulators in Washington state and California are actively working to phase out or identify safer alternatives to 6PPD in tires through their respective Safer Products programs, while the EPA is pursuing federal regulations under the TSCA to address 6PPD and 6PPD-Q concerns. This study presents environmental fate and aquatic toxicity data for N,N'-dicyclohexyl-p-phenylene-1,4-diamine (CCPD, CAS 4175-38-6) and its ozonation product CCPD-Q (CAS 1653-85-6), developed using safe-by-design principles as a 6PPD alternative. The

dataset addresses US and EU hazard criteria and incorporates risk characterisation principles. CCPD exhibited a log Kow of 4.8 and QSAR-estimated BCF/BAF values of 771 and 773, respectively. CCPD demonstrated significant environmental degradation: 61% biodegradation in 28 days (OECD TG 301D), and hydrolytic instability with DT₅₀ values of 2.5 h (pH 7) and 2.0 h (pH 8.5) under OECD TG 111 at 20 °C. Acute toxicity to coho salmon was assessed under flow-through conditions (OECD TG 203). CCPD had a 96h LC₅₀ of 32 µg/L (measured), while CCPD-Q was not toxic at the solubility limit (149 µg/L). CCPD also exhibited comparable toxicity to *Daphnia magna* (48h EC₅₀ = 58.5 µg/L, OECD TG 202). This contrasts sharply with 6PPD-Q, which has a 24h LC₅₀ of 0.095 µg/L to coho salmon. Ongoing flow-through testing in rainbow trout (*Oncorhynchus mykiss*) indicates lower sensitivity compared to coho salmon. Additionally, recognising the relevance of pulsed stormwater exposures, static 48 h tests (EPA OCSPP 850.1075) are underway to simulate transient exposure scenarios, where the instability of CCPD and co-exposure to transformation products will be apparent. Due to hydrolytic instability precluding standard algal tests, a flow-through Lemna growth inhibition test (OECD TG 221) is also in progress. A preliminary environmental risk assessment conducted in accordance with EU REACH indicates safe use of CCPD during manufacture and tire use, when applying the existing operational conditions and risk management measures applied for 6PPD. These studies complete the short-term dataset for classification and hazard assessment. Future long-term studies will further define CCPD's suitability as a safer, functional alternative to 6PPD in tire and rubber goods.

8.04.P-Tu General: Systems Approaches

8.04.P-Tu-133 Advancing sustainable electrode fabrication: A systems approach to safer ingredients through chemical substitution and life cycle assessment

Yunchih Tsai, Chihchi Huang and Mengshan Lee, National Kaohsiung University of Science and Technology, Taiwan

The widespread adoption of membrane capacitive deionization (MCDI) technology is currently hindered by the reliance on hazardous solvents, such as N,N-Dimethylacetamide (DMAc) and N-Methyl-2-pyrrolidone (NMP), used in electrode fabrication, which pose significant environmental and human health risks. This study explores the trade-offs associated with electrode material selection and evaluates greener alternatives to conventional solvents. Specifically, this study presents a comparative life cycle assessment (LCA) of two electrode fabrication recipes: one employing oil-soluble binders and the other using water-soluble binders. The global warming potentials (GWP) of the oil-soluble (polyvinylidene difluoride-based) and water-soluble (carboxymethyl cellulose-based) formulations were estimated at approximately 0.58 kg CO₂-eq and 0.15 kg CO₂-eq per gram removal of salt, respectively. This shift also leads to remarkable reductions in impacts of human carcinogenic toxicity, freshwater ecotoxicity, and marine ecotoxicity. Furthermore, replacing NMP with dimethyl sulfoxide (DMSO) offers additional environmental benefits, including a 65% reduction in terrestrial ecotoxicity, a 58% decrease in water consumption, and a 56% reduction in ozone formation in terrestrial ecosystems. Overall, this study offers valuable insights into balancing technical performance with environmental sustainability. By promoting safer chemical alternatives and minimizing life cycle environmental impacts, this study supports the advancement of greener manufacturing practices in MCDI technology, contributing to more sustainable water treatment solutions and providing practical guidance for industrial implementation. **Disclaimer/Disclosure:** This work is supported by National Science and Technology Council of Taiwan through project 113-2622-E-992 -017.

8.04.P-Tu-134 Developing a regionalized characterization model for water consumption impact

Zih-Ee Lin¹ and Pei-Te Chiueh², (1)National Cheng Kung University, Taiwan, Madagascar, (2)National Taiwan University, Madagascar

The impact of water consumption is highly context-dependent, varying with water availability and competing demands. To address this, our study establishes a regionalized characterization model for water consumption impact within the Life Cycle Assessment (LCA) framework. This model is developed based on the Watershed-

Affected Area (WAA) concept, which delineates the relationship between a watershed and its supply areas. Socio-economic data detailing the distribution of damage receptors within the water supply area are also integrated into the model. Operating in a matrix form, the model accounts for the complex spatial attributes of fate, exposure, and effect factors. We applied this model to three major watersheds in Taiwan: the Tamsui River in the north, the Zhuosui River in the central region, and the Gaoping River in the south. Each watershed presents distinct water resource distributions and user demands. Our results indicate that water consumption leads to the highest economic loss in the domestic sector of the Tamsui River watershed, reaching NTD 3,121/m³ consumed. This is primarily attributable to intense water competition, as reflected by the fate factor. Conversely, the lowest economic loss, NTD 0.6/m³ consumed, is observed in the agricultural sector of the Zhuosui River watershed, largely due to the low economic value of agricultural products (effect factor). To further evaluate the feasibility of the developed characterization factors (CFs) in regional water resources management, we conducted scenario studies on the implementation of wastewater reclamation plants for water supply augmentation. The impact assessment results highlight the consequences of water consumption by incorporating water characteristics data, rather than merely providing volumetric outputs of water supply. Consequently, these impact results offer more comprehensive information to support water resources management, particularly regarding water resource distribution, compared to simple volumetric data.

8.04.P-Tu-135 Life cycle environmental impacts of semi-transparent photovoltaic systems in buildings

Bahareh Pourhosseini Akbarieh and **Ilke Celik**, *Portland State University, Department of Mechanical and Material Engineering*

Buildings account for 40% of energy use in the U.S. and 30% of carbon emissions. According to the Department of Energy, to reach climate goals, emissions from buildings should be reduced by 65% by 2030 and by 90% by 2050. In this study, we evaluate the integration of two types of semi-transparent photovoltaic (STPV) systems into the windows of three-story, medium-sized office building across four distinct U.S. climate zones. We then compare their performance in terms of energy savings, energy generation, and environmental impacts against a reference building with regular windows. To simulate the building's energy use, we apply the optical and thermal properties of the STPVs in OpenStudio. For energy generation, we use the building area and local weather data in HelioScope. Environmental impacts are assessed using outputs from OpenStudio and HelioScope in OpenLCA, utilizing Ecoinvent database. The results show a 20–35% reduction in cooling demand and a 6–38% reduction in heating demand, both relative to the base case with regular windows, with the highest energy savings observed in Los Angeles due to high solar radiation. Including the energy generation from the panels, total energy reduction reaches 27–74%. The global warming potential analysis indicates that integrating STPVs significantly reduces carbon emissions, due to both HVAC energy savings and onsite energy generation. These results highlight the sustainable and environmentally friendly potential of STPV technologies in the studied locations.

8.05.P-Th Late-Breaking Science: Systems Approaches

8.05.P-Th-078 Toward systems-based management of aquatic ecosystems: Integrating multiple contaminants and stressors through feedback-driven approaches

Cheryl A. Murphy, *Bartolomeo Gorgoglione, Rachel Leads, Adrian Deil Manliclic, James Moran and Xiaobo Tan, Michigan State University*

Managing chemical mixtures in ecosystems remains a central challenge for environmental science and policy. Exposures are dynamic, sources are diffuse, and biological responses often emerge from complex interactions that cannot be captured by static, single contaminant dose responses. Traditional assessments typically rely on chemistry-first monitoring and fixed risk benchmarks, overlooking unmeasured inputs, unknown system dynamics, and measurement noise that influence ecological outcomes. To evaluate how far current practice has moved toward adaptive management, we interrogated several case studies addressing mixtures in aquatic and terrestrial ecosystems. Across these examples, we identified recurring patterns: reliance on targeted chemical

monitoring, limited incorporation of biological endpoints, and minimal use of feedback mechanisms to guide intervention. While some frameworks implicitly acknowledged system uncertainty, few explicitly adopted principles consistent with control theory, such as dynamic feedback, state estimation, or adaptive set points. We propose a new framework that applies control theory to management of ecosystems experiencing contaminant mixtures. In this approach, high-throughput biological assays, transcriptomic signatures, and dynamic energy budget models provide measured outputs that represent ecosystem state. Effect-directed chemistry and isotopic fingerprinting address unmeasured drivers and support source attribution, while life cycle assessment overlays ensure sustainability is considered alongside hazards. A controller layer integrates these inputs with management objectives to dynamically adjust monitoring frequency, prioritize interventions, and optimize remediation actions. This synthesis highlights both the limitations of current mixture assessments and practical pathways for improvement. By embedding feedback loops and expanding biological endpoints in monitoring networks, mixture management can evolve from a reactive, chemistry-driven exercise into a proactive, biologically anchored, dynamically optimized system. Such an approach provides a robust foundation for protecting ecosystem health under complex and uncertain conditions.

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- University of Georgia, 2.14.P-Mo-069, 3.03.P-Mo-020, 3.03.P-Mo-022, 3.03.T-04
- University of Georgia, Marine Extension and Georgia Sea Grant, 7.04.P-Tu-131, 7.06.T-07
- University of Georgia, Savannah River Ecology Laboratory, 3.03.P-Mo-021
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- University of Houston, Clear Lake, Environmental Institute of Houston, 3.06.P-Th-040
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- University of Illinois, Chicago, 4.20.P-We-076, 6.04.P-Th-116
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- University of Maryland, Baltimore County, 2.12.T-02, 4.01.B.T-05, 4.17.P-Tu-061, 4.18.A.T-03, 4.20.P-We-074, 6.04.T-01, 6.04.T-05, 7.04.P-Tu-130, 7.05.P-Mo-113
- University of Maryland, Center for Environmental Science, 2.10.P-Tu-009, 2.10.P-Tu-010, 2.11.B.T-01, 3.03.T-01
- University of Maryland, College Park, 1.08.P-Th-114, 5.04.P-Mo-057, 5.06.T-02, 5.13.P-Tu-093, 5.13.T-01
- University of Maryland, Eastern Shore, 1.17.P-Th-013, 2.06.P-We-029, 4.20.P-We-070
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- University of Milano-Bicocca, 2.04.B.T-04
- University of Mines and Technology, 4.17.B.T-06
- University of Minnesota, 1.02.T-03, 1.08.T-04
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