CPRC Annual Spring Meeting
Monday, April 8, 2024
Meeting Program

Time:	Agenda	
8:00 a.m.	***Doors Open***	
8:00-8:40 a.m.	***Check In and Breakfast***	
8:40-9:00 a.m.	***President's Introduction*** - Meredith Bohannon (DCPH-A)	
	Welcome Address - TBA (IMET)	
	Announcements and SETAC Updates - Meredith Bohannon	
	NASAC Announcement - Mara Walters (VIMS)	
	Ice Breaker - Connie Mitchell (HESI)	
9:00-10:20 a.m.	***MORNING PLATFORM PRESENTATIONS***	
9:00-9:15 a.m.	Michella Salvitti (University of Maryland - Eastern Shore) *Per- and Polyfluorinated Substances (PFAS) retention in Atlantic Blue Crabs and the Maryland Coastal Bays*	
9:15-9:30 a.m.	Sabine Malik (University of Maryland - College Park) *Impact of Urban Legacy Contaminants on Reproduction in the Male Mummichog (Fundulus heteroclitus)*	
9:30-9:45 a.m.	Mara Walters (Virginia Institute of Marine Science) *Exposure to UV radiation alters the plastic additive content of microplastics of four different polymer types*	
9:45-10:00 a.m.	Xiaojue Chen (University of Maryland - College Park) *Impacts of Azoles on Anaerobic Ammonium Oxidation (Anammox) Process under Different Substrate*	
10:00-10:20 a.m.	Barnett Rattner (United States Geological Survey) *Building a Safer Mousetrap: Ecological Hazards of Anticoagulant Rodenticides and Potential Solutions*	
10:20-10:50 a.m.	***Coffee Break/Poster Viewing***	
10:50-11:35 a.m.	**KEYNOTE ADDRESS** Dr. Kevin Sowers (Institute for Marine and Environmental Technology) *Bioremediation: A Low Impact, Sustainable Approach for In Situ Treatment of PCB Contaminated Sediments*	
11:35 a.m.-12:20 p.m.	***Group 1: Lab Tour***	***Group 2: Lunch***
12:20-1:05 p.m.	***Group 1: Lunch***	
1:05-1:50 p.m.		***Group 2: Lab Tour***
1:50-3:55 p.m.	***AFTERNOON PLATFORM PRESENTATIONS***	
1:50-2:05 p.m.	Anna Welsh (United States Geological Survey) *Mountaintop coal mining generated aluminum and its potential effects on juvenile crayfishes*	
2:05-2:20 p.m.	Jay Anderson (Photo Thermal) *Measuring weathered/oxidized particles from >50um to <500nm using sub-micron IR spectroscopy*	
2:20-2:35 p.m.	Darius Stanton (American Cleaning Institute) *iSTREEM® (In-STREam Exposure Model)*	
2:35-2:50 p.m.	Eguono Omagamre (University of Maryland - Eastern Shore) *Stressed Soy vs Empowered Armyworm: Perfluorobutanoic Acid Perturbs Insect-Plant Interaction*	
2:50-3:05 p.m.	***Coffee Break/Poster Viewing***	
3:05-3:20 p.m.	Vanessa Wuerthner (United States Environmental Protection Agency) *An Overview of EPA's Approaches to Determining Effects of Pesticides on Federally Listed Species and EPA's Endangered Species Act Workplan*	
3:20-3:35 p.m.	Mary Beth Claude (United States Environmental Protection Agency) *Ecological risk assessment framework and how the existing paradigm accommodates novel approaches*	
3:35-3:55 p.m.	Dean Naujoks (Potomac Riverkeeper Network) *Riverkeeper Coal Ash Investigations*	
4:00-5:30 p.m.	***POSTER SESSION and Poster Judging***	
5:30 p.m.	**Student Awards Announced**	
5:35-6:00 p.m.	***Pictures/Wrap Up***	
6:00-6:15 p.m.	***Walk to National Aquarium***	
6:15-7:00 p.m.	***National Aquarium Walk-Through***	
7:00-9:30 p.m.	***Reception in Dolphin Viewing Area/Jellyfish Exhibit***	

Meeting Sponsors

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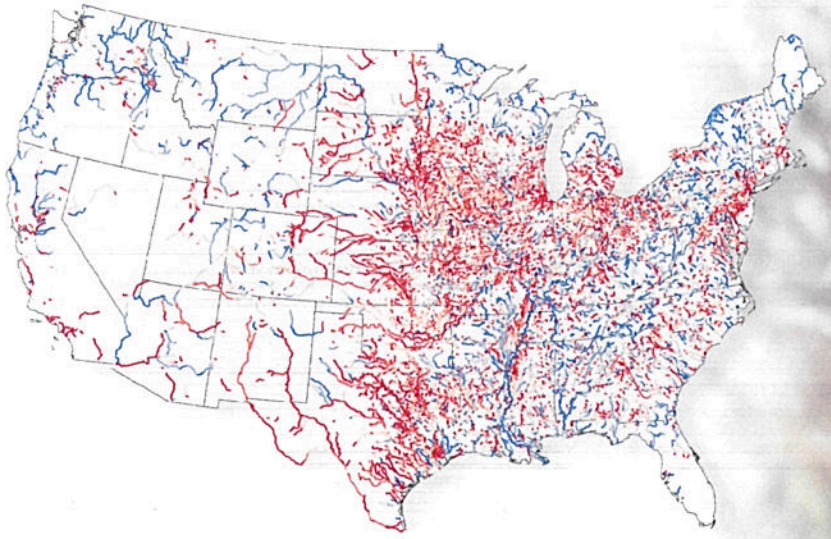

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CPRC MEETING LINKS

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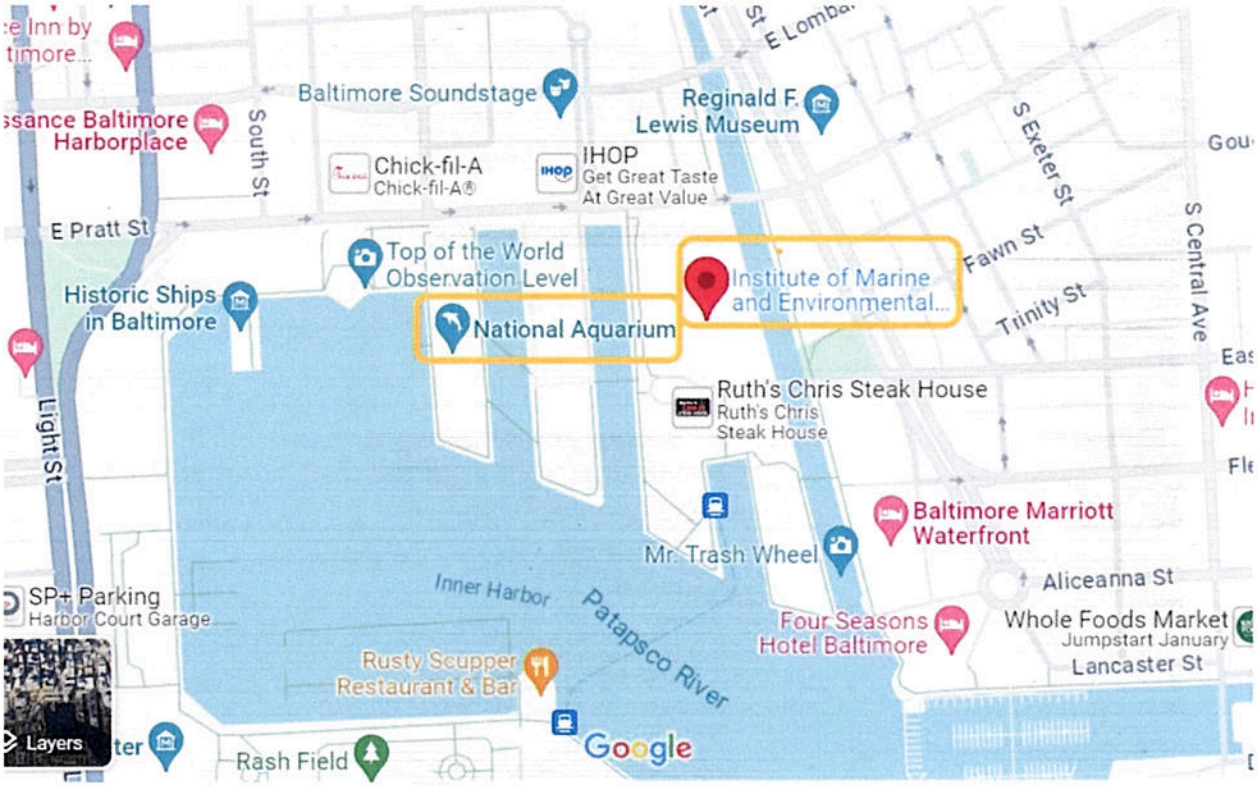
Information Sheet

Location, Aquarium Instructions, Parking, Public Transit, Dining

Location:



Institute of Marine and Environmental Technology – daytime scientific meeting – 9am-6pm
Pier V
701 E. Pratt Street
Baltimore, MD 21202

National Aquarium – evening buffet reception with exhibit visit; 6:15-9:30pm
Pier III
501 E. Pratt Street
Baltimore, MD 21202

<u>Instructions for Aquarium Reception:</u>
Guests will arrive at the Aquarium starting at 6:15 pm at the main entrance and can arrive as late as 6:30. If guests arrive after 6:30, they will be sent directly to the Dolphin Viewing Area without a chance to walk through the Aquarium.
The reception is from 7:00-9:30 pm in the Dolphin Viewing Area with access to the Jellyfish Exhibit. Aquarium staff will make sure everyone arrives at the reception at 7pm.
Each guest will receive two drink tickets.

Parking:

Pier V Parking – 711 E. Pratt Street, Baltimore, MD 21202 – same pier as IMET - parking will be validated at $15 for the entire day. You must pick up a parking voucher from Meredith in order to receive the $15 rate. The garage is open 24 hours however the voucher expires at midnight.

Public Transit:

LightRail – Use the Convention Center stop or the Camden Yards stop to get to IMET from points northbound or southbound – trains run late into the evening – regular fare is $2, student fare is $1.50 one-way, round-trip passes available – station parking is free, suggestion is to park at a stop outside the city - stations at Lutherville and north, and North Linthicum and south, should have ample parking – see https://www.mta.maryland.gov/schedule/lightrail for more information

Dining Near IMET (for lunch):

Inner Harbor:

Docks on the Harbor – Pier IV – near Hard Rock Café
Hard Times Café – Pier IV – near Docks on the Harbor
Blackwall Hitch – Pratt Street, inland side
Chipotle – Pratt Street, inland side
Shake Shack – Pratt Street, inland side
Miss Shirley's Café – Pratt Street, inland side
Cheesecake Factory – Pratt Street Pavilion

Little Italy:

Vaccaro's Italian Pastry Shop
La Tavola
Amicci's
Isabella's Brick Oven Pizza
Dalesio's
Sabatino's
Chiapparelli's
Café Gia Ristorante
Angeli's Pizzeria

Harbor East:

Bambu Baltimore - Vietnamese
James Joyce Irish Pub and Restaurant - Irish
Maximón - Mexican
Taco Fiesta - Mexican
Bambao - Chinese
Lebanese Taverna – Lebanese/Mediterranean
BLK Swan - American
Whole Food Market - Various
RA Sushi Bar - Japanese

CPRC 2024 Annual Spring Meeting
Abstract List

* asterisk after title indicates student presentation competitor
Author name in red indicates presenting author

Keynote Address

Bioremediation: A Low Impact, Sustainable Approach for In Situ Treatment of PCB Contaminated Sediments

Dr. Kevin Sowers

University of Maryland Baltimore County
RemBac Environmental LLC

Polychlorinated biphenyls (PCBs) are a widespread contaminant in sediments in the U.S. and a frequent cause of fish consumption advisories. 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. The innovative aspect of the technology is the application of large numbers of PCB-transforming microorganisms to compensate for the low bioavailability of PCBs required 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 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 loading cell titer and carbon loading rates for each site, we conducted field applications of bioamended AC at sites in several states. The effect of treatments was monitored by measuring total PCB concentrations in sediment cores and in porewater using passive samplers. The field applications show the promise of bioremediation as an in effective *in situ* strategy to help address the widespread need to reduce contamination of the aquatic food web from sediment-bound PCBs.

Platform Abstracts

Measuring weathered/oxidized particles from >50um to <500nm using sub-micron IR spectroscopy

Anderson, Jay; Kansiz, Mustafa; Dillon, Eoghan

Photothermal Spectroscopy Corp

Microplastic or Nanoplastic (MP/NP) contamination has been recognized as a global environmental problem and are found globally in water, air, and soil. These particles are regularly ingested via contaminated water, beverages, food, and by breathing airborne particles. Two workhorse spectroscopic analysis techniques are used to characterize populations of MPs. They included infrared (IR) microscopy, either FTIR or emerging techniques using QCL's. IR microspectroscopy analyses has shown to be limited to >20 µm particles due to issues associated with spatial resolution, scatter artifacts and saturation. Raman microspectroscopy has been suggested to offer better spatial resolution, but suffers from lower sensitivity than IR, autofluorescence, and can burn dark samples easily. Thus in many methods Raman spectroscopy has been limited to similar particles sizes of >20 µm.

A new approach to IR microspectroscopy, called "Optical Photothermal Infrared (O-PTIR)" spectroscopy has demonstrated a unique ability to generate submicron IR spectra without common IR scatter artifacts or limits upon saturation. O-PTIR provides mm to sub-micron size MP/NP characterization, with IR chemical specificity, in a non-contact, reflection geometry, and is not affected by fluorescence. O-PTIR can be used at visible powers of 1/10th of the power needed for Raman, eliminating the potential of burning samples.

Natural fluorescence is a common attribute of many MP/NP samples. This natural phenomenon can be used to highlight particles of interest for further discrimination and offers a substantial time savings and possible misinterpretation of spectral data.

In this study we will focus on measuring MP/NPs <20 µm into the 0.5µm range, to demonstrate the superior IR spatial resolution of O-PTIR but also contrast the measurement of virgin vs oxidized MP's using O-PTIR over Raman. In the example spectra, virgin or oxidized particles can be easily distinguished using O-PTIR. Often the measurement of these MP's attempted with Raman were not interpretable. Using O-PTIR provides a <20 µm solution to measure MP/NP particles in water, air, and soil using a non-contact method offering transmission like spectra that are interpretable and searchable against known standards.

Impacts of Azoles on Anaerobic Ammonium Oxidation (Anammox) Process under Different Substrate Conditions *

Chen, Xiaojue; Li, Guangbin

University of Maryland

Azoles, categorized as emerging organic compounds (EOCs), are extensively used in semiconductor manufacturing, personal care products, pharmaceuticals, flame retardants, surfactants, and certain pesticides. Their presence in the environment, including surface water, groundwater, and wastewater, poses potential ecotoxicological effects at low concentrations (ppb-ppm). This accumulation can adversely affect biological processes, particularly nitrogen-related processes such as nitrification, denitrification, and anaerobic ammonium oxidation (anammox), leading to reduced nitrogen conversion efficiency in natural and engineered systems like wastewater treatment plants (WWTPs).

Previous studies have shown that benzotriazole (BTA) and 5-methyl-benzotriazole (MBTA) at 20 mg/L significantly inhibit (>50%) the anammox process in short-term tests. However, limited information is available on how substrate conditions (nitrogen concentration) impact anammox sensitivity to azoles. This study employed batch bioassays to assess anammox activity and nitrogen removal performance under different substrate conditions in the presence of MBTA and BTA. Three substrate conditions (high, low, and starvation) were tested. Results showed that under low substrate conditions (35 mg N/L) and without azoles, anammox activity decreased compared to high-substrate controls (175 mg N/L). Addition of BTA (18 mg/L) and MBTA (20 mg/L) caused further inhibition under high substrate conditions, with greater inhibition observed under low substrate conditions. After 5 days of starvation, anammox activity decreased, and a lag phase increased, indicating increased sensitivity to azoles. Further assessment will involve longer starvation periods (14 and 21 days) to determine anammox sensitivity to azoles.

Adenosine 5'-triphosphate (ATP) content analysis will elucidate the correlation between cell energy levels and azole sensitivity. Potential biodegradation of azoles by anammox-enriched sludge will also be evaluated. The results from this study will provide insights into anammox process performance and stability in azole-impacted environments, aiding in understanding and mitigating potential risks associated with azole contamination.

Impact of Urban Legacy Contaminants on Reproduction in the Male Mummichog (Fundulus heteroclitus)

Malik, Sabine; Frankel, Tyler; Mitchelmore, Carys; Duncan, Candice; Yonkos, Lance

Department of Environmental Science and Technology, University of Maryland, College Park, USA; Department of Earth and Environmental Sciences, University of Mary Washington, Fredericksburg, VA, USA; Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD, USA; Department of Environmental Science and Technology, University of Maryland, College Park, USA; Department of Environmental Science and Technology, University of Maryland, College Park, USA

Historically contaminated urban rivers harbor legacy pollutants that remain a hazard to human and ecosystem health. Endocrine disrupting compounds are of particular concern, as detriments to reproductive success threaten the viability of native fish populations. Impacts to female reproduction are seen across fish taxa, but descriptions of sperm-related effects are limited. Using the mummichog (Fundulus heteroclitus) as an ecologically relevant model, we developed and validated a field-adaptable method for measuring aquatic sperm quality using three metrics: 1) motility, measured by computer-assisted sperm analysis (CASA); 2) energetic capacity, measured by a firefly-luciferase adenosine triphosphate (ATP) assay; and 3) DNA damage, measured by Comet assay. These methods were applied to field-collected F. heteroclitus from two historically contaminated US rivers (Christina River, DE and Anacostia River, MD) and from a reference location (Wye River, MD). Samples from the Christina River had the most severe DNA damage, moderate ATP reduction, but comparatively normal motility, indicating the potential for genetically damaged sperm to fertilize an egg. In contrast, samples from the Anacostia River had marked decreases in ATP, moderate to severe DNA damage, and poor motility. These results emphasize the importance of multi-faceted testing, as many adverse outcomes are likely overlooked with standard approaches.

Riverkeeper Coal Ash Investigations

Naujoks, Dean

Potomac Riverkeeper Network

Over the past ten years work has been conducted in North Carolina, Virginia, and Maryland uncovering toxic discharges from coal ash ponds, working with EPA Criminal Investigators as well as filing lawsuits and permit challenges against utilities like Duke Energy & GenOn to address improper storage and disposal of toxic coal ash and coal combustion by products. Last year, the EPA elevated coal ash as a top enforcement priority due to "widespread noncompliance" and is currently strengthening the 2015 Coal Combustion Residual (CCR) Rule to require additional cleanup of coal ash landfills, ponds and fill sites

due to a loophole that allowed half a billion tons of coal ash to go unregulated across the country. This talk will cover the basics of coal ash, community action and outreach, and corrective actions being taken to remediate these sites.

Stressed Soy vs Empowered Armyworm: Perfluorobutanoic Acid Perturbs Insect-Plant Interaction

Omagamre, Eguono; Pitula, Joseph

University of Maryland – Eastern Shore

Recent studies indicate that short-chain PFAS primarily accumulate in plant aerial parts, which are crucial for photosynthesis and interactions with insect herbivores and pollinators. Investigating the effects of this accumulation is essential for understanding its impact on insect-plant interactions. Here, we assessed perfluorobutanoic acid (PFBA), a short-chain PFAS with high upward transport in plants, to disrupt soybean-armyworm model systems' interaction. Soybeans were grown using PFBA-spiked water for 5 weeks, and PFBA-containing leaves and artificial diet were used to rear beet and fall armyworms. We conducted phenotypic observations, biochemical characterizations, and transcriptomic analyses for both plants and insects. High PFBA exposure (1 mg/L in irrigation water) had broad toxic effects on plant physiology, including chlorophyll levels, morphology, and height. Both environmentally relevant (100 pg/L to 100 ng/L PFBA in irrigation water) and high PFBA levels induced oxidative stress responses, upregulating reactive oxygen species and stress response genes. PFBA inhibited the plant leaf oxidative stress response pathway, decreasing key stress response enzymes like superoxide dismutase and catalase while enhancing non-enzymatic pathways. The circadian rhythm pathway was significantly enhanced only at environmentally relevant exposure levels. Larvae fed PFBA-containing diet exhibited increased weight gain, higher diet consumption, and faster life cycle transitions, with elevated 20-hydroxyecdysone levels. Transcriptome analysis revealed up to a 13-fold downregulation of genes encoding ecdysone oxidase and ecdysone-like isoform proteins compared to controls. Similarly, several isoforms of juvenile hormone epoxide hydrolase and juvenile hormone esterase genes were downregulated, with fold decreases ranging from 2 to 9 folds. Our data suggest that while inducing oxidative stress in plant leaves, PFBA has the potential to enhance and hasten metamorphosis and leaf area damage in beet armyworm larvae feeding on such plants by perturbing a hormonal pathway.

Ecological risk assessment framework and how the existing paradigm accommodates novel approaches

Pierce, Amanda; Claude, Mary Beth; Mendelsohn, Mike

United States Environmental Protection Agency

Ecological risk assessment combines information from toxicity tests and exposure information to evaluate the likelihood that ecological effects may occur as a result of exposure to a stressor (pesticide). The risk assessment framework is composed of problem formulation, analysis of exposure and effects, and risk characterization. This paradigm is flexible and able to accommodate novel pesticide approaches that may lack a history of safe exposure or prior use. However, novel pesticide approaches will require critical thought in the problem formulation step of ecological risk assessment. Additionally, greater

attention to limitations and assumptions in study design may be necessary to accurately translate toxicity test results to realistic agronomic ecosystem exposures in order to determine risk.

Building a Safer Mousetrap: Ecological Hazards of Anticoagulant Rodenticides and Potential Solutions

Rattner, Barnett

United States Geological Survey, Eastern Ecological Science Center

Anticoagulant rodenticides (ARs) have a long history of successful use in controlling vertebrate pest and invasive species. Unfortunately, non-target companion animals and free-ranging wildlife may be unintentionally exposed to ARs through various trophic pathways, and depending on dose, exposure can result in adverse effects and mortality. To put AR exposure of wildlife into perspective, a 2018 summary of over 40 references documented a 58% exposure rate in nearly 4200 liver samples of animals found dead, representing many wildlife species in more than 10 countries. Since 2018, additional wildlife mortality events linked to AR exposure have been documented throughout Europe, North America and elsewhere. To mitigate AR exposure risks for children, companion animals and non-target wildlife, the USEPA instituted regulatory changes in product labeling and other measures to prevent or limit homeowner use of the more persistent, bioaccumulative and toxic second-generation compounds (i.e., SGARs). It is unclear if these regulatory changes reduced non-target exposure of wildlife. Even stricter regulations have been instituted in some states, with California prohibiting all uses of the SGARs, and more recently expanded the moratorium to include the first-generation AR diphacinone. Nonetheless, the socio-economic consequences of disease and damage caused by rodent pest species support the long-term and expanding need to control commensal rodent populations. It is well-recognized that development of new, innovative, and safer tools to control vertebrate pests is costly and time consuming from a chemical synthesis, efficacy testing, safety evaluation and registration standpoint. Recent findings have demonstrated that bioaccumulation and retention of ARs varies among their isomeric form, with some SGAR diastereoisomers being rapidly and preferentially metabolized by target rodents, posing lower risk to scavenging and predatory non-target wildlife. New laboratory and field research findings that may lead to the development of isomer-specific eco-friendly AR baits will be discussed.

*Per- and Polyfluorinated Substances (PFAS) retention in Atlantic Blue Crabs and the Maryland Coastal Bays **

Salvitti, Michella; Camacho, Camden; Bowden, John; Pitula, Joseph

University of Maryland Eastern Shore; University of Florida; University of Florida; University of Maryland Eastern Shore

Per- and Polyfluorinated substances (PFAS) are a group of synthetic chemicals that are prevalent in everyday consumer use. Widespread use of PFAS has led to PFAS becoming an environmental contaminant of concern. Known mostly for its widespread use in firefighting foams, and presence in soil, sediment, foods such as seafood, and produce. PFAS are a concern because they are a group of chemicals that are challenging to break down, hence "forever chemical" and has been shown to cause detrimental health effects in human populations. The objective of this study was to test surface water for

PFAS at six sites the Maryland Coastal Bays and Callinectes sapidus, the Atlantic Blue Crab, at each site. Callinectes sapidus after collection were dissected, and claw tissue and hepatopancreases tissue were then tested for 35 different PFAS compounds. Results are to be determined. While the biological samples will require further investigation regarding their impacts on the ecosystem, this study will show the concentration of PFAS in the Maryland Coastal Bay Blue Crab and surface waters as preliminary data to then offers guidance for potential remediation techniques of affected areas and future sampling.

iSTREEM® (In-STREam Exposure Model)

Stanton, Darius

American Cleaning Institute

iSTREEM® (In-STREam Exposure Model) is a web-based model (https://www.istreem.org/) designed to estimate the concentrations of down-the-drain (DtD) chemicals in the surface waters of the continental United States. Based on the best publicly available data, the model is focused on the use and disposal of items ranging from personal care & home care products to food additives, pharmaceuticals, & home use pesticides. Identifying the spatial relationship between the United States' river network and waste water treatment plants, iSTREEM is able to calculate the predicted environmental concentrations (PECs) for effluent-impacted streams and rivers. For our presentation, we will discuss the capabilities of ISTREEM, and how the tool is utilized to inform risk evaluations crafted by a wide audience of users, including but not limited to: academia, regulators, and industry scientists.

*Exposure to UV radiation alters the plastic additive content of microplastics of four different polymer types **

Walters, Mara; Song, Bongkeun; Moon, Hyo-Bang; Mok, Sori

Virginia Institute of Marine Science, College of William and Mary

Plastic additives are chemicals mixed into plastics to impart desirable properties such as increased flexibility or decreased susceptibility to oxidation by UV radiation. Thousands of plastic additives are in use by manufacturers globally. Microplastics—plastic particles between 1 µm and 5 mm in size—are important environmental pollutants that can be classified as multistressors, since they are typically composed of plastic polymers and numerous plastic additives. The toxicity of plastic additives is only beginning to be appreciated. However, a common problem is that the additive content of plastics is frequently unknown due to manufacturer reluctance to share proprietary plastic mixtures. Other variables, such as UV exposure, can also alter the concentrations and structures of plastic additives within microplastics in unknown ways. Here, we compare the plastic additive composition of microplastics of four different polymer types before and after UV radiation. A solid-liquid extraction method followed by gas chromatography coupled with quadrupole time-of-flight mass spectrometry was used to quantify a suite of known plastic additives including organophosphate esters, phthalates, and alternative plasticizers. The polyurethane foam microplastics had the most diverse composition of plastic additives, while polylactic acid microplastics contained the lowest number of plastic additives. Treatment with UV radiation significantly changed the concentrations of several plastic additives in all microplastics

tested. This study is one of the first to report how plastic additive content within microplastics changes after UV exposure, demonstrating the potential for UV light to significantly alter microplastic toxicity.

Mountaintop coal mining generated aluminum and its potential effects on juvenile crayfishes

Welsh, Anna; Henry, Paula

United States Geological Survey

Mountaintop coal mining has resulted in the release of sediment, toxins, and heavy metals into the environment, including freshwater streams, potentially making habitats inhospitable for many organisms. Freshwater crayfishes are species of interest because they are both a keystone and flagship species in many ecosystems, and they are often listed as species of special concern or as threatened along the east coast. We evaluated the effects of mountaintop coal mining on freshwater crayfish growth and survival through the exposure of New River riffle crayfish (Cambarus chasmodactylus) juveniles to different concentrations of Aluminum contaminated lake water (control=0 µg/L, low=75 µg/L, medium=750 µg/L, and high=7500 µg/L Al). Juvenile survival and growth were measured through percent mortality per treatment, body mass, carapace length, and instances of shedding. The high dose group showed a decrease to complete loss in righting responses, 10.4% molt-related death syndrome, and an overall 60.4% mortality compared to 4.2% in the controls. Controls and low dosed juveniles showed higher weight gains and completed a greater number of successful shedding events.

An Overview of EPA's Approaches to Determining Effects of Pesticides on Federally Listed Species and EPA's Endangered Species Act Workplan

Wuerthner, Vanessa

United States Environmental Protection Agency

The Endangered Species Act (ESA) directs federal agencies to ensure that their actions are not likely to jeopardize the continued existence of any federally endangered or threatened (listed) species or result in the destruction or adverse modification of their designated critical habitat. In regard to registrations of pesticides, the US Environmental Protection Agency's (EPA) actions includes approval of pesticide products or registration review of pesticide active ingredients. EPA's process for assessing potential effects of pesticide-related actions on listed species has evolved over the years. EPA released a workplan in April 2022 and an update to the workplan in November 2022 that described the Office of Pesticide Programs' (OPP) plans to integrate ESA considerations more efficiently and routinely into its pesticide registration and registration review processes. As an outgrowth of the ESA workplan, the Environmental Fate and Effects Division (EFED) is developing several approaches consistent with the workplan goals. This presentation will provide a brief overview of EPA's developing approaches to better protect listed species under the ESA, including initiatives and strategies outlined in OPP's ESA workplan.

Poster Abstracts

*Monitoring of 40 PFAS in the Water Environment **

Alvi, Dongmei

Occoquan Watershed Monitoring Laboratory

Per- and Polyfluoroalkyl Substances (PFAS) represent a diverse group of over 4,700 synthetic chemicals extensively utilized across various industries, including paints, food packaging, firefighting foam, and non-stick cookware. Their ubiquitous presence raises concerns regarding potential adverse health effects upon exposure. Responding to the EPA's strategic roadmap, the Virginia State Department of Environment Quality (DEQ) conducted a comprehensive PFAS monitoring initiative in 2021, focusing on non-potable water environments statewide. The primary objectives were to assess the prevalence of these substances, establish baseline data, and pinpoint potential hotspots exceeding acceptable levels.

Statistical analysis of 569 water samples revealed a maximum total PFAS concentration of 1102 parts per trillion (ppt), with a median of 1.7ppt and a minimum of 0ppt. Sediment samples, comprising eight specimens, exhibited a maximum total PFAS concentration of 19266ppt, with a median of 5295ppt and a minimum of 4077ppt. Fish tissue analyses, conducted on 77 samples, demonstrated a maximum total PFAS concentration of 98450ppt, a median of 1910ppt, and a minimum of 0ppt.

Overview of Regulations and Frameworks for Environmental Assessment of Human Pharmaceuticals in the US and EU

Burruss, Ben

SafeBridge®

Medicines intended for human use are undeniably beneficial to individual health and society. However, it is well understood that the continual use and disposal of these medicines may lead to unintended environmental exposures and pseudo-persistence. In the United States and the European Union, regulations and guidance have been put forth to require assessments of these biologically active substances to evaluate their impact on the environment. While the regulations and guidance have the same objectives, there are notable differences between them. For example, according to the Food and Drug Administration (FDA) guidance, the maximum expected environmental concentration (MEEC) is determined based on the highest annual production volume in the next five years. In contrast, in the EU under the European Medicines Agency (EMA) guidance, the initial (screening level) environmental exposure is based on the market penetration or prevalence of the disease as determined from epidemiological data. Other notable differences in thresholds for data requirements and tiered testing strategies will be discussed. This presentation provides an overview of the available regulations and guidance from the US and EU regulatory authorities about the environmental assessment of chemicals intended for use as human drugs/medicines and highlights similarities and differences between these two jurisdictions. Furthermore, a case study evaluating the environmental risk assessment of a human pharmaceutical ingredient will be presented.

*Impact of land use development on PCB pollution in soils and stormwater sediments in Chesapeake bay watershed **

Cao, Yongcheng; Kjellerup, Birthe; Davis, Allen

Civil and Environmental Engineering, University of Maryland, College Park

Polychlorinated biphenyls (PCBs) are a group of chlorinated compounds derived from biphenyl which are classified as persistent organic pollutants (POPs). PCBs were banned in 1976 by the Toxic Substance Control Act in the United States but still can be found in numerous water bodies; Total Maximum Daily Loads (TMDLs) have been developed in many cases. Local soils and roadway sediments may be mobilized in stormwater and may contain attached PCBs. Soils and sediments have been collected from different sites in Maryland, with different land uses and eras of development. Concentrations of 209 PCB congeners are determined and analyzed in these soils and sediments samples by gas chromatography/electron capture detector. Non-legacy PCB 11 has been the most frequently detected congener in stormwater sediments and soils and has been found in yellow road paints. Sediment PCB concentrations will be correlated to land use and era of development. It is expected that industrial land uses and pre-1979 developments will have the highest PCB contaminated soils and sediments, relative to residential land uses and post 2005 developments. Information on PCB concentrations in sediments from different land uses can be used as an estimation of PCB load removal in various stormwater controls. Stormwater control measures can be targeted to land uses with the highest sediment PCB concentrations.

*Enhanced Dechlorination of Trichloroethylene in Groundwater: A Six-Year Field **

Cheng, Shih-Huai (Lora); Kjellerup, Birthe; Torrents, Alba

Department of Civil and Environmental Engineering, University of Maryland, College Park

Trichloroethylene (TCE) is a chlorinated volatile organic compound widely detected in groundwater. The persistence of TCE and its ability to migrate over long distances often lead to incomplete dechlorination, resulting in an accumulation of intermediate dechlorination products, e.g., cis-dichloroethylene (cDCE) and vinyl chloride (VC). TCE, cDCE, and VC are carcinogenic to humans. To assess potential factors that may influence TCE dechlorination, we analyzed six-year monitoring data from an in-situ bioremediation site facing incomplete dechlorination. The non-parametric Spearman's rank correlation analysis revealed a positive relationship between sulfate levels and concentrations of TCE, cDCE, and VC. The findings suggest that sulfate-reducing bacteria may affect the efficiency of dechlorinating bacteria in breaking down TCE.

Toxicity Assessment and Fit-for-Purpose Testing of 1-methyl-2,4,5-trinitroimidazole (MTNI)

David-West, Jessica; Adams, Valerie; Reinke, Emily; East, Andrew, Hall, Nicole; Oyler, Karl; and Schneider, Leah

The Defense Centers for Public Health-Aberdeen

The Defense Centers for Public Health-Aberdeen (DCPH-A) Toxicology Directorate is tasked with assessing the toxicity of new and priority compounds for use in weapons systems and other Army components (ARs 70-1, 40-5, 200-1 and MIL-STD 200-1). For the novel compound 1-methyl-2,4,5-trinitroimidazole (MTNI), toxicity and physicochemical estimates were generated using computational modeling based on chemical structure using the software tools: BIOVIA , ECOSAR and EPISuite™. Based on in silico structural analyses, MTNI was identified within the azole antibiotic chemical class. MTNI was then subjected to a four-part in vitro toxicity screen that included bacterial genotoxicity, marine bacterial luminescence test, skin sensitization potential, and an oral LD50 estimate derived from in vitro cytotoxicity data. MTNI was found to be mutagenic, a skin sensitizer, and a predicted rat LD50 = 260.7 mg/kg (using a cell-based acute oral toxicity estimate [CAOTE]). In the marine bacterial luminescence assay, MTNI was highly toxic with an estimated acute aquatic toxicity EC50 of 0.035 mg/L. While undergoing concentration verification for the in vitro assays, MTNI was observed to rapidly degrade in aqueous solutions. In order to address both the concern around the antibiotic properties and determine if the rapid degradation alters MTNI toxicity profiles, additional acute aquatic testing with Daphnia magna was conducted. D. magna toxicity was approximately one order of magnitude lower with fresh MTNI (0.558 mg/L) as compared to the marine bacterial luminescence EC50. Aging of the MTNI for one week in water resulted in an additional 2-orders of magnitude reduction in toxicity, to 18 mg/L. The overall reduction in toxicity from the bacterial to invertebrate assessments was 500-fold. MTNI was used as a proof of principle for the development of a rapid-screening approach for marine bacterial luminescence (MarBL) where the number of test conditions were expanded using a 96 well plate format. The MarBL permitted the simultaneous assessment of MTNI under various aged conditions. These data will be presented and the ramifications for occupational and environmental exposures, in light of MTNI aqueous degradation will be discussed.

Modification of a Nile Red Staining Method for Microplastic Detection in Environmental Media *

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Numerous methods have been developed for microplastics isolation and quantification in various environmental media, many of which require elaborate/expensive analytical equipment and decontaminated lab space. This study seeks to create a reproducible and economical method for the isolation of microplastics in surface water and sediment samples that is reliant on Nile Red staining. We use a Nile Red pre-staining step prior to sample digestion, density separation, and filtration to mitigate downstream in-lab contamination. To test the method generated, we seasonally collected replicate surface water samples from urban streams in the Chesapeake Bay Watershed, USA for one year and quantified microplastic concentrations via fluorescent microscopy. We investigated spatial and temporal microplastic concentrations with the goal of estimating the impact the University of Maryland College Park campus has on microplastic abundance. This method was also applied to sediment samples from the abyssal plain of the Pacific Ocean to see if the method is amenable to the quantification of samples containing substantial and variable sediment loads. The proposed sampling and quantification method found some success in both surface water and marine sediment samples with specific microplastics (≥20 μm) able to be enumerated.

*Assessing the potential interactions between climate change and trace metal toxicity in the embryonic Seminole Ramshorn Snail (Planorbella duryi) **

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Based on projected modeling, increases in surface water temperatures due to climate change will pose a host of new challenges for poikilothermic aquatic organisms. Additionally, little is known about how this added stressor will interact with the impacts of known environmental contaminants. As such, this study was designed to assess the effects of cadmium exposure on Seminole ramshorn snail (Planorbella duryi) embryonic development under varying temperatures based on predictions from the Shared Socioeconomic Pathways (SSPs). Freshly laid (<8hr post-deposit) P. duryi embryonic clutches were harvested from an established adult colony and assessed for viability. Individual clutches were then placed into acid washed 60 × 15 mm glass petri dishes containing 10mL of 0, 5, 10, or 25 ug/L cadmium solution (confirmed using ICP-OES). Plates were then incubated at 20°C (standard assay conditions), 23°C (SSP 2 predictions), or 25°C (SSP5 conditions) for 11 days. During the exposure period, a micrograph of each clutch was obtained under 40x magnification using a mounted USB camera. At the end of the exposure period, one egg from each clutch was selected and assessed for developmental stage (morula, trocophora, veliger, hippo) on days 1, 2, 3, 5, 7, 9, and 11. While this study is still ongoing, we expect to observe a correlation between increased temperature and irregular development, with a subsequent increase in cadmium toxicity at higher temperatures. The results of this study will give a deeper understanding of how sensitivity to trace metal exposure in poikilotherms may be altered by current and future shifts in climate.

Evaluation of Environmental Toxicity of Phototransformed Military Relevant Chemicals

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The Defense Centers for Public Health-Aberdeen

Chemicals used in regular military tasks (e.g. painting, degreasing, munitions, fuels, etc.) have the potential to be released the environment primarily through runoff, training operations, or unintentional release. Once in the environment, these compounds may be exposed to ultraviolet (UV) light, potentially resulting in the transformation that may increase or decrease their toxicity. To evaluate phototransformation and subsequent toxicity, the Defense Centers for Public Health-Aberdeen (DCPH-A) uses a Suntest™ CPS+ (Atlas Material Testing Solutions, Mount Prospect, IL) that integrates a xenon lamp to simulate the entire solar spectrum of light wavelengths. Filters and alterations to the reactor can be applied to adjust for different lighting conditions, decrease heat, and attenuate cytotoxic UVB wavelengths when irradiating cell cultures. It has also been shown photosensitization occurs from environmental releases of polycyclic aromatic compounds, occurring naturally in coal, crude oil, and

gasoline in aquatic organisms. Photosensitization is a combination of two stressors, photodynamic compounds and solar irradiation. Once enough residues have been bioaccumulated and UV activated, free radicals are released, causing oxidative damage, lipid peroxidation and cell death, eventually leading to mortality with sufficient duration and intensity of exposure. To determine if phototransformation is toxic to aquatic organisms, the aqueous chemical solution is irradiated in the Suntest™, Daphnia magna (a commonly used species for aquatic toxicology) are exposed, and toxicity is compared to non-irradiated results. This test –provides additional information regarding aquatic toxicity that augments the bioluminescent bacteria assay using Vibrio fischeri. Ultimately, implementing these phototransformation assays in our laboratory will expand our testing portfolio and assist in making risk-informed decisions for legacy chemicals that may occur in the environment and be useful for evaluating more sustainable "green" chemicals for use in new proposed systems. The mention of any non-federal entity and/or its products is for informational purposes only, and is not to be construed or interpreted, in any manner, as federal endorsement of that non-federal entity or its products.

Floating wetlands as a potential source of microplastics: A case study investigation at the National Aquarium in Baltimore's Inner Harbor

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Microplastics and the potential for toxic compounds to adsorb to them has been a concerning vector for the unintentional insertion of human created chemical compounds into aquatic environments and food chains. A years long proposal to construct a large scale (929m2) floating wetland between Piers 3 & 4 at the National Aquarium began in 2016. The deployment in 2017 of a 37m2 demonstration wetland included the planting or volunteer attraction of almost 40 species. A key design feature of the installation slated for Spring 2024 is the use of spun recycled plastic fiber matts that will provide buoyancy and a permeable medium with which to plant emergent aquatic vegetation. Underwater air bubbler diffusers used below the pilot are expected to be used in the scaled-up system. Working with this material it has been observed that the plastic fibers become brittle over years of exposure to natural sunlight. A UV protective coating is expected to be used to prevent this from occurring. With a stated 40-year life span for the project, it is hypothesized that the breakdown of microfibers will eventually occur on UV exposed surfaces over this time period, while subsurface plastic fibers exposed to continual microbubble explosion abrasion may also occur over time. To test these hypotheses, samples of the benthic sediments in the area of the project is proposed in Spring of 2024 to create a baseline of existing microplastics. Future years of sampling will characterize the sediments to determine if the unique microplastics from the floating wetlands are shedding into Baltimore's Inner Harbor.

*The presence, concentration, and potential ecological impacts of trace metal contaminants in the James River near a coal ash repository (New Canton, VA) **

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Industrial coal combustion residuals (CCRs) are rich in trace metals that infiltrate surface waters via accidental spills, authorized discharges, and leaching from lined or unlined impoundments. Several of these have been shown to act as neurotoxins, hepatotoxins, and/or carcinogens in a variety of vertebrate species. Existing research has primarily focused on the impacts of major catastrophic release events. Thus, this study aimed to evaluate the spatial distribution and biological impacts of trace metal contaminants near the Bremo Power Station (New Canton, VA) which maintains 6.2 million yd^3 of CCRs in its proximal unlined North Ash Pond adjacent to the James River. Surficial sediments and adult panhandle pebblesnails (*Somatogyrus virginicus*) were collected from the James River upstream, downstream, and adjacent to the station. Sediment samples were extracted using aqua regia (1HNO3/3HCl/3H2O) and diluted using nanopure ultra-deionized water. Whole body snail tissues were removed from their shells and combined into pooled samples of five individuals before being extracted using 65% HNO3/ 30% H_2O_2 and diluted using nanopure ultra-deionized water. Extracted samples were then analyzed for 13 CCR-associated elements (Al, As, B, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Se, Zn) using ICP-OES. Surface water samples were collected and assessed for species richness using eDNA MiFish primers (Jonah Ventures, Boulder, CO). Preliminary results indicate enriched Al, Cu, and Pb in surficial sediments downstream from the station as well as B and Mn bioaccumulation in S. virginicus tissues. Environmental DNA analyses have identified the presence of twenty-four fish species, eight of which are commonly consumed by recreational anglers. Our results provide novel insight into the transport, deposition, uptake, and impacts of trace metal contamination near the Bremo Bluff Power Station which can be used for future risk assessment and remediation efforts.

Comparative reproductive and developmental effects in mice exposed to a PFAS-containing AFFF and a PFAS-free firefighting foam

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Although replacements for per-and polyfluroalkyl substances (PFAS) -containing aqueous film-forming foams (AFFFs) should have reduced persistence and bioaccumulation by design, their toxicity has not been extensively evaluated. As such, a variety of studies with a suite of candidate products are underway, including combined repeated-dose toxicity studies and reproductive and developmental toxicity screening tests with CD-1 mice. Herein, developmental data are selectively presented from mice exposed gestationally, via lactation, and post-weaning to Buckeye Platinum Plus C6 MILSPEC 3% (PFAS-containing) and National Foam AvioF3 Green KHC 3% (PFAS-free). Prior to weaning at postnatal day 21 (PND21), endpoints such as litter weight, pinna unfolding, eye opening, and anogenital distance (AGD)

were observed. Starting at PND22, selected F1 animals were dosed also via oral gavage, and body weight gain and attainment of puberty via vaginal opening (VO) and balanopreputial separation (PPS) were assessed. These endpoints are especially valuable for the comparison of replacement products and for informing decision-makers through the transition from use of PFAS-containing AFFFs, especially given that reproduction and development are impacted in some species exposed to PFAS.

Hg toxicity in three Amazonian plant species and soil microbiome

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Mercury is a heavy metal that can enter the environment through anthropogenic activities like Artisanal Gold Mining (ASGM). In Peru, the Amazonian region of Madre de Dios is one of the most impacted by this activity. Exposure to Hg can have adverse effects on human and environmental health. Environmental quality criteria like Soil Screening Levels (SSL) have been developed worldwide to protect environmental and human health from pollutant exposure. In Peru, SSL for Hg in agricultural soil (6.6 mg kg-1) was adopted from the Canadian regulation without considering research with native species and local soil characteristics. We studied the impact of mercury exposure on the health of three Amazonian agricultural plant species, C. chinense (aji dulce), E. foetidum (sacha culantro), and X. sagittifolium L. Schott (uncucha). We also examined the effect of this exposure on their rhizosphere microbial communities. Two experiments were performed following international standardized guidelines. In each experiment, soil collected from a farm non-impacted by ASGM (control soil) was spiked with Hg to the following concentrations: 10, 45, and 100 mg kg-1 dw for the first experiment and 2, 4, 6, and 8 mg kg-1 dw for the second experiment, respectively. In both experiments, plants were grown in plastic pots with Hg-spiked soil. The replicates were ten and 40 pots per Hg concentration tested and per plant species in the first and second experiments. We also grew plants in non-spiked soil (control soil) with the same number of replicates, ten and 40 pots per plant species in the first and second experiments. The duration of the tests was 21 days. At the end of the tests, germination, mortality, chlorosis, and bacterial population in the rhizosphere soil were evaluated. We found adverse health effects in the three plant species after exposure to every Hg concentration tested. The lowest observable concentration of Hg affecting the plant's health was 2 mg kg-1 dw, three times lower than the SSL for Hg in agricultural soil established in the Peruvian regulation. This study aims to generate toxicological data that could be used to review the Peruvian Hg SSL in agricultural soil and develop environmental health risk assessments in areas impacted by ASGM in the Amazon region.

Analysis of Microplastics Sourced from Deep Sea Mining in the Abyssal Plain of the Pacific Ocean

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Microplastics are now ubiquitous throughout our environment; however, microplastics' presence and abundance on the deep ocean floor have not been well-documented. Sediment samples collected from the abyssal plain of the Pacific Ocean as part of a metal mining project provide an opportunity to investigate the occurrence of microplastics in this untouched ecosystem. We use a Nile red staining technique and fluorescence microscopy to identify and describe microplastics present in the marine sediment samples. By using a sequential sieving method we are able to distinguish between different sizes and shapes of microplastics ranging from fibers to particles of varying sizes. Likewise, by employing ZnCl2 density separation at several concentrations, we were able to identify ultra-dense microplastic particles (> 1.5 g/mL) requiring use of ZnCl2 near the maximum achievable density of ~ 2.0 g/mL. To our knowledge, this is the first report of microplastics for sediments of the Pacific abyssal plain. Additional investigations to describe microplastics abundance and variety across multiple deep ocean sediment samples are ongoing.

The Enumeration of Algal Cells Using Flow Cytometry

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Eurofins

The OECD 201 test guideline outlines test procedures for evaluating potential toxicity of test substances to microalgae. Endpoints for the OECD 201 assay used for risk assessment are based on changes in biomass overtime. Current biomass determination methods include the use of electronic particle counters, fluorescence quantification, or manual counts using a hemocytometer and microscope. Algal toxicity tests have been conducted at the ecotoxicology testing facility at Eurofins – Easton, MD for approximately 30 years, and the methodology for the enumeration of algal cells has not been modified substantially. A recent focus has been investigating the potential use of a flow cytometer for cell density determination for commonly tested algal species. Flow cytometry has been used for this purpose in academic research, however, there has been little to no documented use of this technology for algal toxicity tests conducted according to Good Laboratory Practices. The implementation of this technology would be beneficial in improving efficiency in the lab, reducing the occurrence of human error when preparing or enumerating samples, and allowing for automated counts. To quantify cell density using a flow cytometer (CytoFLEX, Beckman Coulter), gates are applied to observed fluorescent peaks, and events per volume (cells/µL) within the peak are determined. This method has indicated that the autofluorescence of the algae used in the investigation produce strong signals that are easily detected using channels APC and PC5.5 on the CytoFLEX instrument. As with other counting methods, calculated cell densities can then be used to determine critical study endpoints, such as EC50 estimates and NOEC values. This method is currently being developed to calculate these values for a previously conducted algal toxicity test, which will compared to the values calculated from data obtained using an electronic particle counter.

MicroTox Bioluminescence Assay Sensitivity to Metals Toxicity in Full Strength Marine Water: Implications for Monitoring Anthropogenic Impacts in the Marine Environment *

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MicroTox in vitro bioluminescence assay with *Aliivibrio fischeri* has been proposed for near real-time monitoring of toxicity in marine waters receiving deep ocean metal mining effluent. However, testing of background marine water samples (35 ppt) against the 2% NaCl diluent provided for the MicroTox assay consistently yields a 20 – 40% increase in microbial metabolic activity and thus light emission relative to the control. This increase in bioluminescence, likely associated with the change in osmotic pressure, serves to mute detection of subtle toxic effects. As a result, 35 ppt pH 7.5 synthetic seawater (SSW) proved more appropriate for use with marine samples. The SSW diluent consistently yielded 10 – 20% increases in light emission following the 15-min exposure period, matching those of field-sourced marine samples whereas 2% NaCl diluent yielded approximately 20% reductions in light emission after the incubation period, explaining the cumulative observed difference of 30 – 40%. Metals toxicity (CuSO4 and ZnSO4) was investigated by testing dilution series produced in each diluent. Resulting dose response curves and calculated EC50s indicate near identical sensitivity of the MicroTox assay to CuSO4 at the two salinities, but significantly reduced sensitivity to ZnSO4 at the higher marine salinity. These toxicity measures were then compared with Rotifera toxicity via Marine RotoTox kits and traditional inland silverside *(Menidia beryllina)* 7-d larval toxicity tests. The RotoTox test was performed in SSW and 2% NaCl diluent to determine salinity effects on metals sensitivity for comparison with MicroTox results. MicroTox proved more sensitive to zinc suggesting it would be protective of marine biota but was significantly less sensitive to copper suggesting that marine biota may still be at risk. In mining effluents we found similar toxicity results across Menidia, Rotifer, and MicroTox tests suggesting that MicroTox can give a comparable result for test organisms of interest, though individual metal contaminant constituents should still be considered.