

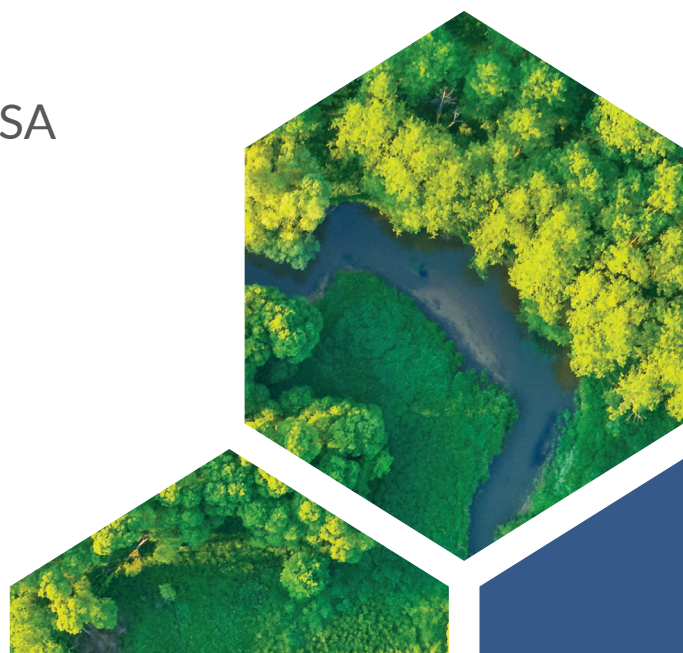
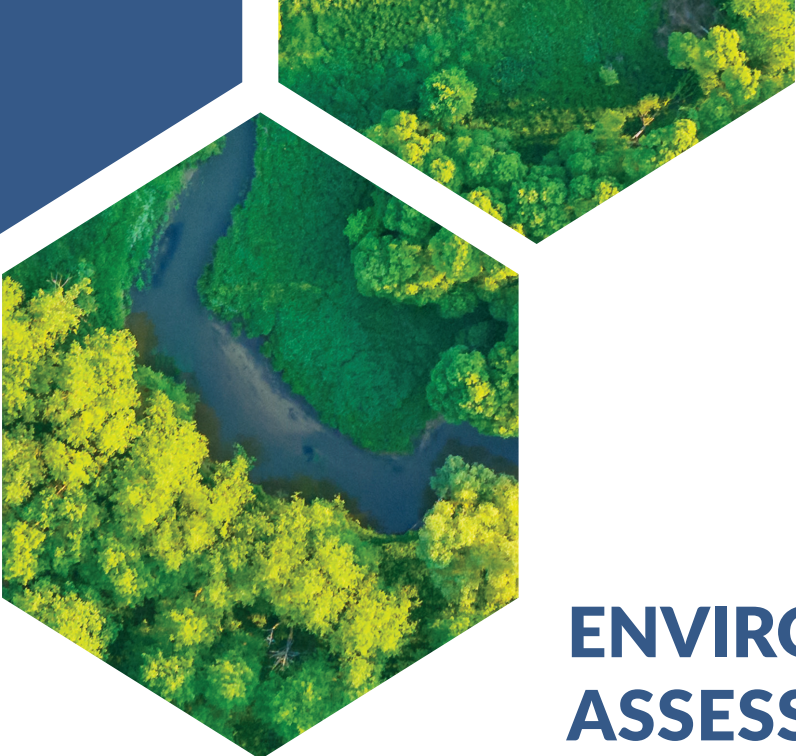


ENVIRONMENTAL RISK ASSESSMENT OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS)

ABSTRACT BOOK

**SETAC North America
Focused Topic Meeting**

12-15 August 2019 | Durham, NC, USA
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This book comprises the abstracts for the platform and poster presentations of the Society of Environmental Toxicology and Chemistry (SETAC) North America Focused Topic Meeting on “Environmental Risk Assessment of Per- and Polyfluoroalkyl Substances (PFAS),” which was held from 12–15 August 2019 at the Durham Convention Center in Durham, North Carolina. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order. In each abstract, the presenting author’s name is underlined.

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SETAC's mission is to support the development of principles and practices for protection, enhancement and management of sustainable environmental quality and ecosystem integrity.

Specific goals of the society are:

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

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1 PFAS in the Environment: Key Challenges and Strategies for Source-Pathway Evaluation and Prioritization

J.L. Guelfo, Texas Tech University / Civil, Environmental, and Construction Engineering

Studies have concluded that millions of people in the U.S. may be exposed to drinking water containing per- and polyfluoroalkyl substances (PFASs) in excess of state standards and federal advisories, and ecosystem impacts may lead to human exposure via diet. Thus, there are an increasing number of local, state and federal agencies, responsible parties, and researchers evaluating occurrence and transport of PFAS in the environment. The objective of some investigations is to determine the source of a known release, and in others it is to determine if a release has occurred. In either scenario, these stakeholders face key challenges related to understanding sources and pathways of PFAS. PFAS have been used widely in products and applications including aqueous film-forming foams, stain-resistant coatings, food paper packaging, and chrome plating, leading to a diversity of facilities that may be associated with PFAS synthesis, use, or disposal. Historical information on these facilities is often unconsolidated or missing. Thus, a primary challenge is identifying potential PFAS source zones and prioritizing potential sources when multiple facilities of concern are present. Another challenge is understanding fate and transport pathways of PFAS following release. PFAS may be released to the subsurface, surface water, and/or air, and the primary pathways of concern will differ by source. Additionally, numerous mechanisms may influence PFAS transport following release including air-water partitioning, sorption, transformation, and impacts of co-contaminants. Of relevance to all of these challenges is the large number of environmentally relevant PFAS, which may vary by type and age of release and elicit different behavior in the environment. This presentation will provide an overview of the challenges associated with evaluating sources and pathways of PFAS release and will also review ongoing work and areas of need related to strategies for prioritizing sources and pathways of concern.

2 TBD

M. Mills, U.S. Environmental Protection Agency / National Risk Management Research Laboratory

TBD

3 PFAS Source-Zones: How to Define Source-Strength?

H. Anderson, U.S. Air Force / AFCEC

PFAS transport within unsaturated source zones is increasingly recognized as dependent on many complex and interrelated factors to include interfacial phase partitioning and hydrophobic and electrostatic interactions with soil organic matter (SOM) and/or mineral surfaces. Bench-scale studies have consistently demonstrated nonlinear sorption with second-order kinetics, PFAS- and soil-specific hysteretic desorption behavior, and to date have largely evaluated interfacial phase partitioning independently from other sorption processes. Practical and universally applicable predictive tools to model PFAS transport within these vadose zones are, thus, operationally challenged by much uncertainty. Moreover, a recent field study consisting of metadata from hundreds of AFFF-contaminated source zones demonstrated that the degree of flushing significantly affected soil-to-groundwater ratios suggesting potential disequilibria, further complicating the application of predictive tools based on equilibrium partitioning. This presentation will discuss these issues and present a potential alternative based on measurements of PFAS concentrations in soil water extracted above the water table (lysimetry). Coupled with similar flux-based measurements in groundwater, source-strength estimates can be generated and will likely provide a defensible basis for soil remediation or lack thereof.

4 Identification of PFAS in the Environment

M.J. Strynar, J. McCord, U.S. Environmental Protection Agency / ORD/NERL/EMMD

Per- and polyfluorinated alkyl substances (PFAS) are a topic of current and growing interest in the global scientific community. For close to two decades many environmental studies have included the environmental occurrence of PFAS such as PFOS and PFOA. Additional studies have reported on a growing number of PFAS with an ever-expanding list of compounds of interest. Hampering the job of the analyst is the growing number of PFAS analytes discovered and reported at a remarkable rate in the published literature. With the introduction of high-resolution mass spectrometry (HRMS) as affordable bench top instrumentation the use in PFAS investigations has led to an ever-increasing portion of the total possible PFAS being detected. Such applications as suspect screening analysis (SSA) from curated PFAS lists have opened the possibility of analyte occurrence discovery. Non-targeted Analysis (NTA) and true compound discovery of additional PFAS has added to these ever-expanding analyte lists. Application of HRMS investigations to industrial use/production sites, and aqueous film forming foam (AFFF) spill/usage sites have continued to unearth novel PFAS in each new application. Using such techniques as negative mass defect, repeating units of a homologous series (CF₂, CF₂O, CF₂CH₂ ...and others), diagnostic PFAS fragment ions and sequential temporal/spatial sampling has allowed for new tentative environmental PFAS discoveries. Hampering much of these international PFAS efforts is the general lack of authentic commercially available standards for unequivocal compound identification. Most studies use HRMS isotope spectral information, and MS/MS data for a weight of evidence for novel PFAS discovered. Missing from most discovered analytes is toxicological data to make informed risk-based decisions. This presentation will focus on the state of the science used for identification of PFAS in the environment and offer suggestions for advancing PFAS research beyond these barriers.

5 Addressing Long-term Transformation of PFAS Precursors in the Environment

L.S. Lee, Purdue University / Department of Agronomy

Per/polyfluoroalkyl substances (PFAS) have been widely used in commercial and industrial products. Their entry into the environment include fire-fighting activities, industrial spills and dumping, leaky landfills, wastewater effluent discharge, land-applied biosolids and storm runoff. PFAS include several subclasses of which many degrade such as those among the telomer-based PFAS subclasses. Degradable PFAS are known as PFAS precursors, because they degrade to other PFAS subclasses. Perfluoroalkyl acids (PFAAs) are the most commonly monitored and regulated terminal products of microbial degradation. PFAAs are also typically more mobile than the PFAS precursors from which they are generated. Degradation rates and metabolite yields are both a function of the specific PFAS precursor subclass as well as other commonly known factors affecting microbial degradation, e.g., oxygen, nutrients and bioavailability. The microbial communities present at a site may also be a factor; however, PFAS precursor degradation does not appear to be unique to a particular microbial community. Most importantly, PFAS precursors degrade substantially more rapidly under aerobic conditions compared to the slow to negligible degradation under anaerobic conditions. Some laboratory-based data suggest some products may form from natural abiotic degradation processes as well, but less has been known on these processes. Sorption to porous media, partitioning into nonaqueous phase liquid phases (NAPLs) and sorption at the air-water and NAPL interfaces are expected to moderate bioavailability, thus degradation rates. Additionally, although PFAAs are terminal metabolites, there are numerous intermediates in the degradation pathway including other telomer-based PFAS, volatile polyfluoroalkyl alcohols, ketones and acids to name a few. Degradation rates and transport will also be impacted by where PFAS precursors and intermediate metabolites may reside, e.g., air, water, and NAPL-filled pores. The relative contribution of PFAS precursors to metabolite and PFAA plumes will also strongly depend on

age of the source zone. This presentation will provide an overview of the processes and pathways that need to be considered; how this information may be used to predict behaviour of PFAS alternatives and possibly help to differentiate PFAS sources; and lastly, identify the knowledge gaps towards improving our ability to predict the long-term generation of more mobile PFAS.

6 Toward a Systematic Characterization and Categorization of PFAS

Z. Wang, Swiss Federal Institute of Technology / Institute of Environmental Engineering

Per- and polyfluoroalkyl substances (PFASs) are a large family of substances that contain at least one perfluoroalkyl (-C_nF_{2n}-) moiety. PFASs are either highly persistent themselves or may transform into highly persistent perfluoroalkyl(ether) acids. Some of them are also (very) bioaccumulative and toxic, and have been detected ubiquitously across the globe. Consequently, PFASs have been increasingly recognized as substances of emerging global concern by governments and stakeholders. Many actions are being taken to address PFASs, starting from understanding their global presence on the market and in the environment. A recent study commissioned by the OECD identified approximately 4700 CAS numbers that are linked to PFASs, including many poorly studied ones. Similarly, an increasing number of “non-targ” studies have identified many previously unknown PFASs in commercial products and in the environment. In contrast, the current development of PFAS terminologies lag much behind, leading to the generation of parallel and often non-intuitive naming and acronyms by different stakeholders. This may further prohibit effective communications among stakeholders and create barriers for synthesizing knowledge. This presentation intends to provide an overview of an ongoing international initiative under the OECD/UNEP Global PFC Group to develop a comprehensive set of guidance and tools that would allow stakeholders to use a common system to systematically characterize, categorize and communicate PFASs. In addition, case studies of major groups of PFASs will be presented to illustrate such a system.

7 Classification and Grouping of PFAS for Environmental Risk Assessment

J.M. Conder, J. Arblaster, Geosyntec Consultants, Inc.; E. Larson, Geosyntec Consultants

Per and polyfluoroalkyl substances (PFAS) comprise several thousand different compounds that span an incredibly wide range of exposure and hazard profiles. This presentation will provide an overview of the major PFAS groupings and highlight some of the key exposure scenarios that have received attention with regards to risk-based decision-making. Key to this review are the extremely large differences in fate, exposure, and potencies among the PFAS groups. A key focus of this presentation will include a case study on the assessment and management of key perfluoroalkyl acids (PFAAs) and other PFAS associated with site-specific releases of aqueous film-forming foam (AFFF) used in the fighting of Class B hydrocarbon fires. The wide variety of exposures and risks among the various PFAS associated with AFFF risks illustrates the extreme diversity of the various PFAS groups in terms of their exposure and effect potential. With regards to understanding risk, PFAS are not a monolith, and there are currently no shortcuts or easy answers.

Exposure

8 From Emission Inventories to the Environment: Prospects for Estimating Exposures To PFAS at the Global Scale

J.M. Armitage, AES Armitage Environmental Sciences, Inc / Physical and Environmental Sciences

Understanding human and ecological exposure to per- and polyfluoroalkyl substances (PFAS) and the associated risks has been the subject of extensive regulatory and scientific effort since the early 2000s. Critical to all risk assessments are accurate and representative estimates of exposure

that take key sources and pathways into account. In the absence of sufficient (bio)monitoring data, exposure and risk assessment can be conducted using a combination of fate/transport and bioaccumulation models. However, a key challenge to the model-based approach for any chemical is the derivation of reliable emission inventories. Besides the typical limitations related to the public availability of required data (e.g., production estimates, use patterns, formulations), the task of estimating emissions for PFAS is further complicated by the production, use and release of chemicals that are degraded to the PFAS of interest in the environment (i.e., “direct” vs “indirect” sources). The first global-scale emission inventories for PFAS focused on direct and indirect (i.e., precursor) sources of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). Emission inventories for direct sources of longer-chain length perfluoroalkyl acids (PFAAs, C₉-C₁₃) were also estimated around the same time. These efforts were driven in part by the detection of PFAS in the Arctic environment and biota and the subsequent debate around the dominant source of these exposures. The main objectives of this presentation are to i) review past and present efforts to develop emission inventories for PFAS and ii) discuss the reliability of these estimates as revealed through simulations of the fate/transport of PFAAs in the global environment. Although the main focus of this presentation is on ecological exposures and fate/transport at the global scale, efforts to quantify other exposure scenarios (e.g., at local scales) will also be discussed briefly to inform a wider discussion of the strengths and weaknesses of current approaches to emission estimation and exposure/risk assessment as it relates to PFAS. This broader perspective is particularly important to consider because of the evolving landscape of PFAS production and use (e.g., introduction of substitutes) and the logistical and financial constraints that limit our ability to generate monitoring data to support every relevant exposure/risk assessment scenario.

9 Human Exposure to PFAS in Drinking Water: Knowledge Gaps and Where Do We Go from Here

Xindi Hu, Harvard University

Poly- and Perfluoroalkyl Substances (PFASs) are detectable in virtually all Americans and have been linked to a suite of adverse health outcomes, including developmental, metabolic and immunotoxic effects. Recent drinking water PFAS contamination near highly contaminated sites has drawn nationwide attention; however, away from point sources the relative importance of drinking water to human exposure to PFASs is largely undefined. This poses challenges as environmental regulators are working toward setting drinking water standards, and communities are grappling with the potential health consequences of the contaminated drinking water source. In this talk, I will link environmental sources of PFAS, the occurrence of PFAS in drinking water and human exposure modeling to understand the relative importance of drinking water as a source for human exposure. Using diverse information, including the latest national-level occurrence data and several large U.S.-based prospective cohort studies, I will present on the prevalence of drinking water contamination by PFAS in the U.S. and key factors that can explain the differences among human exposure to PFAS through drinking water. Three important knowledge gaps and potential solutions will be discussed in the end: 1) lack of monitoring data for approximately one third of the U.S. population, 2) lack of temporally resolved exposure data, 3) lack of methodology to estimate the contribution of drinking water to the more than 4000 PFAS. Human health risk assessment needs to address all these gaps to be effective and health protective.

10 Dietary Exposure to PFAS

T.F. Webster, Boston University School of Public Health / Department of Environmental Health

The major routes of exposure to PFAS are generally considered to be diet, water and the indoor environment. Diet is estimated to be the major route of exposure of PFAS to general populations, i.e., non-occupationally exposed people living in areas without major water contamination.

Dietary exposure is typically estimated in two ways: 1) measurements of PFAS in food in market basket studies multiplied by food consumption rates; 2) regression of PFAS serum concentrations against dietary data as measured by food frequency questionnaires and other methods. Each approach has its strengths and weaknesses, including representativeness of samples and analytical issues (e.g., detection limits) for the first method, significant measurement error for food frequency questionnaires for the second. PFAS may be found in food from both accumulation in the environment and contamination from food packaging or during food processing. In this paper we will review recent research on this subject, including work conducted in the USA.

11 Indoor Environmental Exposure to PFAS

A. Kärrman, Örebro University / Man-Technology-Environment (MTM) Research Centre, School of Science and Technology

Humans are exposed to per- and polyfluoroalkyl substances (PFASs) through multiple exposure pathways. The indoor environment is important since people spend more than 90 percent of their time indoors, and ingestion of indoor dust have been concluded to be a major pathway for toddlers. This presentation will review indoor environmental exposure pathways and the current knowledge on the links between external intakes of PFASs from indoor sources and the circulating levels in human blood. Sources of PFASs in the indoor environment are numerous. The indoor environment holds many materials that contain PFASs, from textiles to personal care products. Emissions of volatile substances and dust particles from household products, carpets, fibres from textiles and paper, human and animal skin- and hair fragments, but also air and particles from the outside environment are all contributing to the exposure. Subsequent pathways for exposure that will be discussed are inhalation of indoor air, ingestion of dust, dermal absorption and hand-to-mouth contact. Numerous studies have indicated that indirect exposure of PFASs is of importance in the indoor environment. Exposure for precursor compounds that generally are not detected in humans but transforms to stable and persistent end-products have been shown to be significant sources for the stable end-products detected in human blood. Polyfluoroalkyl phosphates (PAPs) is one class of precursor compounds that have been frequently detected in the indoor environment and that can be biotransformed to persistent carboxylic acids. Indoor sources are complex and can vary substantial with time depending on input of PFAS-containing materials. Samples from the indoor environment can therefore also be used as a marker for PFASs currently used in consumer products. For example house dust have been suggested as an indicator for PFASs used in consumer products, together with waste water and sludge. The variation in PFAS concentrations is also depending on the sampling method and dust in particular can be difficult to sample in a representative way due to its heterogeneous character. Results need therefore to be interpreted by caution.

12 PFAS Exposures to Humans and Wildlife at Contaminated Sites

T. Bruton, Green Science Policy Institute

PFAS are ubiquitous in the global environment, but environmental concentrations and human and wildlife exposures to these substances are typically highest at contaminated sites. The wide range of applications in which PFAS have been used has led to a number of distinct types of localized PFAS contamination. In this talk, I will summarize what is known about different sources of contamination, including primary (fluorochemical) and secondary (other) manufacturing facilities, the uncontrolled use of PFAS-containing firefighting foam, and land-application of biosolids. I will review the prevalence and geographic distribution of the different types of contaminated sites, as well as the environmental transport pathways that are relevant for different sources. I will provide an overview of relative environmental concentrations of PFAS observed at contaminated sites and how those concentrations relate to exposure for humans and wildlife. I will conclude with recommendations for further research,

including investigating exposure pathways other than drinking water and expanding lists of target analytes to capture the PFAS that are most relevant for a given type of contaminated site.

13 Methods for Assessing Total Exposure to PFAS

M. Sun, University of North Carolina at Charlotte / Civil and Environmental Engineering; D. Knappe, C. Zhang, North Carolina State University / Civil, Construction, and Environmental Engineering; Y. Han, V. Pulikkal, University of North Carolina at Charlotte / Civil and Environmental Engineering

Thousands of per- and polyfluoroalkyl substances (PFAS) species are registered and can potentially enter the environment. Their identification and quantification are challenging because structural information and analytical standards are often lacking. Surrogate methods, such as the total oxidizable precursor (TOP) assay and combustion ion chromatography (CIC), were developed to estimate total PFAS in environmental and biological samples as well as in consumer products. This talk will provide an overview of total PFAS methods and highlight ongoing efforts to improve our understanding of strengths and weaknesses of each method. In the TOP assay, samples are treated with persulfate at high pH and high temperature, and precursors of perfluoroalkyl carboxylic and sulfonic acids (PFCA and PFSA) are oxidized to PFCA and PFSA for analysis. Current TOP assay does not capture recently identified per- and polyfluoroalkyl ether acids. Perfluoroalkyl ether acids and F-53B, a polyfluorinated ether with a terminal -CF₂Cl group, are stable in the TOP assay and thus should be added to the analyte list targeted as end products of the TOP assay. Polyfluoroalkyl ether acids with a -O-CFH- moiety are oxidized in the TOP assay with unidentified end products. In CIC, all forms of fluorine are mineralized to fluoride via combustion, and the fluoride level is subsequently determined to assess the total fluorine content. This CIC method are modified to mitigate background fluoride interference as either extractable organofluorine (by enriching organofluorine through solid-phase extraction and combusting the eluent) or adsorbable organofluorine (by enriching organofluorine through a carbonaceous sorbent and combusting the sorbent). Both TOP and CIC methods, after modification, were applied in environmental samples, and the results were compared with these from liquid chromatography–mass spectrometry to cover fluorine mass balance and provide more comprehensive information on PFAS occurrence and exposure. Other approaches for analyzing total fluorine include particle-induced gamma ray emission, nuclear magnetic resonance spectroscopy, X-ray photoelectron spectroscopy, continuum source molecular absorption spectrometry, and instrumental neutron activation analysis. These methods, together with TOP and CIC, are compared for their advantages and limitations when applied to different matrices, and a discussion is provided on how to choose proper methods for different applications.

14 A Chemical-Activity–Based Approach for Assessing the Bioaccumulation Potential of PFAS

B.C. Kelly, Simon Fraser University / School of Resource and Environmental Management

Per- and poly-fluoroalkyl substances (PFAS) are an important class of organic contaminants due to increasing evidence of environmental persistence, bioaccumulation potential and toxicity. This presentation will provide an overview of the current state of knowledge regarding PFAS bioaccumulation potential, including governing mechanisms and modelling approaches. We utilize a chemical activity-based modelling approach to further evaluate PFAS bioaccumulation in aquatic and terrestrial food webs. Chemical activity (a , unitless) in a given medium is related to concentration (C , mol/m³) by the corresponding solubility (S , mol/m³), (i.e., $a = C/S$). The available data show that bioaccumulation potential of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSA) is highly related to perfluoroalkyl chain length. For example, bioconcentration factors (BCFs) are relatively low for C₈-C₁₁ PFCAs (4.0-4,900 L/kg), whereas BCFs of longer-chain PFASs (C₁₂-C₁₄ PFCAs) range between 18,000-40,000 L/kg. Recent studies have also

demonstrated relatively high bioconcentration potential of other classes of PFAS, including perfluoroalkyl phosphinic acids (PFPIAs) and per- and polyfluoroalkyl ether acid compounds. Field-based studies have shown trophic magnification factors (TMFs) of PFCAs and PFASs in aquatic food webs are typically < 1 , indicating no biomagnification. Conversely, TMFs in food webs containing birds and mammals generally exceed 1, indicating biomagnification in those air-breathing animals. Previous modelling studies have highlighted protein-water and membrane-water distribution coefficients (D_{pw}, D_{mw}) are key parameters governing PFAS bioaccumulation potential. Application of a chemical activity-based model shows steady-state activities of PFCAs and PFASs in aquatic organisms at all trophic levels are equivalent to those in surface water, indicating equilibrium rather than biomagnification. Conversely, the model shows that steady-state PFCA and PFSA activities in birds, mammals and reptiles are elevated above those in environmental media and prey organisms, primarily due to efficient gastrointestinal uptake and negligible elimination via key depuration pathways (i.e., urinary, biliary, respiratory, biotransformation). Lastly, key uncertainties and knowledge gaps related to PFAS bioaccumulation behavior and exposure assessment will be highlighted and discussed.

15 PFAS Toxicokinetics: From External Exposure to Unique Internal Distributions

C.A. Ng, University of Pittsburgh / Civil & Environmental Engineering

The worldwide environmental and, especially, biological accumulation of per- and polyfluorinated alkyl substances (PFAS) took scientists, regulators, and communities largely by surprise. How did these substances fly under the radar for so long? This talk will provide a general overview of the unique uptake, tissue distribution, and biological half-life data on PFAS and how these have been used by a number of research groups to better understand their fate in biological systems, as well as to build new types of bioaccumulation and toxicokinetic models. In particular, I will discuss the potentially synergistic roles of protein binding and phospholipid interactions in determining tissue distribution in different organisms, and how membrane transporters play a role in determining biological half-lives that lead to differences not only among species but also between genders. Data availability and needs for parameterizing reliable physiologically based toxicokinetic (PBTK) models in different species, including humans, will be discussed. Both *in vitro* and *in silico* strategies for gathering these data will be introduced, with their particular strengths and weaknesses for *in vitro-in vivo* and *in silico-in vivo* extrapolation (IVIVE and ISIVE). Finally, persistent data gaps on understanding how exposure route impacts toxicokinetics will be introduced, in the context of understanding the implications of ongoing chronic exposure to PFAS mixtures in both water and food. With an estimated 4000+ PFAS potentially in use, what factors are holding back action on PFAS, and how can toxicokinetic models help?

Ecological Toxicity

16 Overview of Ecotoxicity of PFAS

B.E. Sample, Ecological Risk, Inc; C. McCarthy, Jacobs; J.L. Newsted, OBG, Part of Ramboll / Environmental and Health Sciences

Per- and polyfluoroalkyl substances (PFAS) have been detected in multiple media and tissues of biota in aquatic and terrestrial environments worldwide. Historically, toxicity studies on PFAS have focused on human health evaluations. Although ecotoxicity data are available for the older, more prevalent compounds (i.e., PFOS and PFOA) conducted with common laboratory test species, data are lacking for many compounds and taxa. Because PFAS are a large and complex group of analytes with variable fate and transport characteristics and toxicity mechanisms, data for one compound and laboratory test species may not be relevant to another compound or receptor species. Additionally, PFAS are present in the environment as mixtures of precursor and terminal products that can influence estimates of exposure. Evaluation of the ecological risk from PFAS requires data that are representative of appropriate effect thresholds

(i.e., organ-level effects in addition to growth, reproduction, and survival), exposure pathways (i.e., direct uptake, dietary exposure, and tissue residues), and test species that are suitable surrogates for wild species. This presentation will provide an overview of the current state of knowledge concerning the ecotoxicology of PFAS. For example, while there are substantial data for mammals, data for birds are more limited, and although PFAS residues have been measured in reptiles, reptile toxicity data are currently lacking (but in development). Data for amphibians are also limited. In aquatic environments, the most robust data are for common test species. Most test species have been evaluated for only one or two PFAS and most PFAS have been tested with only a few species. Data suggest PFOS is the most directly toxic PFAS in aquatic environments. PFAS ecotoxicity data for benthic invertebrates exposed to sediments are virtually lacking. Some data are available on the ecotoxicity of PFAS in soil to plants and invertebrates, however the focus has been primarily on PFOS and PFOA in agricultural systems. Overall, although some data are available, significant data gaps exist. These gaps include data for legacy and newer/replacement compounds, mixture effects, modes of action, greater number of test species, and data for multiple exposure pathways to support ecological risk evaluations. New methods (as will be discussed in this session) will help address some of these data gap challenges (i.e., cross-species and cross-chemical extrapolation).

17 PFAS as Novel Materials: Challenges in Toxicity Testing

R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology

As a large group of chemicals with unique physical/chemical properties, PFAS exhibit both hydrophobic and lipophobic properties, presenting a number of difficulties for conducting toxicity tests. While bioaccumulation appears to be related to fluorocarbon chain length and functional group (e.g., sulfonate vs carboxylate), standard log K_{ow}-based bioaccumulation models have not proved useful in predicting bioaccumulation. Tissue residue-based models combined with toxicokinetics data may be useful in developing critical body residue models for assessing the effects of PFAS bioaccumulation. Aqueous exposure routes have dominated PFAS toxicity testing, with fewer tests conducted in sediment or soil systems. Similar issues are likely for estimating partitioning to solid phases in soils and sediments, as equilibrium partitioning approaches have not been useful in predicting toxicity of PFAS. The unique properties of PFAS also contribute to difficulties in expressing exposure dose and potency in toxicity tests. While mass/volume (e.g., ug/L) concentrations may be appropriate in aqueous exposure systems, without a better understanding of partitioning to solid phases, it is not clear whether a simple mass/mass (e.g., ug/kg) expression is appropriate or whether dose should be normalized to the organic carbon content of sediments and soils. Similarly, lipid-normalized bioaccumulation estimates are not appropriate since PFAS do not partition preferentially to lipids. PFAS also present practical challenges for conducting standard toxicity tests such as the effects of carrier solvents, test chamber composition (plastic vs glass), and test system design (flow-through vs static-renewal). In order to accurately and precisely quantify PFAS exposure, individual PFAS or mixtures of verified purity must be readily available in sufficient quantity for conducting toxicity tests. The lack of sensitive, standard analytical techniques for PFAS exposure verification at low or environmentally-relevant concentrations in toxicity tests is also problematic. In some situations, ambient PFAS concentrations present a significant contribution to exposure, so how should this be dealt with? PFAS present challenges in conducting standard toxicity tests, both in the exposure of organisms and the interpretation of data. This presentation will provide an overview of the major issues encountered in conducting toxicity tests with PFAS and discuss possible solutions.

18 Toxicity of PFAS to Invertebrates in Laboratory and Field Studies: Conventional and Emergent Toxicity Effects

S. Valsecchi, Water Research Institute - Italian National Research Council IRSA-CNR

Invertebrates act as the main component of the food web base and play a key role in the dynamics of bioaccumulation and biomagnification. Because many PFAS, especially perfluoroalkyl acids (PFAA), are highly persistent, a longer-term exposure of invertebrates may occur in the environment. The definition of the hazards of these substances for a correct risk assessment requires knowledge of effect levels and a mechanistic understanding of the interaction between toxicants and biological systems, which might cause adverse effects. Ecotoxicity data for PFAS in invertebrates were primarily identified for aquatic organisms. Ecotoxicity tests are mostly limited to a few PFAS, primarily PFOS and PFOA, and the dataset is small in comparison to established pollutants. The available data on conventional acute and chronic endpoints (survival, growth, reproduction) conducted in accordance to standardised laboratory test protocols, show low or moderate toxicity for aquatic invertebrates. The effective concentrations (EC50, EC20) and No Observed Effect Concentrations (NOEC) are largely in the order of mg/L. Generally, there is a lack of studies on longer-term exposure and sub-lethal responses with realistic environmental concentrations, considering the persistence and the wide environmental occurrence of PFAS. On the other hand, recently many toxicity studies have shifted their focus to more sub-lethal metrics such as oxidative stress biomarkers, general toxic stress response, detoxification mechanisms, and endocrine disruptive effects, to obtain a better understanding of how contaminants alter the physiology of these organisms. Some evidence of alteration of biological pathways after exposure to PFAS have been detected in invertebrates such as mussel, midge and cladoceran species but, again, these studies have been conducted only for regulated PFAS and no data are available for new or emerging PFAS of environmental concern. This presentation will show an overview of the conventional toxicological data on PFAS for invertebrate (aquatic and terrestrial) species. Both laboratory and field studies will be considered in order to identify possible intra-class or inter-species relationships. Additionally, mechanistic ecotoxicological data as well as novel approaches, such as evolutionary ecotoxicology studies with invertebrates exposed to PFAS in order to identify effects at environmentally-relevant concentrations will be described.

19 Toxicity of PFAS in Fish

M.S. Sepulveda, Purdue University / Department of Forestry and Natural Resources

PFAS are highly persistent, bioaccumulative, and potentially toxic. The exact number of PFAS is unknown and it is estimated that it could be in the thousands with new PFAS constantly being detected in the environment. Although environmental concentrations can reach hundreds of parts per billion in areas close to Aqueous Fire Fight Foaming sites and PFAS manufacturing plants, the majority of environmental media contains levels in the parts per trillion. It is well known that fish are currently being exposed to PFAS and thus, there is a need to assess the potential impacts of this exposure in both freshwater and marine environments. This presentation will summarize the state of knowledge on the toxic effects and mechanism of toxicity in model and non-model fish species. Since 2003, 161 manuscripts have been published assessing the toxic effects of PFAS to fish, with 20% of these studies published last year alone. Close to 70% of these studies have been conducted in model fish species, mostly zebrafish (42%). In addition, only nine PFAS have been studied, with PFOA and PFOS alone representing 50% and 25% of the toxicity data available for this taxonomic group, respectively. PFAS are not overtly toxic to fish and lethal toxicity values are in the tens to hundreds of parts per million. Exposure of fish to sublethal doses of PFAS has resulted in a myriad of effects including endocrine disruption (thyroid and estrogenic effects mostly reported); alterations in metabolism (disruption of lipid metabolism); developmental and reproductive toxicity (delayed hatching and decreased fecundity); and behavioral changes (altered

swimming). Studies focused on understanding mechanisms of toxicity lag behind, with a majority reporting oxidative stress leading to genotoxicity and mitotoxicity. Mechanisms behind endocrine disruption have been the focus of several recent studies; however, results are inconclusive. Three major gaps were identified: little to no data from marine fishes; an almost complete lack of mixture studies; and inconsistent mechanistic data with an overall lack of fundamental knowledge on the mechanisms of toxicity for this class of chemicals. In conclusion, current PFAS fish toxicity data is inadequate for assessing ecological risk. A full characterization of effects is therefore needed for building robust predictive ecological risk models at the hundreds of PFAS-contaminated sites across the United States.

20 A Current Perspective on the Acute and Chronic Effects of PFAS to Birds, Mammals and Reptiles

J.L. Newsted, OBG, Part of Ramboll / Environmental and Health Sciences

Perfluoroalkyl substances (PFAS) are synthetic fluorinated organic compounds that can be released into the environment during manufacturing processes, from commercial products and applications, or indirectly via oxidation of precursor molecules containing fluoroalkyl chains. Currently over 500 PFAS have been identified in environmental biotic and abiotic media that can include perfluoroalkyl carboxylates (PFCAs), perfluoroalkyl sulfonates (PFSAs), perfluoroalkyl phosphonic acids (PFPA) and fluorotelomer alcohols (FTOHs). To date, while PFAS have received increased attention in federal and state monitoring programs, the focus of these efforts has primarily been on aspects related to human exposure and health. However numerous studies have demonstrated the bioaccumulation of PFAS into aquatic organisms and avian and mammalian wildlife. The significance of these concentration data relative to the risk they pose to wildlife is difficult to ascertain due to the lack ecotoxicity data. This issue is especially relevant for avian and mammalian species where few laboratory studies have been conducted and of which most have focused on either perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). To provide insight into the scope of this issue, an overview wildlife laboratory studies and field studies will be conducted where apical endpoints (survival, growth and reproduction) and other indices of health have been evaluated. The primary focus will be on avian dietary acute and chronic toxicity studies as well as egg injection studies, and an overview of relevant mammalian toxicity data collected in rodent species that could be used surrogate data for mammalian wildlife (marine mammal, mustelids, feral rodent species). Field studies with avian and marine mammal species that relate exposure to potential adverse health outcomes will also be discussed. Finally, these data will be evaluated relative to their potential utility in deriving scientifically useful and defensible toxicity reference values (TRVs) along with insights into what is still needed to provide a greater understanding of potential risks that PFAS pose to wildlife.

21 The Aquatic Toxicology of PFAS: What Has Been Learned from the Development of Water Quality Guidelines in Australia?

A. Kumar, CSIRO / Land and Water; Program- Environmental Contaminant Mitigation and Biotechnology

In Australia, perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorohexane sulfonate (PFHxS) are recognised as primary indicators of a broad range of PFAS, including short chain and other long chain perfluoroalkyl carboxylic acids (PFCA) and perfluoroalkyl sulfonic acids (PFSA). The draft 2015 freshwater default guideline values (DGVs) for PFOS and PFOA in Australia are based on chronic studies that directly link laboratory exposure to adverse effects in aquatic organisms. An exceedance of the DGVs in a water body or inputs to it may trigger further investigation such as site-specific risk assessment to refine the possible risk as opposed to the assumption that harm will have occurred. The development of DGVs for PFOS and PFOA in Australia has highlighted the need for a suite of tests that are reliable and relevant for this class of toxicant. One of the significant conclusions for persistent and bioaccumulative chemicals such as PFOS and PFOA is that there is a need for

multigenerational experiments using vertebrate species and invertebrates with improved experimental design. This will provide a larger number of reliable apical toxicological end-points and add value by linking with emerging mechanistic data. Multigenerational studies could be an advantage in understanding the plausible cumulative adverse effects caused by bioaccumulative contaminants such as long-chain PFASs at environmentally relevant concentrations. Other gaps in knowledge include (i) influence of environmental variables in the toxicity of PFAS; (ii) exposure through water versus exposure through food; (iii) linking body burden measures with ecotoxicological endpoints; (iv) toxicity evaluation and DGVs for other PFAS (including PFAS other than PFOS, PFOA and PFHxS) and their mixtures; (v) sediment DGVs and ecotoxicological data on sediment dwelling species (including multigenerational scenarios); and (vi) implementation of DGVs for beneficial reuse or discharge of effluents from wastewater treatment plants, and for land application of biosolids contaminated with PFAS. By establishing a robust evaluation framework for the biological effects and responses to PFAS using multiple lines of evidence (including mechanistic insights), we will reduce uncertainty in environmental management decisions for managing risks associated with PFAS contamination in the environment.

22 A Canadian Perspective on Evaluating Ecological Hazards and Thresholds for PFAS

M. Houde, Environment and Climate Change Canada / Aquatic Contaminants Research Division; J. Kurias, Environment and Climate Change Canada / Department of Environment and Climate Change; P. Cureton, Environment and Climate Change Canada / National Guidelines and Standards Office, Science and Technology Branch

The Canadian Environmental Protection Act (CEPA, 1999) is the backbone of Canadian environmental legislation. Under CEPA, Health Canada and Environment and Climate Change Canada (ECCC) conduct risk assessments of substances in commerce in Canada (existing substances) or proposed for introduction to the Canadian market (new substances) to determine whether these substances may present a risk to the environment and/or to human health. Risk assessments conducted under CEPA for PFOS, PFOA and long-chain (C9-C20) PFCA s concluded that these substances are harmful to the environment. Concerns have been raised internationally that short-chain (C4-C7) PFAS may have similar impacts to PFOA and PFOS. However, there are significant assessment challenges and ecological data gaps for this large class of substances. ECCC is presently developing a state of science report outlining these gaps and their relevance to evaluation of ecological risk or development of guidelines. Using the information generated by Canadian scientists and others worldwide, environmental quality guidelines have been established for some PFAS. In 2018, Federal Environmental Quality Guidelines (FEQGs) for PFOS in water, fish tissue, wildlife diet (for protection of mammalian and avian consumers of aquatic biota) and bird eggs were published. The FEQGs are benchmarks for the quality of the ambient environment and are based on toxicological effects data. These federal guidelines are developed when environmental quality guidelines have not been established by the Canadian Council of Ministers of the Environment (CCME). The CCME, a minister-led intergovernmental forum for collective action on environmental issues, has developed draft ecological quality guidelines for PFOS in soil for four land uses (agricultural, residential/parkland, commercial, and industrial) and for groundwater considering ecological and human receptors. The CCME guidelines have been developed for the assessment and remediation of contaminated sites and are based on toxicological effects data. The province of British Columbia has also legislated regulatory limits for PFOS, PFOA and PFBS in water and the two sulfonates in soil. Moreover, maximum acceptable concentration for PFOS in drinking water and screening values for nine PFAS have been established by Health Canada for human-related exposure. Considering the large spectrum of PFAS chemicals, data gaps to guideline development will be discussed.

23 PFAS in the Environment: A Survey of Occurrence, Relevant Concentrations, and Observed Effects

C.J. Salice, Towson University / Environmental Science & Studies Biology; A. East, Towson University / Environmental Sciences; H. Anderson, U.S. Air Force / AFCEC

Per- and polyfluoroalkyl substances (PFAS) remain a complex environmental contamination and management issue. Challenges in evaluating risk of PFAS to ecological receptors lie in the shortage of toxicity data as well as a lack of understanding or consensus concerning the most relevant chemicals and/or mixtures. While there are many uncertainties regarding PFAS, there are monitoring data and some field studies that can be used to inform our perspective on ecological risk of PFAS and guide future research efforts. We analyzed a large data set consisting of PFAS measurements in soil and surface waters near U.S. Air Force Installations that have a history of using PFAS-containing Aqueous Film Forming Foams (AFFF) for fire suppression. Using data exploration and prioritization methods, results indicated that two PFAS occurred at the highest frequency and at the highest concentrations: perfluorooctane sulfonate (PFOS) followed by perfluorohexane sulfonate (PFHxS). This was true for both soil and surface water samples with most concentrations in the low parts-per-billion and high concentrations in the low parts-per-million. Other field monitoring efforts have similarly shown predominance of PFOS and PFHxS in surface water, but in some cases, PFAS other than PFOS and PFHxS can dominate environmental samples. Collectively, the data on PFAS in the environment suggest that the source of contamination has a strong influence on which PFAS occur in soil and surface waters. PFAS have also been measured in tissues in a wide variety of faunal species confirming that exposures to ecological receptors occur frequently. Far fewer data, however, are available concerning the effects of PFAS on wildlife and natural fish populations and existing results are equivocal. For example, some studies on avian reproduction and PFAS tissue concentrations show apparent deleterious effects on reproduction while other studies have shown no effect at similar concentrations. Collectively, the data on PFAS in environmental media seem to point to some helpful generalities while the data related to exposure and effects of PFAS in wild species are far fewer and somewhat ambiguous. Ongoing research to better understand occurrence, exposure and effects of PFAS on ecological receptors should be a priority.

24 Pathway-Based Predictive Approaches for Assessing the Ecological Effects of PFAS

G.T. Ankley, U.S. Environmental Protection Agency / Mid-Continent Ecology Division

Emphasis to date concerning PFAS has been mostly on human health, but there is growing realization that these chemicals also are of substantial concern in terms of potential ecological effects. While some ecotoxicity data exist for high-visibility PFAS like PFOS and PFOA, there is a lack of information suitable for assessing ecological risks of the great majority of PFAS that might enter the environment. Further, most testing that has been done with PFAS has been limited to just a few fish and (mostly aquatic) invertebrate species. Given the large number of PFAS and biological species of potential ecological concern, it is not reasonable to expect that knowledge gaps can be adequately addressed through collection of empirical data from whole animal tests. This is not a challenge limited to PFAS. There is an increasing awareness in regulatory toxicology of the need to develop and implement time/cost-effective predictive approaches to support chemical safety assessment. These predictive approaches feature generation of data from tools such as computational models, pathway-based in vitro systems (including high-throughput assays), and short-term in vivo tests with molecular/biochemical endpoints indicative of perturbation of biological pathways/processes of concern. While these types of tools can produce response data more efficiently and rapidly than conventional whole-animal tests, there remains the task of translating this mechanistic information into apical endpoints (survival, growth, reproduction) meaningful to risk assessors/managers. To help address this translation challenge the adverse outcome pathway (AOP) concept was

proposed about a decade ago. The AOP framework provides a transparent depiction of casual linkages between initial chemical perturbation of a biological system (the molecular initiating event) and subsequent measurable changes at progressively higher levels of biological organization (key events), that ultimately lead to an adverse outcome in individuals or populations. This presentation will describe how mechanistic data developed and evaluated in the context of the AOP framework can help address key knowledge gaps in assessing the ecological effects of PFAS, including lack of substantive *in vivo* toxicity data for most PFAS structures of concern, and comparatively little information concerning cross-species susceptibility/sensitivity to the chemicals. The contents of this talk do not reflect USEPA policy.

Human Toxicity

25 Challenges in Understanding the Dose–Effect Changes in Serum Lipids in Laboratory Animals and Humans with Exposure to PFOA and PFOS

S. Chang, 3M Company

While the hypolipidemic mechanism with high doses of PFOS and PFOA have been elucidated using transgenic mouse models, a mode-of-action regarding environmental level PFOA / PFOS concentrations and higher serum cholesterol in humans has not been demonstrated. The dichotomy of these opposite observations is a challenge with the on-going regulatory activities. This presentation will summarize the key observations and highlight several possible non-causal explanations regarding the positive lipid associations with exposure to environmental levels of PFOA and/or PFOS. As science of the various perfluoroalkyl and polyfluoroalkyl substances (PFAS) continues to develop, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have been the anchor points for the research investigations and regulatory activities. In toxicological studies of PFOA and PFOS, the liver is the primary target organ where nuclear receptor activations mediate many biological responses. This includes the PPAR γ -mediated hypolipidemia response typically observed at high doses of PFOA or PFOS in rodents that are several orders of magnitude higher than levels measured in the general human population. These observations are in direct contrast with several cross-sectional epidemiological studies that have reported positive associations between serum non-HDL cholesterol and PFOA / PFOS. Most recently, EFSA adopted a provisional opinion based on benchmark response models for PFOA and PFOS with increased serum total cholesterol using these epidemiology data.

26 Immunotoxicological Findings of PFAS: Consistency of Effects Between Humans and Rodent Models

J.C. DeWitt, S. Vance, T. Woodlief, Q. Hu, East Carolina University / Pharmacology and Toxicology

A toxic insult to the immune system can result in suppression, enhancement, or dysfunction of immune responses that may present as clinically-defined diseases within the immune system or in other systems or may result in sub-clinical outcomes that impact quality of life and disease risk. To date, evidence supports that some of the “legacy” long-chain perfluorinated carboxylic and sulfonic acids within the family of compounds known as per- and polyfluoroalkyl substances (PFAS) are immunotoxic, being able to both suppress and enhance specific immunological responses. This conclusion is supported by data from epidemiological studies of exposed human populations and toxicological studies of rodent models and emphasizes that when such studies are considered jointly, PFAS-induced immunotoxicity is biologically plausible. This talk will summarize the major immunotoxicological findings of legacy PFAS to date, including suppression of the vaccine response in humans and the analogous T cell-dependent antibody response in rodents, enhanced immunological responses that result in allergies, asthma, and autoimmune responses, and dysfunctional inflammatory responses associated with specific disease pathways and outcomes. Putative mechanisms underlying immunotoxicity of the legacy PFAS and evolving

immunotoxicological findings associated with “emerging” PFAS being newly discovered in the environment will be presented. Finally directions for future immunotoxicological research with PFAS will be proposed.

27 A Snapshot of Reproductive and Developmental Toxicity of PFAS

C. Lau, U.S. Environmental Protection Agency / Toxicity Assessment Division/NHEERL/ORD

Concerns for health effects of PFAS, primarily the perfluoroalkyl acids (PFAA) such as perfluoroalkyl sulfonates and perfluorocarboxylates have been raised for the past two decades. In response, a substantial body of literature is now available addressing the potential reproductive and developmental toxicity of these chemicals (of various carbon chain-lengths and functional groups). These reports include laboratory studies with rodents and occasionally monkeys, as well as epidemiological surveys of general population and targeted cohorts. By and large, descriptions of altered reproductive function in adults from exposure to PFAA are inconsistent and inconclusive in both animal and human studies. In contrast, adverse pregnancy outcomes ranging from early pregnancy loss to neonatal mortality in the offspring were detected in rodents from gestational exposure to high doses of long-chain PFAA (such as PFOS, PFOA and PFNA), and altered trajectories of growth and development were seen at lower doses. These effects corresponded well to both toxicokinetics and potency of each PFAA, and appeared to involve the nuclear receptor Peroxisome Proliferator-Activated Receptor-alpha (PPAR α). Recent reports suggest that developmental toxicity of legacy PFAA is recapitulated by some of the replacement PFAS (such as polyfluoroethers). The impacts of PFAA exposure on reproductive and developmental outcomes in humans are less certain and inconsistent among studies. Importantly, the effects of PFAS on development of physiological and metabolic functions and long-term sequelae from *in utero* and early life exposure have been rarely examined (with possible exception of immune responses), which provides opportunities for future investigation. This abstract does not necessarily reflect US EPA policy.

28 Human Health Outcomes of Perfluoroalkyl Acid (PFAA) Exposure: Physiology and Toxicology Inform the Epidemiology Findings

A. Ducatman, WVU School of Public Health

Multi-population prevalence and longitudinal studies associate PFAAs to adverse clinically “coded” lab diagnoses including liver function, lipid, uric acid and thyroid hormone abnormality in children and adults. Outcomes are clearer in studies with wider exposure ranges, revealing dose-responses at general population exposure levels. Non-lab (e.g. health survey) outcomes rely on smaller numerators, requiring large data sets. A sufficient-sized data set found associations to pregnancy-induced hypertension, ulcerative colitis, testicular and renal cancer. Questions (limited evidence) pertain to prostate, bladder, ovarian cancer, and bone/joint disease. Maternal-child and childhood longitudinal studies show consistent associations to altered immune response (Z28.3, -vaccine uptake), childhood infections (and possibly childhood asthma). Childhood studies are inconsistent regarding birthweight and more consistent for subsequent developmental obesity or inability to lose weight, with conflicting neurodevelopmental evidence. Reproductive studies indicate delayed time to pregnancy, altered sperm motility/morphology, and decreased duration of breast feeding. Recent work reveals an “inverted-U” relationship of PFAAs to human stages of renal failure, with crescendo negative relationship to albuminuria, analogous to cadmium-associated excretion patterns. Disparate human outcomes are informed by physiology and toxicology studies. The triad of Liver/lipid/uric acid associations are seen in human steatosis, yet we lack human liver histology following PFAA exposure. However, lipid droplet accumulation found in early human steatosis is seen in PFAA-exposed fish, amphibians, rodents, and monkeys; most species also exhibit hepatocyte enlargement. Abnormal lipid handling in humans alters sterol hormone pathways, consistent with (obesogenic) estradiol/testosterone patterns seen in PFAA-exposed humans, while PFAAs cause altered testicular stromal cells, sterol hormone synthesis,

and testicular stromal cell cancer in animals. Future studies should consider critical windows of exposure in humans, and toxicologic studies focusing on unifying elements of consistent epidemiologic findings would be valuable. The liver/lipid axis will predictably be informed by more careful evaluation of lipid fractions, and by steatosis risk calculations, both poorly represented in standard clinical datasets. Human outcome data pertain to 2-6 PFAAs; means to compare class-wide mechanisms are needed.

29 Integrating Molecular and Toxicokinetic Models for Insight into the Fate and Effects of Diverse PFAS

C.A. Ng, University of Pittsburgh / Civil & Environmental Engineering

Per- and polyfluorinated alkyl substances (PFAS) are a large and diverse group of substances, used in a multitude of consumer and industrial applications and widely detected in the environment and in organisms. Given the extreme persistence of perfluorinated substances (which are also the terminal degradation products of many less persistent polyfluorinated chemicals) there is growing concern about ongoing human exposure to these substances. We now know that exposure to “legacy” long-chain perfluorinated acids is a multi-generational phenomenon, and recent findings of many “emerging” PFAS such as fluoroethers associated with fluoropolymer production are sparking regional contamination crises. Yet of the more than 4000 different PFAS substances that have been potentially used and released into the environment, reliable toxicological data are available for only a small handful, mostly the perfluorinated carboxylic and sulfonic acids. Given ongoing exposures, an experimental substance-by-substance approach to evaluating these substances is untenable. In this talk, model-based approaches to gaining insight into the biological fate and possible impacts of the many untested PFAS will be discussed. Specifically, I will introduce toxicokinetic modeling as an important link between external exposure and the internal distribution of PFAS to sites of toxic action, and what existing models tell us about similarities and differences across different PFAS. In addition, the use of molecular modeling to predict PFAS-protein interactions will be illustrated, with discussion of how such an approach can be used to identify potential toxic mechanisms for PFAS. Finally, I will touch on how molecular modeling can help in the evaluation of whether model organisms are appropriate for predicting PFAS fate and effects in humans.

30 Toxicity and Toxicokinetic Testing and Screening of PFAS

M. DeVito, (NIEHS) National Institute of Environmental Health Sciences / NTP Laboratory

PFAS are industrial chemicals used for a variety of products including, non-stick cookware, stain resistant fabrics, food packaging and firefighting foams. Their widespread use and persistence in the environment and human tissues has led to considerable concern over their safety. The prototype PFAS are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Due to their toxicity, persistence and bioaccumulation, PFOA and PFOS have been replaced by other PFAS compounds. It is estimated that there are hundreds of PFAS chemicals released into the environment as either the products, their impurities, or breakdown products. Little is known about the toxicity of these PFOA/PFOS substitutes, impurities and breakdown products. Clearly traditional methods of toxicity testing will not provide actionable information in a timely manner on hundreds of PFAS. In response to the need to evaluate these chemicals, the National Toxicology Program and the U.S. Environmental Protection Agency have developed a collaborative effort that incorporates in vitro screening efforts followed up by select in vivo studies. Initial assay selection was based on our understanding of the bioactivity of PFOA and PFOS and focused on in vitro assays for hepatic function, transcription factor activation, neuroactivity, and developmental effects. However, it is noted that many of the PFAS found in the environment differ structurally from PFOS and PFOA and are unlikely to have identical bioactivity. Assays for whole genome transcriptional changes in several cell lines will also be included in these screens to increase coverage of bioactivity that is not included in the assays selected based on the prototype chemicals. Finally,

the in vitro bioactivity data will be contextualized by applying high throughput toxicokinetic approaches to estimate the oral equivalent dose that results in blood concentrations equivalent to the media concentrations at the AC50. This in vitro exposure estimate can be combined with estimates of human exposures to provide initial margin of exposure estimates to aid in prioritizing chemicals for further evaluation. Initial studies have evaluated the bioactivity of these chemicals and their metabolic stability. This collaboration represents one of the first large scale efforts to incorporate new alternative methods into an evaluation of a large class of chemicals. This abstract does not necessarily reflect NIH policy.

31 A Chemical Category-Based Approach for Selecting and Screening PFAS for Toxicity and Toxicokinetic Testing

R.S. Thomas, U.S. Environmental Protection Agency / Office of Research and Development

Per- and polyfluoroalkyl substances (PFAS) are a group of fluorinated substances of interest to researchers, regulators, and the public due to their widespread presence in the environment. A few PFAS have comparatively extensive amounts of human epidemiological, exposure, and experimental animal toxicity data (e.g., perfluorooctanoic acid), whereas little toxicity and exposure information exists for much of the broader set of PFAS. Given that traditional approaches to generate toxicity information are resource intensive, new approach methods, including in vitro high throughput toxicity (HTT) and toxicokinetic (HTTK) testing, are being employed by the U.S. Environmental Protection Agency (EPA) and the National Toxicology Program (NTP) to inform further (in vivo) testing of PFAS. The HTT methods include in vitro assays for transcription factor activation, neuronal function, zebrafish development, transcriptomic changes, and high-content imaging for cellular phenotypic changes. The HTTK methods include plasma protein binding and hepatic clearance. A total of 75 PFAS were initially selected for HTT and HTTK testing based on interest to the EPA, compounds within targeted categories, structural diversity, exposure considerations, procurability and testability, and availability of existing toxicity data. An additional 75 PFAS were added to the screening effort at a later date using similar selection considerations. Preliminary data have been collected on the first 75 PFAS and are being cross-referenced with analytical chemistry evaluation of the stock solutions. Generating HTT and HTTK data for PFAS represents a new approach for informing priority setting for these substances. This abstract does not necessarily reflect U.S. EPA policy.

Risk Assessment and Characterization

32 Human Health Risk Characterization: A North American Perspective

M. Dourson, Toxicology Excellence for Risk Assessment

Federal agencies in the United States and Canada have assessed the risk to PFAS chemicals and made some determinations of environmental criteria. State and provincial agencies have also wrestled with similar assessments. These criteria are widely disparate due in part to differences in year of evaluation, available information, assumptions in the absence of critical data, or differences in underlying approaches to criteria development. Salient features of these federal level assessments will be shown and contrasted. New human information will also be shown that might allay the use of some underlying assumptions and perhaps set the stage for harmonization.

33 Human Health Risk Characterisation: An Australian Perspective

A. Hinwood, Environment Protection Authority Victoria / Office of the Chief Environmental Scientist; J. Martin, Environment Protection Authority Victoria / Environmental Public Health Unit; R. McKenzie, Environment Protection Authority Victoria / Environmental Public Health Unit

Human health risk characterisation in Australia is generally based on tolerable daily intakes (TDIs) for PFOA, PFOS and PFHxS concentrations. In 2017 the peak body for establishing Australian Food Standards

Australia and New Zealand (FSANZ) were asked to derive a human based guidance value for the three common PFAS found in Australia. They determined that there was insufficient epidemiological evidence to do so and set out to establish tolerable daily intake (TDI) values which took account of international criteria, the application of mammalian toxicology studies and two Australian pieces of work on pharmacokinetic modelling and a review of immunomodulation. TDIs were derived for PFOS and PFOA. The TDI for PFOS applies to the sum of PFOS and PFHxS. Trigger points for investigation for fish, meat, fruit and vegetables have also been derived by FSANZ using assumed body weights from national data and for children using the 90th percentile for consumption. Each food type is evaluated as 100% of the TDI with no background considered. Drinking water and recreational water quality criteria have been set by the Department of Health based on the TDIs for the same three PFAS. Australian health and environmental regulators use these values to characterise risk using differing assumptions, exposure scenarios and hence advice can differ on the potential risks leading to inconsistent messages about the significance of exposures by communities across Australia.

34 Human Health Risk Characterization: A European Perspective

T.I. Halldorsson, University of Iceland / Faculty of Food Science and Nutrition, School of Health Sciences

In 2008 the European Food Safety Authority (EFSA) established a Health Based Guidance Value (HBGV) for PFOS of 150 ng/kg bw/day based on changes in lipids and thyroid hormones in monkeys; and of 1500 ng/kg bw/day for PFOA based on increased liver weights in rats. Since then several European agencies have published opinions on polyfluoroalkyl substances (PFAS). As an example, in 2016 the National Institute for Public Health and the Environment (RIVM), Netherlands, set a HBGV of 12.5 ng/kg bw/day for PFOA using the same critical study as EFSA in 2008. Relative potency factors for several PFAS using the same endpoint were also derived. A new EFSA opinion was published in 2018 where increase in liver enzymes, serum lipids and immune suppression were identified as critical outcomes based on human studies. A HBGV of 13 ng/kg bw/week for PFOS and 6 ng/kg bw/week for PFOA was derived based on changes in serum lipids. These 100-1000-fold lower values are partly in line with the approach taken by the German Human Biomonitoring (HBM) Commission that derived HBM-I values for PFOA and PFOS in plasma of 2 ng/mL and 5 ng/mL using human studies. The EFSA 2018 opinion ended in a formal divergency process with several European agencies, leading to ongoing revisions of the opinion. Publicly available documents from that process nicely summarize the conflicting views that exist between different European agencies. In this presentation the main points of friction in this ongoing saga will be addressed briefly.

35 Human Health Risk Characterization: A States Perspective

Gloria Post, New Jersey Department of Environmental Protection

Several U.S. states have developed drinking water criteria for PFOA and PFOS that are more stringent than the U.S. Environmental Protection Agency (USEPA) Lifetime Health Advisories (LHAs) of 70 ng/L, while other states rely on the USEPA LHAs. Drinking water criteria for other PFAS that do not have USEPA criteria (e.g. PFBA, PFBS, PFHxS, PFNA, GenX) have been developed by several states, and some states have PFAS criteria for other environmental media (e.g. ground water, surface water, soil) and/or PFAS fish consumption trigger levels. In some states, criteria apply to individual PFAS, while in others, they apply to the total concentration of multiple PFAS. While states generally rely on USEPA risk assessment guidance, development of PFAS criteria involves multiple technically complex decisions for both toxicity evaluation and exposure assumptions, and these choices substantially impact the criterion value. Current state criteria for PFAS are primarily based on non-cancer effects from animal toxicology studies (i.e. Reference Doses; RfDs); some states also evaluate cancer risk from chronic animal study data. RfD development requires choice of critical endpoint (which should be well established, adverse or precursor to adverse, and relevant to humans), point of departure (Benchmark Dose, No Observed Adverse Effect Level,

or Lowest Observed Adverse Effect Level), and uncertainty factors. Additionally, an interspecies extrapolation approach is needed to account for the much higher internal dose (i.e. blood serum PFAS level) in humans than animals from the same administered dose. The choice of exposure assumptions also impacts the criterion. Using drinking water as an example, exposure considerations include the appropriate water ingestion rate (e.g. default adult, lactating woman, infant) or alternative approach (e.g. toxicokinetic model to predict internal dose in breast-fed and formula-fed infants), and a Relative Source Contribution factor to account for the portion of the RfD from non-drinking water exposure sources. To further characterize the potential for human health risk, blood serum PFAS levels predicted from exposure at the criterion level are compared to serum PFAS levels associated with health effects in humans by some states. Examples of choices made by different states in the development of PFAS criteria and the impact of these choices on the numerical values of their PFAS criteria will be presented.

36 An Ecological Perspective: Assessment Challenges/Data Gaps for Short-Chain (C4 - C7) PFCAs/ PFASs and Long-Chain (C9 - C20) PFASs

J. Kurias, Environment and Climate Change Canada / Department of Environment and Climate Change

Risk assessments conducted under the Canadian Environmental Protection Act, 1999 for PFOS, PFOA and long-chain (C9-C20) PFCAs have concluded that these substances are harmful to the environment. Concerns have been raised internationally that short-chain (C4-C7) PFCAs/PFSAs and long-chain (C9-C20) PFSAs may have similar ecological impacts as PFOA and PFOS. These substances are not in commerce, manufactured or produced in Canada; however, these substances are found in biota and in the Canadian environment, such as the Canadian Arctic and the Great Lakes. There are significant assessment challenges including ecological data gaps for these substances. Environment and Climate Change Canada is presently developing a report outlining data gaps and their relevance to the evaluation of ecological risk to encourage data generation. Some key points include: Air-breathing marine/terrestrial mammals and birds are the species most bioaccumulative for PFAS and therefore, of most ecological concern.;PFAS bioaccumulation data based on fish, daphnia and algae alone cannot be used to reliably predict food web bioaccumulation for marine/terrestrial mammals or birds; Species differences result in different rates of bioaccumulation making extrapolations between species and between PFAS chain length difficult;Short-chain PFCAs/PFSAs and long-chain PFSAs are QSAR-difficult. Estimates of log Kow and/or using log Kow of neutral species can result in unreliable predictions for bioaccumulation. Some bioaccumulation models incorporate key characteristics of PFAS (i.e., ionizing and preferential protein partitioning). However, model results remain unreliable as training sets are based on freshwater fish, few empirical data or use either protein-partitioning or lipid-partitioning, but not both; Low acute/chronic ecotoxicity in fish, daphnia and algae is expected as these species have generally low rates of PFAS bioaccumulation. Short-chain PFCAs/PFSAs ecotoxicity values ranged from 110 to 20 250 mg/L. Similar results were seen for PFOA, PFOS, and long-chain PFCAs; New approach methodology data can be helpful to evaluate endocrine related effects if links from molecular/cellular level can be made to a mode of action and/or gross effect (i.e., reproduction, growth and development), and if modes of action are conserved across species (and if so, which modes of action and for which species) to allow for read-across to marine/terrestrial mammals, birds or saltwater fish

37 Development of Canadian Environmental Quality Guidelines for Perfluorinated Substances

P. Cureton, Environment and Climate Change Canada / National Guidelines and Standards Office, Science and Technology Branch; D. Spry, Environment Canada / National Guidelines and Standards Office, Science and Risk Assessment Directorate; S. Backus, Environment and Climate Change Canada; C. Garron, Environment and Climate Change Canada / Water Quality Monitoring and Surveillance; R.J. Letcher, Environment and Climate Change Canada / Ecotoxicology and Wildlife Health Division; P.A. Martin, Environment Canada / Ecotoxicology and Wildlife Health Division; D.J. McGoldrick, Environment and Climate Change Canada / Water Quality Monitoring and Surveillance

Under Canada's Chemicals Management Plan, perfluorooctane sulfonate (PFOS) was declared toxic to the environment (2006). Since then various risk management regulations have been implemented to limit use and release of PFOS, its salts and its precursors in Canada. Internationally, PFOS and 96 PFOS-related substances were added to the Stockholm Convention on Persistent Organic Pollutants (2009) with the purpose to reduce risks associated with the substance. We will describe development of Canadian Council of Ministers of the Environment (CCME) draft Canadian Environmental Quality Guidelines (CEQGs) for soil and groundwater for the protection of the environment for four land uses: agricultural, residential/parkland, commercial and industrial for use in contaminated site assessment and remediation. Several ecological exposure pathways are considered, namely: direct contact of plants and invertebrates to contaminated soil, terrestrial food chain exposure, off-site migration and groundwater movement. Federal Environmental Quality Guidelines (FEQGs) will also be presented for the ambient environment to protect freshwater aquatic species, fish tissue, wildlife diet (mammalian and avian), and bird egg. FEQGs can aid in preventing pollution by providing targets for acceptable environmental quality; can assist in evaluating the significance of environmental monitoring; and can serve as performance measures of the success of risk management activities. The use of CEQGs and FEQGs is voluntary unless prescribed in permits or other regulatory tools. A comprehensive national multi-media monitoring plan, together with archived samples, has provided information on PFOS in water, air, sediments, fish and wildlife and wastewater in the Great Lakes from 1979 to 2014. These data are being used under the Canadian Environmental Sustainability Indicators and Federal Sustainable Development Strategy for reporting on fish and water. Concentrations in water and in fish tissue remained below the FEQG for both the protection of freshwater life (6.8 ug/L) and in fish tissue (9.4 mg/kg ww) since 1979. However, levels of PFOS in lake trout in Lake Ontario exceeded FEQGs for mammalian and avian wildlife diet and could be of concern for fish-eating wildlife.

Environmental Sources, Chemistry, Fate and Transport

MP001 EPA, ASTM, ISO and Modified Methods, Oh My! Navigating Analytical Method Options for PFAS

T. Mcknight, Eurofins TestAmerica

The chemistry and analysis of PFAS is unique and challenging. There is currently no consensus best method for all environmental matrices. The USEPA published Method 537 in 2008 with a recent update in 2018. This method is applied to drinking water matrices only. Several other methods have been published for PFAS in non-potable and solid matrices including ISO Method 25101, ASTM D7979 and D7968. EPA is in the process of validating a number of SW-846 methods with various analytical techniques and parameters for different matrices. The EPA is also in the process of finalizing an additional drinking water method for a more comprehensive list of compounds utilizing different analytical techniques than method 537. The Department of Defense (DoD) has compiled rigorous quality assurance criteria and required parameters for PFAS testing under Table B-15 of the Quality Systems Manual (QSM). In order to meet client needs, environmental laboratories have adopted Method 537 and developed significant modifications to address non-potable water and solid matrices. In general, the broader modifications applied are similar between labs, but the opportunity exists for labs to make unique and unqualified modifications. The inconsistent adoption of these modifications yields opportunity for data variability over time and between labs. This soup of non-validated methods and analytical approaches has left stakeholders with the challenging job of navigating their options and making the right choice for their project objectives. This presentation aims to provide clarity through a comparison of methods and a discussion about analytical parameters and their potential impact on data quality. An update on what is currently available in terms of published methods versus methods under development will be provided as well.

MP002 Development of an Organofluoride Method to Quantify Total Fluorine present at Per- and Polyfluoroalkylated Substances (PFAS) Contaminated Site

K. Dasu, Battelle / National Risk Management Research Laboratory; C. Cucksey, Battelle

Per- and polyfluoroalkyl substances (PFAS) are a huge class of chemicals which are widely used for many commercial and industrial applications. There are more than 4000 PFAS reported to be in the market till date. Out of these, the known quantifiable PFAS account for very small fraction and only limited number of analytes can be quantified using the targeted analysis. Hence there is a need for a method which can be used to quantify the total fluorine present in the environmental samples. The current study investigates a proof of concept approach to breakdown the total PFAS into free fluoride and hence to quantify the fluoride generated using an ion selective electrode and ion chromatography techniques. A rapid screening method is developed to measure the free fluoride generated by the quick reductive defluorination of PFAS in the environmental samples. Different quantitative methods were tested to quantify the free fluoride formed. Fluoride quantification was performed using an ion selective electrode, ion chromatography techniques, and a quantitative ^{19}F NMR technique for accurate quantification and confirmation of free fluoride concentrations. In addition, non-targeted analysis was also performed to understand the mechanistic details of the fluoride generation from the parent compound using different conditions. This technique was tested on both the shorter and longer chain perfluorinated carboxylic acids, sulfonates and precursor chemicals. The results show that this novel total organofluoride method can be used as rapid screening tool to measure the free fluoride generated in the environmental samples. Consistent results were obtained from different fluoride quantitation methods. This method can be used as a quick tool to identify the total fluorine present in the samples, which can be applied for site characterization as well as the site remediation of PFAS contaminated site.

MP003 Development and Application of Forensic Approach for Per- and Polyfluoroalkyl Substances (PFAS) Source Tracking

K. Dasu, Battelle / National Risk Management Research Laboratory; L.A. Mullins, Battelle Memorial Institute / CBRNE Defense; T. Klupinski, D. Friedenber, L. Luan, Battelle; J.R. Thorn, Battelle / Applied Research and Laboratory Operations

Per- and polyfluoroalkyl substances (PFAS) are widely used for many commercial and industrial applications. PFAS contamination at Department of Defense bases and sites is mainly attributed to use and release of aqueous film forming foams (AFFF). PFAS ground water contamination plumes often cover large areas and may include multiple source areas. To better understand sources of PFAS contamination in ground water and differentiate responsible parties, there is a need for forensic techniques. Most PFAS-containing products use a proprietary, technical grade mixture of PFAS designed to impart specific performance-based characteristics to the products (e.g., heat resistance, surfactant properties). This is especially complicated if there are multiple sources of PFAS. As the number of PFAS contaminated sites are on the rise. There is an immediate need to investigate these sites and identify the sources of PFAS contamination using some forensic approaches. Currently, Battelle is developing an approach based on the concentration and composition trends of different PFAS from various sources of contamination scenarios to find correlation between PFAS homologue profiles and their sources. The chemical signature of the degradation products and some of the commonly found perfluorinated alkyl acid precursors varies based on the manufacturers, intended applications and the age of the contamination. This approach is being developed using the high-resolution mass spectrometry techniques, (ultra-performance liquid chromatography coupled to a quadrupole time-of-flight (QTOF) mass spectrometry) in combination with PFAS targeted analysis and the advanced statistical analysis. Our initial study to identify different signatures from the samples of known sources shows clear differentiation of sources in both the environmental samples and the AFFF formulations. This approach will be very helpful for the better understanding of PFAS profiles in differentiating sources of contamination at PFAS contaminated sites.

MP004 How Dirty Is Clean - Patterns and Trends in Real-World PFAS Quality Assurance Data

D.S. Jones, AECOM; J. Collins, AECOM / DCS Remediation; M. Aucoin, K.L. Davis, AECOM

Per- and Polyfluoroalkyl Substances (PFAS) present unique and challenging cross-contamination issues when collecting and handling environmental samples. Investigations of potential environmental contamination require low-level analytical chemistry methods to meet the stringent criteria and benchmarks for these bioaccumulative and persistent compounds. Unlike many environmental contaminants, PFAS are often used to enhance commonly available products and materials. Sampling procedures emphasize avoiding sources of PFAS contamination, including non-stick materials, water- and stain-proof coatings, and various personal care products. There is relatively limited research on how various products potentially used by field personnel may affect analytical results, and the extent to which conservative sampling protocols reduce cross contamination. However, blank and rinsate samples are routinely collected from sampling and personal protective equipment during environmental sampling events. This meta-study evaluated PFAS quality assurance data to help characterize this inherent source of uncertainty. A dataset was assembled from a broad suite of projects, locations, and events. The data were anonymized, aggregated, and statistically evaluated to identify patterns and trends. The results help quantify, to the extent feasible, potential cross-contamination sources of PFAS encountered during routine sampling events. This meta-analysis demonstrates that staff awareness, thorough training, and conscientious adherence to rigorous sampling protocols produce high quality data and help increase confidence in the findings of PFAS environmental investigations.

MP005 Forensic Tools for Assessing PFAS Sources*E. Houtz, ARCADIS US Inc. / Environmental*

Due to the widespread use of per- and polyfluoroalkyl substances (PFASs), there is considerable concern around correctly associating PFAS detections with the sources from which they were released, a topic often referred to as PFAS forensics. While PFASs are known to be a large class of chemicals, many of the commonly measured PFASs like perfluorooctane sulfonate (PFOS) are associated with many industries and applications. Some of the tools that can be used to assess sources of other compounds of concern, namely compound specific isotope analysis (CSIA), are currently infeasible for PFASs. In spite of these limitations, there are a number of tools that can be used to understand PFAS forensic signatures. Using routine PFAS data collected from analysis via methods like US EPA 537 and those accredited by DoD Quality Systems Manual 5.1 and later, PFAS sources can be differentiated via the relative amounts of the individual PFASs, i.e., PFAS profiles, and in some cases, their chromatographic branching patterns. Assessing these PFAS data features in the context of the spatial distribution of samples and an understanding of the local hydrogeology where the samples were collected can allow for a robust assessment of unique, discrete PFAS sources, even for similar analytes. Tools such as high resolution mass spectrometry can provide an additional level of confidence in differentiating among different PFAS sources by identifying unique PFASs that are outside the scope of typical commercial laboratory PFAS methods. This presentation will review the tools that are available for PFAS forensics analysis and demonstrate the use of these tools at characterized PFAS-impacted sites.

MP006 Targeted and Non-Targeted Analysis of Per- and Polyfluoroalkyl Substances in Wet Deposition Events*R. Mott, University of North Carolina, Wilmington*

A series of wet and dry deposition samples are being analyzed for the presence of long chained per- and polyfluoroalkyl substances (PFAS). Precipitation samples are being collected on an event basis at the University of North Carolina Wilmington atmospheric collection station for a period of one year. Additional seasonal samples (3 during winter and 3 during summer) are being collected at various sites throughout the state of North Carolina including Bald head Island at the mouth of the Cape Fear River, University of North Carolina at Chapel Hill, University of North Carolina at Charlotte, East Carolina University, and Western Carolina University. PFAS are extracted via weak anion exchange cartridges with acceptable recoveries of 13C-PFOA of 70-120%. Travel blanks, field blanks, and procedural blanks show non-detects of PFMOPrA, PFMOBA, PRPOPPrA, PFOA, and PFOS with concentrations falling below the level of quantification. Preliminary results indicate that there is a significant influence of air mass back trajectory on PFAS abundance and distribution where events coming from coastal origins have very low to non-detectable concentrations of analytes whereas terrestrial dominated events result in higher presence of these compounds. Terrestrial rain events (n=2) indicated PFPOPPrA concentrations ranging from 24 ng/l to >500 ng/L. Marine and coastal rain events (n=3) showed non-detects for PFMOPrA, PRPOPPrA, PFOA, and PFOS. Results of this study are significant because they suggest that PFAS are present in precipitation in North Carolina. Data also indicate that these anthropogenic contaminants have the potential to travel significant distances from where they are emitted.

MP007 Developing Database of PFAS (Per- and Polyfluoroalkyl Substances) Transformation Pathway with the Aid of Prediction Tool*Y. Choi, C.P. Higgins, Colorado School of Mines / Department of Civil and Environmental Engineering*

AFFFs (Aqueous Film Forming Foams) are complex proprietary formulations that contain % levels of PFAS as the ingredients of products containing proprietary fluorinated surfactants to the public, elucidating the composition of PFAS is important for environmental and health concerns. Due to the knowledge gap from the limited degradation

pathway and intermediates reported, many potential interim compounds of degradation are not well aligned with newly analyzed PFAS classes to understand the transformation status of the precursors. With the aid of reliable prediction program, the predicted compounds will assist prediction/modeling of PFAS compound. The database was developed by compiling data of transformations and pathways including parent compounds, observed transformation products, and putative transformation products identified in the literature and through software prediction. The comprehensive pathway data will help the management of PFAS impacted site including source allocation of, delineating contamination plumes, and determining the status of degradation.

MP008 Targeted and Non-Targeted Analysis of Per- and Polyfluoroalkyl Substances in North Carolina Drinking Water Sources*N. DeStefano, North Carolina State University; Z. Hopkins, North Carolina State University / Civil, Construction and Environmental Engineering; A.S. Joyce, Duke University / Civil and Environmental Engineering; Y. Han, M. Sun, University of North Carolina at Charlotte / Civil and Environmental Engineering; P. Ferguson, Duke University / Civil and Environmental Engineering; D. Knappe, North Carolina State University / Civil, Construction and Environmental Engineering*

Per- and polyfluoroalkyl substances (PFAS) are a group of man-made compounds produced and used commercially for their nonstick and heat resistant properties. They are commonly used in food containers, household products, electronics manufacturing, and firefighting foam. Several PFAS, such as perfluorooctanoic acid (PFOA), are ubiquitous globally and can harm human health through commercial and environmental exposure. In addition to the suite of known compounds, thousands of new PFAS are being produced and released into environmental matrices, limiting our ability to reliably assess true human exposure potential. For example, the compound known as "GenX" was recently discovered in high concentrations in the Cape Fear River in North Carolina (NC), downstream of a fluorochemical manufacturing plant. GenX and related compounds passed through drinking water treatment plants unaffected into finished water taps, and the resulting impacts on human health are largely unknown. As a result of the discovery of GenX and related fluoroethers, residents in other NC communities are wondering whether poorly understood PFAS are present in their drinking water. Therefore, the overarching aim of this study is to gain a better understanding of PFAS occurrence in North Carolina drinking water sources. A combination of targeted and non-targeted workflows is being performed to comprehensively identify PFAS in raw drinking water from public water systems throughout NC. Also, adsorbable organic fluorine (AOF) is being quantified in each water sample by combustion ion chromatography to understand the total burden of fluorinated compounds. Known PFAS compounds are quantified using available authentic standards and compared to AOF data to determine the proportion of PFAS that remains uncharacterized. Non-targeted PFAS identification is being performed by spectral library interrogation, isotope rationalization, in situ fragmentation, generation of fragmentation trees, and comprehensive literature and patent review.

MP009 Isotope Dilution-Direct Injection (DI-LC/MS/MS) Method for the Analysis of PFAS in Drinking Water, Groundwater, and Wastewater*S.T. Wolf, 3M / EHS Environmental Lab; C. Grochow, 3M Company / 3M EHS Laboratory; B.T. Mader, 3M / Environmental Laboratory*

An isotope-dilution-direct injection-liquid chromatography tandem mass spectrometry (DI-LC/MS/MS) analytical method is routinely used in our laboratory to measure the concentration of perfluoroalkyl substances (PFAS) in drinking water, groundwater, non-contact cooling water, and wastewater treatment plant effluent samples. This method incorporates the use of stable isotope internal standards (ISs) and surrogate recovery standards (SRSSs) that are added to sample bottles prior to sample collection and enables correction for PFAS adsorption losses. The use of stable isotopes, and the direct injection of sample onto the LC instrument,

results in significant reductions in sample preparation time and laboratory waste generation while also improving the precision of the analysis. The method was first described in *Analytical Methods*, 2013, 5(10), 2444-2454 and since that time the method has been added to the 3M EHS Laboratory ISO 17025 accreditation and the scope expanded from thirteen target analytes and four SRSs ([2,3,4-¹³C₃]-PFBA, [1,2,3,4-¹³C₄]-PFOA, [1,2,3,4-¹³C₄]-PFOS, and [1,2-¹³C₂]-PFUnA) to more than twenty-five target analytes. Over the last seven years, this method has been used to analyse more than 15,000 water samples that originated from a variety of different locations. In this presentation we will summarize the quality assurance data from the analysis of laboratory control samples and matrix spike samples and demonstrate the robustness and overall uncertainty of the method. Target analytes: PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrA, PFTeA, PFBSA, PFOSA, N-MeFOSA, N-EtFOSA, N-MeFOSAA, N-EtFOSAA, N-MeFOSE, N-EtFOSE, FtS 4:2, FtS 6:2, and FtS 8:2.

MP010 Validation of SW-846 8327 PFAS in Non-Potable Waters

L. Gaines, U.S. Environmental Protection Agency / OLEM OSRTI SPB

EPA is validating an analytical method for 24 PFAS in non-potable waters. Draft method SW-846 8327 is a direct inject LCMSMS method being validated for groundwater, surface water, and wastewater effluent. Statistical results from the validation study will be presented including mean and range of recovery and relative standard deviation for the different analytes. Intra-laboratory and inter-laboratory statistical analysis results will be discussed. An update on other PFAS methods to be validated will also be discussed. The views of the authors of this abstract are those of the authors and do not represent Agency policy or endorsement.

MP011 Novel Bubble-Based Analytical Techniques for Concentrating and Detecting PFAS

L. Luo, R. Ranaweera, Y. Cao, Wayne State University / Chemistry

Here, we present two novel bubble-based techniques for PFAS analysis. The first technique is for detecting PFAS in water. The detection mechanism is the high surface activity of PFAS lowers the energy barrier for bubble nucleation, which is then reported as the change of electrical current by an ultrasensitive electrochemical transducer. The limit of detection of our detection method is 20 nM (or ~10 µg/L) with a dynamic range of over three orders of magnitude. With an additional preconcentration step, our method achieves the limit of detection of interest: 14 pM or 70 ng/L, which is recommended by the U.S. Environmental Protection Agency for PFOS and PFOA in drinking water individually or combined. This detection method does not respond to common matrix interferences such as humic acid and proteins. The second technique is for preconcentrating PFAS in water prior to detection. The preconcentration was achieved by bursting electrogenerated H₂ bubbles to form PFAS-enriched aerosol. Our method achieves over 1,000-fold enrichment of PFAS in ~10 min.

MP012 Tentative Identification of New-generation Per- and polyfluoroalkyl Substances in Industrial Products

M. Davis, C. Rosal, M. Henderson, B. Acrey, R. Zepp, J. Washington, U.S. Environmental Protection Agency / Office of Research and Development

Per- and polyfluoroalkyl substances (PFAS) are contaminants of emerging concern, with trace amounts being found in even the most isolated ecosystems across the globe. Properly identifying and classifying these substances is essential for determining their fate in the environment and their toxicity to humans and ecosystems. However, the rapid development and employment of new PFAS chemistries in industrial processes and products has made this task an increasingly significant challenge. Here, we examined the composition and fate of 13 industrial dispersions and surfactants. Non-targeted analysis of samples was conducted using high mass-resolution liquid chromatography/mass spectrometry (LC/MS) to tentatively identify new-generation PFAS as well as non-fluorinated additives. Targeted analysis via conventional mass-resolution

gas chromatography/mass spectrometry (GC/MS) was used to screen for suspected compounds, providing a more complete compositional analysis. In tentatively identifying new PFAS being employed by the chemical industry and studying their environmental fate, we provide further insight into which compounds should be targeted for further investigation into environmental exposures and hazards.

MP013 Chemometric Analysis of a Collective Perfluoroalkyl Acid Dataset from 14 Site Investigations of Aqueous Film-Forming Foam Impacted Sites

Z. Neigh, AECOM / Environment

Fluorosurfactant-based foams, such as aqueous film-forming foam (AFFF), have been used to fight fires since the 1960s and have created a long legacy of per- and polyfluoroalkyl substance (PFAS) contamination at defense sites. To investigate this legacy PFAS contamination, 14 Site Investigations of similar scope were performed at active defense sites across the United States focused on potential AFFF release locations. The potentially impacted areas were grouped into ten common categories for all sites, (1) nozzle testing area, (2) fire training area, (3) former crash site, (4) fire department building, (5) hangar, (6) aircraft apron, (7) vehicle maintenance or wash bay, (8) stormwater drainage basin or outfall, (9) wastewater treatment or biosolids application, and (10) boundary monitoring wells. At each location, samples were collected from soil, sediment, groundwater, and/or surface water, producing a collective total of 1,281 samples. The samples were analyzed by United States Environmental Protection Agency (USEPA) Method 537 Modified consistent with the modifications discussed in Table B-15 of the Department of Defense Quality Systems Manual 5.1 for the six PFAS analytes listed in the USEPA's Third Unregulated Contaminant Monitoring Rule (UCMR3): Perfluorobutane Sulfonic Acid (PFBS: 375-73-5), Perfluorohexane Sulfonic Acid (PFHxS: 355-46-4), Perfluoroheptanoic Acid (PFHpA: 375-85-9), Perfluorooctanoic Acid (PFOA: 335-67-1), Perfluorooctane Sulfonic Acid (PFOS: 1763-23-1), and Perfluorononanoic Acid (PFNA: 375-95-1). Using readily available multivariate chemometric methods such as principal component analysis, cluster analysis, and discriminant functions, we aimed to maximize the amount of information extracted from this shortened analyte list regarding the releases themselves and the environmental fate and transport behavior of these PFAS mixtures. This information will be critical in developing an effective approach to the sites that advance to a Remedial Investigation. We observed general associations between the sites and developed unique mixture profiles based on release location category and spatial distribution data such as soil sample depth. Additionally, by comparing the release information and mixture data of the six UCMR3 PFAS analytes to available AFFF data with extended analyte lists, we were able to extrapolate additional PFAS constituents which could be present in these mixtures.

MP014 The Legacy of Long-Chained Poly- and Perfluoroalkyl Substances in Seafood: Implications for Risk-Based Guidelines

Clifton Dassuncao, Eastern Research Group, Inc. / Environmental Health; Adela Chovancova, Harvard University; Andrea Tokranov, U.S. Geological Survey / New England Water Science Center; Heidi Pickard, Elsie Sunderland, Harvard University / Harvard John A. Paulson School of Engineering and Applied Sciences

Despite a phase-out in production, it is unclear if levels of long-chained PFASs are decreasing in freshwater fish near contaminated sources. The European Food Safety Authority estimates that upwards of 86% of PFOS exposure comes from fish and other seafood. Paired surface water and freshwater fish samples were collected at nine locations across the Merrimack river watershed in New Hampshire and analyzed for 24 PFASs. Bioaccumulation factors (BAFs) for 18 compounds were calculated in eight freshwater fish species (Bluegill, Pumpkinseed, Yellow Perch, Lake Whitefish, Brown Bullhead, Smallmouth bass, Largemouth bass, Chain Pickerel). BAFs rapidly increased with trophic level and carbon chain length with Smallmouth Bass having the greatest BAF. The highest logBAFs were measured for the long-chain PFASs: PFOS

(2.8 - 3.8), PFDA (2.3 - 3.6), PFUA (2.8 - 4.3), and PFDoA (3.2 - 5.1). PFOA (1.7 - 2.1) and PFNA (1.5 - 2.1) bioaccumulated to a less extent. The longest-chained compounds (PFDS, PFTrA, and PFTA) were detected in fish but infrequently detected in water, and so BAFs were unable to be calculated. A risk-based approach was used to develop fish advisory guidelines for this watershed including for the often less considered long-chained compounds.

MP015 In Situ and Laboratory Characterization of Two Novel Passive Samplers for PFAS Detection

J. Becanova, C. Gardiner, M. Dunn, R. Lohmann, University of Rhode Island / Graduate School of Oceanography

An essential step to prevent future PFAS exposure is to establish robust monitoring tools for the detection of PFAS at potentially contaminated sites, in combination with subsequent corrective action. The use of passive samplers, which provide a time-weighted average contaminant concentration, is convenient to determine the dissolved PFAS concentrations in water and assess the PFAS bioavailability in various matrixes. We tested and characterized two passive sampling approaches; i) a solid-phase microextraction (SPME) fiber sampler to determine the PFAS in porewater and ii) a microporous polyethylene (PE) tube sampler to assess PFAS in surface water. The polyacrylate (PA) SPME fibers were used to determine the equilibrium partitioning constant (KPA-W) and factors affecting the partitioning process. SPME fibers were exposed to 21 dissolved PFAS for various time periods (0h to 48h). After exposure, the PFAS concentration in water and fiber extracts were analyzed, and apparent equilibrium was reached within 24 hours. The calculated log KPA_W increase with increasing length of the carbon chain and vary with PFAS functional group. Follow-up studies were conducted to detail how i) water properties (pH and salinity) and ii) PFAS concentrations effect PFAS sorption and characterize the robustness of the fiber application. The PE tube samplers were characterized using a tank-based laboratory method and field deployments. The laboratory validation consists of exposing the samplers to a known concentration of 21 PFAS in water, under environmentally relevant conditions (flow rate, temperature, and salinity). During the exposure period, the PFAS concentration in water was monitored to guarantee the stability of PFAS uptake. After exposure, the PFAS concentration in the samplers was analyzed and the laboratory sampling rates (RS) were calculated. Additionally, to characterize in-situ RS for PE tubes samplers, two field sampling campaigns were conducted across nine sites in a well-mixed estuary and at two wastewater treatment plant (WWTP) effluents. 25 PFAS were measured across all sites in the passive samplers, as well as analogous water samples. In the estuary, the PE tube samplers accumulated a sum PFAS of 2 to 15 ng/sampler, and in the WWTP effluent accumulated 60 to 70 ng/sampler. These preliminary studies suggest the successful use of these passive samplers for PFAS, and future utility as a monitoring device in aquatic environments.

MP016 Rapid Small-Scale Column Tests to Predict Per- and Polyfluoroalkyl Substances (PFAS) Removal by Anion Exchange Resins

L. Cheng, D. Knappe, North Carolina State University / Civil, Construction and Environmental Engineering

Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants with adverse environmental and public health effects. For drinking water, the EPA issued a health advisory level for the sum concentration of PFOA and PFOS at 70 ng/L. Furthermore, the North Carolina health goal for the perfluoroalkyl ether acid (PFEA) GenX is 140 ng/L. Conventional drinking water treatment processes are ineffective for PFAS removal. Ion exchange (IX) resins have gained attention as a treatment alternative for PFAS removal because they can be regenerated onsite and may be more effective for short-chain PFAS removal than granular activated carbon. Goals of this research are to evaluate whether PFAS diffusion inside of IX resin varies with particle size, and how IX resins properties, coexisting PFAS, background water matrix and empty bed contact time affect PFAS removal efficiency, IX resin use rates, and scale-up. To

date, suitable approaches for the scale-up of bench-scale IX data have not been developed. To fill this knowledge gap, we are exploring a range of design options for the rapid small-scale column test (RSSCT) to simulate PFAS removal observed in a recently completed pilot study. RSSCTs can significantly reduce time and cost to determine IX design and operating parameters. RSSCTs are based on principles of similitude and are conducted with crushed IX resin. Using a proportional diffusivity design for scale-up, PFHxA breakthrough curves from the RSSCT and a pilot study conducted in Wilmington, NC, were in good agreement. IX resin use rate decreased with increasing PFAS chain length. Short-chain PFAS with 2 and 3 perfluorinated carbon atoms, such as PFMOAA, PMPA, and PFBA, started breakthrough after processing about 10,000 bed volumes (BVs) of water through the IX resin bed. PFAS with 5 perfluorinated carbon atoms, such as GenX and PFHxA, reached breakthrough after ~60,000 BVs. Recently identified PFEA, such as HydroEVE and NVHOS, reached breakthrough after ~100,000BV. This research will support the design of future IX treatment processes in the context of remediation and drinking water treatment.

MP017 A Holding Time Evaluation Study for the Analysis of PFAS in Aqueous Samples

C. Neslund, Eurofins Lancaster Laboratories Environmental, LLC

The presentation will describe the results of this holding time study and will look to compare container type, preservative versus no-preservative and whole container versus a subsampled container. Results will be compared for a range of compounds broader than the EPA Method 537 list and will attempt to determine the optimum holding time for each of the scenarios presented previously. Additionally, the results of a client supplied proficiency sample that was prepared in sample matrix at two different points approximately 6 months apart will be shared. EPA Method 537 requires the use of polypropylene containers and specifically admonishes glass. This is purportedly due to adsorption of PFAS compounds on the glass walls. In addition, a preservative is added as a chlorine scavenger, Trizma, which also is purported to have some buffered capacity that could potentially improve holding time. Trials were prepared with spiked laboratory water in glass and high density polyethylene (HDPE), and with and without Trizma. Additionally, since Method 537 requires the use of the entire sample container and a rinse of the container, trials with whole container and partial container (subsample) were prepared. Containers were pulled from storage at consistent intervals over 3 months. Perfluoroalkyl and polyfluoroalkyl substances are considered persistent organic pollutants (POPs). As such our expectation is that they remain in the environment for years and in many cases are not biodegradable. However, the lone EPA method for the analysis, EPA Method 537, version 1.1, stipulates a holding time of 14 days. Fourteen days is the same holding time used for EPA methods like SW-846 3510/8270 where the target compound lists include compounds known to be susceptible to microbial action or other enhanced oxidation technologies. Is the 14 day holding time for POPs like PFAS overkill?

MP018 Employing Unique High-Resolution Mass Spectrometry Strategies for Analysis of PFASs Including the Novel PFOA-Replacement Compound, GenX

K.C. Hyland, Sciex / Environmental Food and Beverage

GenX is a compound of recent, intense regulatory attention due to human health concerns and detection in surface water. Using the X500R QTOF system both SWATH(R) and MRM-HR acquisition methods were developed. MRM-HR is advantageous as a high-resolution product ion transition is monitored, resulting in enhanced specificity for the target in complex matrices. In contrast, SWATH(R) acquisition allowed for the collection of the MS/MS fragmentation pattern for library searching and compound confirmation. This Data Independent Acquisition technique is unique in its ability to tailor precursor isolation window ranges as well as combine its acquisition with MRM-HR or with a Data Independent Acquisition Technique, to optimize the quantitative an qualitative method

performance and data quality. Finally, LOD/LOQs values will be presented as well as other performance metrics such as method precision and linear dynamic range.

MP019 Legacy and Emerging Perfluorinated Alkyl Substances in Water: Developing an SPE Method for LC-MS/MS Analysis

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Perfluoroalkyl substances (PFAS) are a group of commonly used compounds in industrial and consumer goods. Recent interest in the occurrence of these persistent and bioaccumulative anthropogenic pollutants has been observed. Many countries recommend some of the legacy compounds, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) be monitored in water, requiring challenging detection limits at sub to low ng/L (ppt) levels. Surface water, ground water, influent waste water, and effluent waste water were collected. Sample extraction was adapted from ISO 25101. The method was adjusted to allow for an extended list of PFAS. Water samples were acidified, and 250 mL were extracted using weak anion exchange solid phase extraction cartridges. A 22 minute LC gradient was employed on a 100 mm C18 analytical column to provide retention and separation of 65 PFAS analytes. Best practices to minimize background were employed in this work, where all laboratory supplies were checked before use and trace residue LC components (PEEK tubing to replace the traditional Teflon coated solvent lines) and solvents (bottled in a manner to reduce residual background) were sourced. This was important to do because of the number of perfluorinated substances, including Gen-X, target. The method was found to be accurate, sensitive, quantitative and reproducible for the variety of water types analyzed. A certified standard was used as a QC for instrument performance. The system performed within the designated Acceptance Limits, with the average error of 15% from the certified values. Performance of the sample preparation method is summarized in the recovery values, where the majority of the PFAS fell within the range of 75% to 130%. Repeatability of the method (n=6) was assessed where all PFAS had a %RSD below 15%. Excellent linearity ($R^2 > 0.995$ and residuals $< 20\%$) was achieved for all analytes over satisfactory working range. Detection limits achieved in-sample were in the range of < 0.01 to 2 ng/L, where the majority of PFAS yielded sub-ng/L (ppt), reaching to the pg/L (ppq) levels, while a few of the less water-soluble compounds had ng/L (ppt) detection limits. The method was used to monitor the occurrence of PFAS analytes in ground, surface, influent waste and effluent waste waters. Of the compounds targeted, 27 were detected across the samples. All samples contained legacy and emerging PFAS compounds.

MP020 Ultra-Short-Chain Perfluoroalkyl Substances Including Trifluoroacetic Acid in Water Connected to Known and Suspected Point Sources in Sweden

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Ultra-short-chain perfluoroalkyl substances, including trifluoroacetic acid, are highly persistent and highly polar perfluorinated substances with 1-3 carbon atoms. Trifluoroacetic acid has been found globally and a well-known source is the atmospheric degradation of hydrofluorocarbons and hydrochlorofluorocarbons. Data reporting environmental occurrence of other ultra-short-chain perfluoroalkyl substances is scarce and potential sources are not well studied. Sources other than the atmospheric degradation of hydrofluorocarbons and hydrochlorofluorocarbons may also be relevant for the occurrence of high concentrations of trifluoroacetic acid in the environment. In the present study, trifluoroacetic acid along with other ultra-short-chain perfluoroalkyl substances, namely perfluoropropanoic acid, trifluoromethane sulfonic acid, perfluoroethane sulfonic acid and perfluoropropane sulfonic acid, was measured in water connected to known and suspected point sources in Sweden. In addition, correlations

between the ultra-short-chain perfluoroalkyl substances and short- and long-chain perfluoroalkyl substances were investigated. For this purpose, a method based on supercritical fluid chromatography coupled to tandem mass spectrometry was used. Water samples (n=34) were collected in connection to sites where aqueous film forming foams have been used, landfills and a hazardous waste management facility. Ultra-short-chain perfluoroalkyl substances were found in all samples analyzed, at a total concentration up to 84 000 ng/L. Trifluoroacetic acid and perfluoropropanoic acid were detected at concentrations up to 14 000 ng/L and 53 000 ng/L and contributed with $>30\%$ in 12 and 5 samples, respectively. Trifluoromethane sulfonic acid, perfluoroethane sulfonic acid and perfluoropropane sulfonic acid were detected at concentrations up to 940 ng/L, 1 700 ng/L and 15 000 ng/L, respectively, and contributed with $< 30\%$. These findings reveal the presence of high concentrations of ultra-short-chain perfluoroalkyl substances released into the environment from various sources and emphasizes the large contribution of ultra-short-chain perfluoroalkyl substances to the total amount of perfluoroalkyl substances in water.

MP021 Atmospheric Chemistry of a Novel Polyfluorinated Alcohol: Reactions with OH and Cl Radicals

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Many commercially used PFASs are synthesized from alcohol starting materials, such as the fluorotelomer alcohols (FTOHs). These alcohols can enter the environment as residuals or can be reformed as the commercial materials degrade. FTOHs and other polyfluorinated alcohols are semi-volatile, and thus their atmospheric fate must be considered. As PFAS phase-outs and bans have occurred, there has been intelligent approaches to designing polyfluoroalkyl substances that will degrade to non-bioaccumulative components under ambient environmental conditions. We investigated the atmospheric fate of a new polyfluorinated alcohol analogous to FTOHs with the general structure R-O-CHF-CF₂-S-CH₂-CH₂-OH. As oxidation is the primary reaction that occurs in the atmosphere, we performed competitive kinetic experiments with both Cl and OH radicals. Preliminary results report reaction kinetics faster than the FTOHs. Final reaction rates will be determined in time for the meeting after additional control experiments are performed. The atmospheric lifetime of this alcohol is about 1-3 days with respect to reaction with OH. The IR spectrum showed at least two different aldehydes as primary products (R-O-CHF-CF₂-S-CH₂-CH=O and R-O-CHF-CF₂-S-CH=O), and terminal products including COF₂, SO₂ and a prominent acyl fluoride (R-O-C(=O)F). Mechanistic studies confirmed that oxidation occurs by hydrogen abstraction at hydrogens both alpha and beta to the alcohol. Direct oxidation of the sulfur atom could not be confirmed. Offline product experiments were also performed under various atmospheric conditions, and offline samples were collected in a bubbler and analyzed for acid degradation products. Two larger acids were identified as primary degradation products, and two smaller acids that appear to be terminal degradation products, one of which is perfluoropropanoic acid.

MP022 Bench-Scale Evaluation of Perfluoroalkyl Acids Adsorption from Landfill Groundwater by Mixed Media Containing Biochar

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Groundwater is an important migration pathway in the transport of perfluoroalkyl acids (PFAAs), a subset of perfluoroalkyl substances (PFAS), from an industrial landfill to a nearby surface water body. Annual monitoring data show that PFAAs that surpass surface water criteria have been decreasing after the groundwater passes through a permeable barrier made of soil and wood chips. The objective of this study was to evaluate PFAAs adsorption from groundwater by media containing soil, wood and biochar, a cost-effective, carbon-rich product

obtained by thermal decomposition of organic biomass. A two-phase bench-scale study was conducted with emphasis on perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfonic acid (PFOS). Phase 1 batch experiments screened for adsorption capacities by media with different compositions. The first experiment determined adsorption of PFAAs at four different initial concentrations and four different mixture ratios, and a second experiment evaluated media optimization at the six different ratios. Phase 2 consisted of a column experiment to determine the retention of PFAAs at four different media ratios. Results from Phase 1 first experiment indicated that media containing biochar outperformed the other mixtures with >99% adsorption of PFOS and PFOA. Second batch experiment results indicated that the best media composition had 15% of wood shavings, 5% of biochar, and 80% of soil with adsorption of PFOA and PFOS at 98.5% and 96.5%, respectively. Results from the Phase 2 column experiment showed that the media containing 5% biochar and 95% soil had the highest retention of PFAAs; breakthrough for PFOA and PFOS was at more than 6.7 pore volumes and an adsorption capacity of 2.23 g/m³ and 0.22 g/m³ for PFOA and PFOS, respectively. This indicated that biochar increases the partitioning of PFAAs into the solid matrix. Follow-up treatability studies are currently underway using different media and to inform the conceptual site model for the site that could be used to perform contaminant transport simulations and determine the longevity of the permeable barrier.

MP023 Fluorine Mass Balance and Emerging PFASs in Beluga Whales from the Arctic in Canada

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Many Inuit living in the Arctic value subsistence foods for nutrition and culture, which include harvested beluga whales (*Delphinapterus leucas*). Common dietary preparation includes maktaaq (fresh, fried, frozen, boiled, fermented blubber with skin), nikuk (dried meat/intestines), throat/trachea, misaraq (fermented and liquefied blubber), and cartilage. Contaminant monitoring in traditional foods is a priority of the Northern Contaminants Program in Canada and includes assessment of Arctic temporal trends and long-range transport of contaminants. Biomagnification of certain per/polyfluoroalkyl substances (PFAS) has been confirmed in Arctic marine and freshwater food webs. In this study, we quantified 23 PFASs (including perfluoroalkylcarboxylates (PFCAs), perfluoroalkylsulfonates (PFASs), and perfluorooctanesulfonamide (FOSA)) and extractable organic fluorine (EOF) in beluga livers from three different areas of Arctic Canada: Hendrickson Island (HI) in the Beaufort Sea, Sanikiluaq (SQ) in eastern Hudson Bay, and Pangnirtung (PG) in Cumberland Sound, Baffin Island. Samples found to have unaccounted EOF were subjected to additional suspect screening using high resolution mass spectrometry (HRMS). Total targeted PFAS ($\sum \text{PFAS}_{\text{target}}$ = $\sum \text{PFCAs} + \sum \text{PFASs} + \text{FOSA}$) corresponded to 8179 ng/g wet weight in HI, 6278 ng/g in PG, and 8878 ng/g in SQ. Approximately 40-60% of $\sum \text{PFAS}_{\text{target}}$ was accounted for by perfluoroundecanoic acid (PFUnDA) and perfluorooctane sulfonate (PFOS). However, a shift from historical dominance of PFOS was apparent such that PFUnDA \gg PFOS in 2018 beluga. The PFOS:PFUnDA concentration ratio was 0.47 \pm 0.03 in HI, 1.16 \pm 0.06 in PG, and 1.21 \pm 0.18 in SQ. Emerging PFAS including monochloro-perfluorooctane sulfonate (Cl(CF₂)₈SO₃-, 8Cl-PFOS) and the major monochloropolyfluoroalkyl ether sulfonate component in F53B (Cl(CF₂)₆OCF₂CF₂SO₃-, 9Cl-PF3ONS) was site-specific: 0.64 \pm 0.27 ng/g 8Cl-PFOS in HI and 0.11 \pm 0.01 ng/g 9Cl-PF3ONS in PG. The fraction of EOF accounted for by $\sum \text{PFAS}_{\text{target}}$ corresponded to 50 \pm 4% in HI, 42 \pm 5% in PG and 87 \pm 11% in SQ, suggesting that further study of the unknown

organofluorine in HI and PG beluga is warranted. The authors gratefully acknowledge the provision of beluga samples by participating Indigenous communities and the Northern Contaminants Program funded by the federal department, Crown-Indigenous Relations and Northern Affairs, in Canada.

MP024 Analysis of Legacy and Emerging Per- and Polyfluoroalkyl Substances in Drinking, Ground, Surface and Wastewater Using Minimal Sample Preparation

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Per and polyfluoroalkyl substances (PFAS) are chemicals used in consumer products and industry due to the unique and desirable chemical properties. Due to widespread usage and environmental persistence, legacy PFAS are ubiquitous in the environment and new fluoro-chemicals are being found in the environment frequently. Public interest has increased pressure to develop comprehensive methods for sensitive analysis in different types of water. EPA has released a new drinking water analysis method, The method utilizes solid phase extraction and is updated to include 4 new fluorochemicals. ASTM 7979 (draft SW-846 Method 8327 has the same sample prep procedure) can be employed for non-drinking water samples. Sample preparation consists only of dilution with methanol and filtration. We present data collected with these two methods with optimized analytical procedures that include all analytes listed in the methods plus several new emerging fluorochemicals. Method robustness, range, and detection levels will be presented. As the state and federal regulations continue to evolve in this field, robust analytical methods that can be adjusted to encompass new analytes will be important for confidence the public water supply.

MP025 Poly- and Perfluoroalkyl Substances (PFAS) in AFFF-Impacted Groundwater Using Non-Target Screening by High Resolution Mass Spectrometry

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Poly- and perfluoroalkyl substances (PFAS) are well-known contaminants in the abiotic and biotic environment. The dominant PFAS exposure source remains unclear but known sources include the ingestion of household dust, inhalation of volatile compounds, and consumption through the diet and drinking water. A recent study from the United States showed the widespread contamination of public drinking water supplies with PFOA and PFOS levels exceeding that of the updated US EPA's lifetime health advisory of 70 ng/L. Aqueous film-forming foam (AFFF) - typically used for the suppression of hydrocarbon-based fires at civilian and military airports - is known to contaminate local surface and groundwater, as well as biota. AFFF is complex mixture of hydrocarbon and fluorocarbon surfactants, and recent efforts have made considerable progress characterizing the PFAS composition. Similarly, AFFF-impacted groundwater is comprised of a very diverse class of PFAS compounds. Further, a significant proportion of the PFAS groundwater content may be precursors that degrade to the very persistent perfluorinated carboxylates and sulfonates (e.g. PFOS and PFOA). Recently, high resolution mass spectrometry (HRMS) techniques with collision induced dissociation have been used to identify legacy and novel PFAS compounds in groundwater. The overall goal of this study was to perform targeted and non-targeted screening of novel PFAS compounds in AFFF-contaminated groundwater. Previously developed large volume injection techniques were used to concentrate and separate target analytes. Samples were analyzed using the SCIEX X500R QTOF system using SWATH(R) acquisition and high resolution MRM (MRM-HR). Data was processing was performed using the new SCIEX OS software. Analytes were confirmed by comparing obtained MS/MS spectra to those in a newly developed mass spectral library. The advantages of PFAS quantification with MRM-HR in the groundwater matrix

were explored. Finally, non-target screening was performed to identify novel PFAS compounds; several of which were not on typical regulatory monitoring lists

MP026 Default Cold Storage Conditions Are Not Adequate for PFAS Storage Stability: Results From a 6-Month Study

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Sample, extract and standard stability information is critical for the accurate measurement of contaminants. In the present study, we measured the effect of sample type and storage temperature on the stability of per- and polyfluorinated alkyl substances (PFAS) in water. Spiked reagent water, surface water, and effluent samples were stored in HDPE container at +20°C, 4°C, and -20°C over a period of 180 days. C4-C14 perfluorinated carboxylates, C4-C10 and C12 perfluorinated sulfonates, 4:2, 6:2 and 8:2 fluorotelomer sulfonates, three perfluorooctane sulfonamides (PFOSA, N-MeFOSA, N-EtFOSA), two perfluorooctane sulfonamide ethanols (N-MeFOSE and N-EtFOSE), and two perfluorooctane sulfonamideacetic acids ((N-MeFOSAA and EtFOSAA) were included in the study. All samples were spiked with isotopically labeled surrogate standards, extracted using weak anion exchange and analyzed using isotope-dilution LC-MS/MS. Results from the study confirmed the overall stability of perfluoroalkyl acids with no significant concentration changes in reagent water, surface water and effluent samples at 4°C and -20°C storage. However, there was a steady increase in the concentrations of NMeFOSAA and NEtFOSAA in both surface water and effluent samples over the study period at +20°C, and 4°C with significant changes observed at the first stability time point (7 days). The behavior was not observed in reagent water samples and indicated the impact of sample matrix on the stability of polyfluorinated compounds during storage. In addition, the increases in concentrations of NMeFOSAA and NEtFOSAA tracked decreases in the concentrations of N-MeFOSE and N-MeFOSA, N-EtFOSE and N-EtFOSA respectively indicating analyte conversion during cold sample storage. Freezing of samples at -20°C showed long term stability of all analytes over 180 days. This study underscores the importance of testing effects of default storage conditions in samples using real-world samples rather than in spiked clean water.

MP027 Identifying Unknown PFAS Sources in WWTP Biosolids Using the TOP Assay

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Many PFAS can transform into perfluorinated carboxylates/sulfonates (PFCA/PFSA) such as PFOA and PFOS in the environment from exposure to sunlight, microbial activity, and through the wastewater treatment process. While the transformation of PFAS into perfluoroalkyl acids (PFAA) through the wastewater treatment process has been studied extensively, this study has involved the measurement of select precursors and target PFAAs. So, understanding the mass balance of all PFAS through the process, and the risks involved in the use of the resultant effluents/biosolids is unclear. Measurement of an exhaustive suite of PFAS is challenging/impracticable because of the number and chemical diversity of the compounds, with many of them yet uncharacterized or without available quantitative analytical standards. The total oxidizable precursor assay (TOP) uses an oxidative approach to convert these precursors into PFCAs, hence quantifying the potential contribution of precursors to current and future potential PFAA loads. In this study, we used a standards-enhanced TOP assay coupled with isotope dilution LC-MS/MS to estimate unknown PFAS in WWTP influent, effluent and biosolids. TOP assay enhancements included the use of isotopically labeled PFAS to monitor reaction completeness and losses during reaction, a standardized spiking mixture to assess ongoing precision and recovery, and other measures. Solid samples were spiked with an isotopically labeled reaction monitoring and reaction control standard and extracted using ammoniacal methanol. Aqueous samples and solid extracts were oxidized using potassium persulfate and sodium hydroxide for 6 hours at 85 °C. Samples

were then quenched, neutralized, spiked with an isotopically labeled surrogate mixture, and extracted and analyzed using LC-MS/MS. Paired samples that did not undergo oxidation were also analyzed using LC-MS/MS. Preliminary results from the biosolids show very large increases in PFAAs after oxidation. Samples showed >400% increase in measurable PFAAs with carbon chain lengths of C4-C14 being measured. The chain length pattern could not be traced back to any single known small molecule PFAS. While this study identifies a large PFAS source in biosolids that is amenable to PFAA formation from hydroxyl radical reaction, the PFAS source, and immediate risk of PFAA formation in the environment requires further study.

MP028 Targeted Method for Quantitating PFAS from Biological Samples Using Ultra-High-Performance Liquid Chromatography-Tandem Mass Spectrometry

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A sensitive quantitative method using ultra-high-performance liquid chromatography (UPLC) combined with triple quadrupole tandem mass spectrometry (MS/MS) was developed to determine per- and polyfluoroalkyl substances (PFAS) in biological samples, specifically using human plasma to determine various toxicokinetic (TK) measures. This method was applied to more than 20 PFAS of different classes such as perfluoroalkyl carboxylic acids and perfluoroalkane sulfonic acids, as well as more unique PFAS that contain ether linkages and sulfonamides. Additionally, a dozen 13C- and 2H-labelled PFAS were included as internal standards in this method. Detection limits were in the low to sub-pg/?L range depending on the analyte employing a Waters Xevo TQ-S micro system and an ACQUITY I-Class UPLC with Waters PFC Analysis Kit. All compounds were included within this rapid method (8.5 min total run time) using a mobile phase consisting of water, acetonitrile, and 2.5 mM ammonium acetate. For application, this method evaluated the presence and quality of PFAS standards in DMSO. Assessment through simultaneous MS full scan acquisition and multiple reaction monitoring (MRM) mode, a technique known as RADAR(C), allowed for monitoring of impurities and degradants without impacting instrument sensitivity. Our analysis indicated that most carboxylic acid-containing PFAS were of high quality, while some of the sulfonate-bearing compounds presented as a mixture of linear and branched isomers. These findings assisted with our targeted analyses to monitor for TK properties, identifying troublesome compounds that may require additional optimization or sample preparation. Subsequently, plasma protein binding assays by ultracentrifugation and rapid equilibrium dialysis were analyzed with this developed approach. Measured plasma protein binding indicated very high binding rates for most studied PFAS, attesting to the sensitive detection capabilities of this developed UPLC-MS/MS method. Using this method, we plan to continue hazard and exposure evaluation of various TK measures across a broad group of PFAS. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

MP029 Biotransformation of Amphoteric Foamer Surfactants Present in a Legacy Aqueous Film-Forming Foam (AFFF) Product

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Aqueous film-forming foams (AFFFs) are synthetic Class-B fire extinguishing agents critical for extinguishing hydrocarbon fuel fires. AFFFs utilize fluoroaliphatic surfactants to create a stable foam for fire extinguishment and to form an aqueous-surfactant film layer on the surface of the fuel that acts as a vapor barrier to prevent flashbacks. The fluorochemical composition of AFFFs are complex with many different manufacturers, formulations and products sold over the last 50 years. Some AFFF products manufactured by 3M contained perfluorooctane

sulfonate (PFOS) film forming agent and separate fluoroaliphatic amphoteric foaming agent. The fluoroaliphatic amphoteric foaming agent in one AFFF product, Lightwater™ FC-203CF, was primarily a mixture of perfluorohexanesulfonamido-amine (PFHxSaAm), perfluorohexanesulfonamido-amino carboxylate (PFHxSaAmA) and perfluorohexanesulfonamido amino dicarboxylate (PFHxSaAmDA) and described in a 1992 3M patent. We investigated the aerobic and anaerobic biodegradation of these foaming agent substances and will present proposed biodegradation pathways, biotransformation products, and the results of a quantitative mass balance.

MP030 Leaching and Transport of PFAS From Unsaturated Soil at a Firefighting Training Facility Under Cold Climatic Conditions

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The contaminant situation at a Norwegian firefighting training facility (FTF) was investigated 15 years after the use of perfluorooctanesulfonic acid (PFOS) based aqueous film forming foams (AFFF) products had ceased. Detailed mapping of the soil and groundwater at the FTF field site in 2016, revealed high concentrations of per- and polyfluoroalkyl substances (PFAS). PFOS accounted for 96 % of the total PFAS concentration in the soil with concentrations ranging from < 0.3 ug/kg to 6500 ug/kg. The average concentration of PFOS in the groundwater down-gradient of the site was 22 ug/l (6.5-44.4 ug/l), accounting for 71 % of the total PFAS concentration. Unsaturated column studies were performed with pristine soil with a similar texture and mineralogy as found at the FTF and the same PFOS containing AFFF used at the site. Transport and attenuation processes governing PFAS behavior were studied with focus on cold climate conditions and infiltration during snow melting, the main groundwater recharge process at the FTF. Based on the field study, retardation factors for the average vertical transport of PFOS in the unsaturated zone were estimated to be 33-42 and 16-21 for the areas with a low and high AFFF impact, respectively. The estimated retardation factors for the column experiments were much lower at 6.5 and 5.8 for low and high infiltration, respectively. This study showed that PFOS is strongly attenuated in the unsaturated zone and mobility is dependent on infiltration rate. The results also suggest that the attenuation rate increases with time.

MP031 Metabolism of 6:2 Fluorotelomer Sulfonate in an Amphibian Ecosystem

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6:2 fluorotelomer sulfonate (6:2 FTS) has been shown to degrade through microbial degradation generating shorter chain alcohols, ketones, and poly- and perfluoroalkyl acids (PFAA). Little is known about metabolism of 6:2 FTS in animals. We probed the degradation and metabolism of 6:2 FTS after dermal and ingestion exposures studies designed to quantify bioaccumulation. For the dermal studies, juvenile amphibians (Northern leopard frogs, Eastern tiger salamanders, and American toads) were exposed dermally for one month through moss (sediment bedding substitute) soaked in 10, 100, or 1,000 ppb of 6:2 FTS in water. In the ingestion exposures, salamanders were fed 6:2 FTS-dosed crickets, which were fed media dosed at 100, 1,000, or 10,000 ppb. Fecal samples were collected at the conclusion of the exposure. Similar experiments were conducted with PFOA, PFOS and PFHxS individually, which are non-biologically degradable PFAA. These three PFAA and 6:2 FTS were quantified in moss, crickets, fecal samples, and amphibians (whole body for anurans and livers for salamanders due to their size). After 30 days of dermal exposure, 6:2 FTS levels in moss were one to two orders of magnitude lower than the initial concentrations, while moss PFAA concentrations were stable over time. In amphibian tissues, 6:2 FTS was only detected in dermally-dosed toads with up to 85 ng/g dry weight, dw (highest exposure level) and PFAA levels were 3 to 133 times higher compared to 6:2 FTS. Similar PFAA levels were measured in frogs and salamanders. Livers of

salamanders dosed via ingestion had very low content of 6:2 FTS (< 10 ng/g dw), while crickets and fecal samples had concentrations up to 4,400 and 1,400 ng/g dw, respectively. Additional PFAA known to be terminal microbial metabolites of 6:2 FTS were quantified. Perfluoroheptanoic acid (PFHpA) and perfluorohexanoic acid (PFHxA) were the two major target PFAA metabolites quantified in moss and amphibian tissue following dermal exposure. Crickets, salamander livers and fecal samples from the ingestion experiment showed only background levels of these PFAA. Screening of non-target fluorinated metabolites was also performed. Numerous non-target compounds were detected in moss, a few were also detected in crickets, fecal samples, whole frog and toad tissues, but even fewer in salamander livers of either experiment.

MP032 Developing a Predictive Understanding of PFAS Bioaccumulation with Environmental Complexity

M.F. Simcik, University of Minnesota / Division of Environmental Health Sciences; B.W. Brooks, Baylor University / Environmental Science This presentation is an introduction to a project recently selected for funding by the Strategic Environmental Research and Development Program (SERDP). The objective of this project is to determine bioaccumulation dynamics (uptake, elimination) of individual PFAS from complex mixtures across representative environmental conditions. Uptake and elimination rates of PFAS in the benthic organism, *Hyalella azteca*, and a common fish model (*Pimephales promelas*) will be determined when exposed to simulated groundwater while varying environmental complexity (e.g., pH, salinity). These bioaccumulation dynamics will be modeled using a non-linear one-component uptake equation or two compartment model. The results will be determined for both whole-body accumulation and plasma accumulation. Analysis of PFAS will include branched and linear congeners as well as a suite of target PFAS analytes. Successful completion of this project will provide improved predictive models to estimate uptake and elimination of PFAS in widely distributed, ecologically important invertebrate and fish models across environmentally relevant gradients.

MP033 Exploring Empirical Data and Biological Relevance of Polyfluoroalkyl Substance Environmental Degradation and In Vivo Metabolism

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Environmental and in vivo degradation products of polyfluoroalkyl substances, such as fluorotelomers and other chemically distinct polyfluoroalkyl substances with modified side-chain fluorinated and non-fluorinated functional groups, are an important consideration when it comes to prioritizing the evaluation of polyfluoroalkyl substances for potential toxicological effects. Perfluorinated alkyl acids (PFAAs) can be a potential degradant, both environmentally and in vivo, of polyfluoroalkyl substances, and it is often assumed that polyfluoroalkyl substances and side-chain fluorinated polymers may be a source of PFAA exposure. The non-fluorinated carbon bonds in polyfluoroalkyl substances are potential points along carbon chains that may be susceptible to biotic or abiotic degradation and/or metabolism. As a result, polyfluoroalkyl substances that contain a fully fluorinated carbon chain have the potential to be PFAA precursor compounds. Some degradation and metabolic pathways for polyfluoroalkyl precursors have been proposed, however, available studies primarily consist of laboratory experiments or modeling efforts, not environmental or biological empirical data. The objective of the work herein is to conduct a review focusing on mechanisms of biotransformation, including potential pathways and intermediate and final degradation products, to ascertain the potential biological relevance of available models and results for humans. Critical knowledge gaps will also be identified, as will major pathways of potential degradation. In addition, any distinction between environmental and in vivo degradation pathways, and which pathways are potentially favored or not, will also be discussed. Assessment of analytical methods used in biological and environmental analysis and their impact on potential degradation pathways will be reviewed. By way of example, our analysis will discuss

the potential terminal degradation products of fluorotelomer alcohols (FTOHs). The overall yield of perfluorooctanoic acid (PFOA) is low following 8:2 FTOH metabolism, presumably due to conjugation reactions and excretion. This is consistent with the overall yield of perfluorohexanoic acid (PFHxA) being low following 6:2 FTOH metabolism.

MP034 Uptake and Bioaccumulation/Biomagnification of Subsurface-Derived PFAS by Stream Food Webs

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Environmental contamination of per- and polyfluoroalkyl substances (PFAS) is a major problem at hundreds of sites with historical use of aqueous film forming foam (AFFF). Transport of this contamination into aquatic environments via stormwater runoff and contaminated groundwater plumes, coupled with the mobility and persistence of PFAS in aquatic systems, means it is critical to understand the ecological and human health risks associated with the presence of PFAS in aquatic habitats. There exists growing evidence that some PFAS compounds bioaccumulate, however, the extent and mechanisms of bioaccumulation and biomagnification of PFAS in food webs, as well as their bioavailability are not well characterized nor understood. Our project will investigate the extent, pathways and rates by which subsurface-derived PFAS are taken up by and transferred through stream food webs. Three complementary studies are planned: 1) a field study evaluating the bioavailability, bioaccumulation and biomagnification of various PFAS constituents throughout the aquatic food web of a warm water stream contaminated with PFAS attributed to AFFF-impacted groundwaters; 2) a controlled experiment assessing the effect of key physical and geochemical factors on the bioavailability and uptake rates of PFAS from sediment and water to select lower trophic-order organisms; and 3) a controlled trophic experiment investigating the rates of PFAS transfer along controlled primary uptake and trophic transfer pathways. The results of this project will support quantitative prediction and assessment of the risks of PFAS to aquatic wildlife and humans, including by providing estimates of trophic magnification, bioconcentration and bioaccumulation factors of various PFAS under different physical and geochemical conditions.

MP035 Investigation of Perfluoroalkyl and Polyfluoroalkyl Substances: Transfer from Contaminated Feed Into Eggs of Laying Hens

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A feeding study was performed with 12 laying hens. Hens were fed with a PFAS contaminated feed for 25 days. Afterwards, hens were fed with uncontaminated feed for 42 days. During the duration of the study, feed intake was recorded daily and eggs were collected every two days. Eggs were separated into egg yolk and egg white and pooled for further analysis. Feed and egg samples were analysed for a wide spectrum of PFAS including several precursors. To assess the presence and relevance of unknown precursors, a Total Oxidizable Precursor (TOP) assay was applied. Up to eight PFAAs of different chain length (C4-C8) were detected in feed and egg yolk. The comparison of PFAA uptake from the feed and PFAA concentrations in the eggs indicated apparent excretion rates of more than 100% for PFHpS and PFOS via egg yolk. Application of the TOP assay for the feed showed an increase of PFCA (C5-C8)

concentrations between 230 and 790%. These results serve as evidence for the presence of precursors and were confirmed by target analysis of the feed. While perfluorooctane sulfonamid (FOSA) and perfluorooctane sulfonamidoacetic acid (FOSAA) were quantified at concentrations of 4.8 ug/kg and 4.3 ug/kg, respectively, the alkylated forms of FOSAA showed significantly higher concentrations of 41.3 ug/kg (methyl-FOSAA) and 33.0 ug/kg (ethyl-FOSAA). Quantitative analysis of the egg yolk samples showed the highest PFAS concentrations directly after the exposure period and a steady decrease of PFAS concentrations during the depuration phase. Oxidation of precursors in egg yolk using the TOP assay led to an increase of PFCA-levels, e.g. 647% for PFOA in the day 26 sample. The target analysis of precursors showed substantial amounts of FOSA, FOSAA, methyl-FOSAA, and ethyl-FOSAA. The results demonstrate the transfer of PFAA and precursor compounds from feed to egg. The different patterns of FOSAA, methyl-FOSAA, and ethyl-FOSAA in feed and egg yolk can be explained by i) an extended transfer of FOSAA compared to its alkyl-analogues into the egg yolk and/or ii) a dealkylation of methyl- and ethyl-FOSAA in the animal metabolism leading to the formation of FOSAA (and possibly FOSA). The study highlights the significant contribution of PFAA precursors to the total PFAS burden in the investigated feed and eggs. In a second poster the modelling of laying hen biotransformation of PFAS including PFAA-precursors in eggs will be presented.

MP036 Atmospheric Deposition of PFAS via Precipitation at Selected Locations Across the United States

M.M. Shafer, University of Wisconsin, Madison / School of Medicine and Public Health; C. Zhang, University of Wisconsin-Madison / State Laboratory of Hygiene

The importance of precipitation as a critical vector of a wide range of environmental contaminants to both aquatic and terrestrial ecosystems, where exposures to humans may then take place, is well documented. Precipitation is also a valuable sentinel of atmospheric contaminant pools and atmospheric processing and therefore monitoring provides key insights into the status and trends of contaminant levels and fluxes, at point, regional and global scales. The atmospheric transformation PFAS precursor molecules likely contributes to the production of more stable PFAS, which are then more susceptible to regional and global transport and eventually deposition in precipitation. Modeling efforts and recent field data show measurable levels of a broad-range of PFAS species in precipitation, though the scale and scope of those studies was limited. Information on short-chain (C3-C7) PFAS species, which may preferentially enter atmospheric cycles, is particularly sparse. Precipitation may reflect washout of both water-soluble species and particulate-associated PFAS. In a study designed to address the efficacy of National Atmospheric Deposition Program (NADP) precipitation samples for PFAS deposition determinations, and to provide new data on levels of PFAS in precipitation across the US, we carried-out a pilot study, performing PFAS measurements on geographically diverse precipitation samples. Precipitation samples were obtained from the NADP central laboratory at the University of Wisconsin-Madison; sub-sampled and stabilized with Trizma immediately upon receipt of the precipitation sample at the laboratory. Processing and analysis of the precipitation samples for 18 PFAS species at the UW-Madison, State Laboratory of Hygiene strictly followed EPA method 537.1. Concentrations of the detectable PFAS species were low, generally less than 1 ng/L. PFNA was the most frequently detected species, closely followed by PFOS and PFOA. The greatest number of PFAS species was detected in precipitation samples from NADP sites in North Carolina (NC41 near Raleigh) and New Jersey (NJ99, Mercer Co). The number of PFAS species addressable in precipitation will soon be doubled with application of ISO method 21675. Planned field and laboratory experiments will examine whether the NADP/NTN sampling network as currently configured (or with certain modifications) would support using this large network of 270 sites as a national PFAS sampling network longer-term.

MP037 Biodegradation Rates of Perfluoro-2-propoxypropanoic Acid in Sediments of the Cape Fear River Estuary*K. Tito, University of North Carolina, Wilmington*

The release of perfluoro-2-propoxypropanoic acid (PFPrOPrA), commonly known as “GenX”, into the Cape Fear River Estuary has recently become of great concern in North Carolina. This study investigates for the first time the biogeochemical cycling of this compound over a 32-week time series in sediments collected near the Chemours facility located near Fayetteville North Carolina. Results reveal an average decrease of recoverable PFPrOPrA of 45% after the first week of storage at room temperature, followed by an average 68% decrease during the second week after which there was no significant difference in the abundance of PFPrOPrA recovered. There was no significant difference between recoverable PFPrOPrA in autoclaved and bioactive mud over the course of 6 weeks, suggesting that biodegradation was not predominately responsible for the decrease in recoverable GenX during the first two weeks of incubation. Future research will explore the mechanisms responsible for the decrease in recoverable PFPrOPrA in sediments focusing on the potential formation of molecular aggregates which may not be as amenable to current extraction techniques and/or chemical degradation pathways. Results of this study are important because they highlight potentially important processes in the biochemical cycling of PFPrOPrA in anthropogenically impacted sedimentary environments.

MP038 Fate of Per- and Polyfluoroalkyl Ether Acids in the Total Oxidizable Precursor Assay*C. Zhang, Z. Hopkins, D. Knappe, North Carolina State University / Civil, Construction and Environmental Engineering*

Per- and polyfluoroalkyl substances (PFAS) are widely used anthropogenic chemicals. The unique properties of PFAS have led to a variety of applications including firefighting foams, stain repellents, food contact paper, and production of fluoropolymers. Two long-chain PFAS, perfluorooctane sulfonate and perfluorooctanoic acid, were historically produced in large quantities, but a manufacturing shift towards short-chain PFAS and fluorinated replacements started in the early 2000s due to health implications. Many new PFAS are proprietary and include functional groups that can degrade to dead-end products such as perfluoroalkyl carboxylic and sulfonic acids (PFCA and PFSA). To determine concentrations of unidentified PFCA and PFSA precursors, the total oxidizable precursor (TOP) assay was developed. In the process, many precursor compounds are oxidized to PFCA and PFSA that can be readily quantified by existing analytical methods. To date, little information is available about the fate of a recently discovered perfluoroalkyl ether acids (PFEA) in the TOP assay. The aims of this research were to (1) determine the fate of individual PFEA in the TOP assay, including six perfluoroalkyl mono-ether carboxylic acids, four perfluoroalkyl multi-ether carboxylic acids and five polyfluoroalkyl ether acids; (2) identify possible new terminal-end products in the TOP assay; and (3) apply the TOP assay to surface water samples impacted by a wastewater discharge containing PFEA. The ten tested perfluoroalkyl ether acids and F-53B, a polyfluorinated ether compound with a terminal -CF₂Cl group, were stable in the TOP assay and represent new terminal-end products. Thus, adding perfluoroalkyl ether acids and F-53B to the list of target analytes for the TOP assay can enhance our ability to capture a larger percentage of the total PFAS concentration in environmental samples. In contrast, polyfluoroalkyl ether acids containing a -O-CFH- moiety were oxidized during the TOP assay, and the majority of the resulting oxidation products could not be identified by liquid chromatography-high resolution mass spectrometry. Application of the TOP assay to PFAS-impacted water revealed the presence of PFCA precursors, but PFEA precursors were not present at measurable levels.

MP039 Screening of Novel Per- and Polyfluorinated Alkyl Substances (PFAS) in Surface Sediments of Southeastern North Carolina*R. Sanchez; M. Shimizu, G.B. Avery, R. Mead, R.J. Kieber, S. Skrabal, University of North Carolina at Wilmington / Department of Chemistry and Biochemistry*

Replacement poly- and perfluorinated alkyl substances (PFASs) are shorter chain compounds and ether polymers that are similar in structure and function to highly regulated legacy compounds such as PFOA and PFOS. Unlike legacy PFAS, replacement PFAS are not regulated or studied nearly as much. Recent studies revealed that unregulated replacement PFASs were present in river water used for drinking water, downstream of a fluorochemical manufacturer, in the Cape Fear River watershed in NC. However, sediments from the Cape Fear River have not been analyzed for novel PFAS. Sediments are a natural sink for organic contaminants and may also serve as a secondary source. This study investigates replacement PFASs in surface sediments from the middle to lower Cape Fear River. The aim of this ongoing study is to identify novel PFAS and determine spatial and temporal distributions of these compounds. Q-TOF high resolution mass spectrometry was used for suspect screening and non-targeted analysis of novel PFAS. A suspect screening identified 15 replacement PFASs including that of GenX, Nafion byproducts, NVHOS, and various chlorinated PFASs along with other PFECAS and PFESAS. By continuing to identify unknown PFAS in the region this study will provide an awareness of the occurrence of these contaminants and possible sources.

MP040 GenX in the Environment and Waste Streams in the Netherlands 2013–2018*W. Bil, T.D. Kort, National Institute for Public Health and the Environment (RIVM) / Centre for Safety of Substances and Products; M. Beekman, National Institute for Public Health and the Environment (RIVM) / Centre for Safety of Substances and Products, Bureau REACH; J. Ng-A-Tham, National Institute for Public Health and the Environment (RIVM) / Centre for Safety of Products and Substances*

Hexafluoropropylene oxide-dimer acid (HFPO-DA), also known as GenX and FRD-902/FRD-903, is a perfluorinated substance used in the production of fluoropolymer resins in Dordrecht, the Netherlands. This involves (permitted) air and wastewater emissions. GenX is under scrutiny of national and European authorities. The European Chemicals Agency (ECHA) is evaluating GenX as the accumulation/half-life in the human body is unclear and the substance is possibly carcinogenic to humans. ECHA is also considering including GenX in the European Candidate List of substances of very high concern (SVHC) for its very high persistence and very high mobility in the environment and concerns for effects on human health. For the former reason, GenX is included in the Dutch list of potential SVHCs (ZZS). The National Institute for Public Health and the Environment (RIVM) assembled available data on the presence of GenX in the environment and waste streams in the Netherlands (2013-2018). Data were mainly obtained from governmental organizations (e.g., Waterboards, regional environment agencies, inspectorates). Data were collected for surface, waste and ground water, soil, crops, and waste streams. GenX was found in concentrations above the level of quantification (LOQ) in all investigated compartments. Highest environmental concentrations were found in soil and groundwater (1.3 mg/kg dw and 36 µg/L, resp.), and in surface water (27.4 µg/L), all on the site of a subcontracting company processing fluoropolymer resins. Run-off water in a storm drain on the same site, and waste water from the production facility transported to a municipal waste water treatment plant contained the highest concentrations (6.7 and 4.2 mg/L, resp.). In addition to waste streams related to the production facility, the Human Environment and Transport inspectorate found high concentrations of GenX in waste streams not related to the production facility, up to 3 mg/L. Investigations on the source(s) of GenX in these waste streams are ongoing.

MP041 How Much Chemicals with PFAS Do We Use in Norway? A Data Register Study Based on Data From 2009-2017

M. Grung, S. Rannekleiv, E. Hovland Steindal, NIVA; K. Petersen, Norwegian Institute for Water Research

A data register study was performed to identify the amounts of PFAS in chemicals imported to Norway during 2009-2017. The Product Register in Norway is the Norwegian authorities' official register of chemicals imported and produced in Norway. According to legislation, manufacturers or importers who produce and/or place on the market 100 kg/y or more of a chemical classified as hazardous are obliged to submit a declaration to the Product register. The data from the years 2009-2017 was searched for substances from PFAS on the OECD list and on the KEMI list (short chain PFAS). During the period, 72 CAS from the OECD list and 5 from the KEMI list was used in products in Norway. Most of the PFAS were telomers, sulfonyls precursors or polymers. The tonnages imported (since Norway has no production) were between 15-28 tons/y and was 250 tons in the period. During the years, the amount of telomers was highest (150 tons), but also the amounts of polymers, short chain PFAS and precursors were high (51, 26 and 16 tons respectively). Since 2014, the yearly percentage of use of telomers have decreased from >75 to 25% of yearly import, while the use of polymers, short chain PFAS and precursors have increased. A telomer with chain length of 6 was the PFAS used in highest amount (110 tons) in the period for fire fighting purposes, followed by PTFE (49 tons) used in very different products such as e.g. paint, reducing friction, lubricant and more. A silicon with 9 Fluor atoms was used as an antifoam compound by the oil industry. The use of PFAS compounds was quite diverse, but fire-fighting purposes was using most PFAS (130 tons). However, more than 71 tons of PFAS in the period was used in paint, as antifoam compounds, friction reducers and paper industry (each group >14 tons). During the years, the yearly percentage of PFAS used for fire fighting purposes has been reduced (>50% to 25%) while other uses have increased. We believe that the amounts that we have reported are minimum data since Norway currently do not have an obligation to declare PFAS to the product register. We recommend Norwegian authorities to amend legislation to include such obligation in line with Sweden. Our conclusions are that the Product Registry is a very useful tool showing that PFASs are used in high amounts. Fire-fighting purposes has been the major use, but PFAS compounds used for other purposes are increasing.

MP042 Overview of Per- and Poly- Fluoroalkyl Substances (PFAS) in the United Kingdom

J. Lymer, S. Martin, Environment Agency / Chemical Assessment Unit; S. Dungey, Environment Agency / Chemical Assessment Unit; P.K. Curtis-Jackson, Environment Agency (England and Wales) / Chemical Assessment Unit

Per- and poly- fluoroalkyl substances (PFAS) are extremely persistent in the environment. It is therefore inevitable that people and wildlife will be subjected to long-term exposure to these substances if they are released. By 2020 both perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) will be subjected to strict regulation at an international level due to concerns about their human health and environmental impacts. Consequently, PFOS and PFOA are being replaced with alternative PFAS, many of which have either similar or additional human health and/or environmental concerns. According to the OECD nearly 5000 PFAS were identified to be in worldwide use in 2018. In contrast, only 114 were registered in Europe under the Registration, Evaluation, Authorisation and restriction of Chemicals (REACH) Regulation by the end of 2018 (imported or manufactured at greater than 1 tonne/year/registrant). With the exception of PFOS and PFOA, data on PFAS concentration in environmental media in the UK are limited. Therefore, the Environment Agency initiated a review to build an understanding of PFAS at a national level. Information in this presentation is the output of the first phase of work. It is based on existing information held by the Environment Agency and other UK government bodies, sourced from interrogating regulatory databases i.e. Environment Agency monitoring database, the European Chemical Agency's (ECHA) REACH-IT system, the world wide web.

This presentation will include current regulatory information about PFAS manufactured and marketed in the UK, identified sources and pathways of environmental exposure, monitoring data and recommendation for further work and investigation

MP043 Investigating Recycled Water Use as a Diffuse Source of Per- and Polyfluoroalkyl Substances (PFAS) To Groundwater in Melbourne, Australia

D. Szabo, RMIT University / School of Applied Science; T.L. Coggan, RMIT University; T. Robson, Australian Contaminated Land Consultants Association; M. Currell, RMIT University; B. Clarke, RMIT University / School of Science

The purpose of this study was to investigate the contribution of per- and polyfluoroalkyl substances (PFAS) to groundwater at a location where recycled water from a wastewater treatment plant (WWTP) is used to irrigate crops. Groundwater from Werribee South, located west of Melbourne, Australia, was sampled over two campaigns in 2017 and 2018, extracted using solid phase extraction (SPE) and analysed with liquid chromatography-tandem mass spectrometry (LC-MS/MS-QQQ). PFASs were detected in 100% of the groundwater samples. The sum total of twenty PFAS compounds (20PFASs) for all sites in the study ranged from 0.03 to 74 ng/L (n = 28) and the highest levels of which were observed in the centre of the irrigation district. Perfluorooctanesulfonic acid (PFOS) was the most detected compound overall (96%) with a mean concentration of 11 ng/L (0.03-34 ng/L), followed by perfluorobutanesulfonic acid (PFBS; 86%, 4.4 ng/L), perfluorooctanoic PFOS acid (PFOA; 82%, 2.2 ng/L) and perfluorobutanoic acid (PFBA; 77%, 6.1 ng/L). Concentrations of PFASs found in this study are greater than background levels of PFASs detected in groundwater and are in the range of concentrations typically detected in wastewater effluent. This study presents evidence that the use of recycled water can be a source of PFAS contamination to groundwater. Please see <https://doi.org/10.1016/j.scitotenv.2018.07.048> for further information.

MP044 Levels of PFAS and Precursor Compounds in Australian Biosolids

D.T. Moodie, RMIT University, Melbourne; T.L. Coggan, RMIT University; S.M. Reichman, RMIT University / School of Engineering; D. Nugogoda, B. Clarke, RMIT University / School of Science

Per- and Poly-fluorinated compounds (PFAS) are an increasing area of concern for terrestrial and aquatic ecosystems. PFAS are introduced to waste water treatment streams from domestic and industrial sources. Biosolids are the end user product for the waste water solid fraction and as such have the potential to accumulate PFAS adsorbed to solids during the treatment process. Field collected biosolid samples from 19 different Australian treatment plants that serve a mixed variety of users were collected and analyzed for 45 PFAS compounds using a novel alkaline extraction technique coupled with analysis by LC MS MS to determine the levels of PFAS adsorbed to biosolids. PFAS was detected in 100 % of the samples analyzed. Perfluorooctanoic acid (PFOA) was detected in 85% of the sample replicates and Perfluorooctanesulfonic acid (PFOS) was detected in 92% biosolid replicates. The highest concentration for PFOS in the biosolids was 27 ng/g and 21 ng/g for PFOA. The highest PFAS concentration was for perfluorodecanoic acid (PFDA) at 40 ng/g, PFDA was detected in 83% of the replicates. Precursor compounds were detected in 100% of the biosolid samples analyzed with the extended optimized method. The highest concentration among the analyzed precursor compounds was for 6-2, 8-2 diPAP at 430 ng/g. The second highest observed precursor concentration was for 6-2 diPAP at 300 ng/g. 8-2 diPAP was also detected with a maximum concentration of 77 ng/g. To the authors knowledge this is the first recorded detections of diPAP's in Australian biosolids. The relative abundance of detected precursor compounds associated with longer chain even numbered carboxylic and sulfonic acids may be indicative of the potential for biodegradation and transformation of PFAS in Australian biosolids.

MP045 Occurrence and Distribution of PFASs in Drinking Water and Various Industrial Wastewater in South Korea

J. Oh, K. Kim, Pusan National University / Department of Civil and Environmental Engineering

Perfluoroalkylated substances (PFASs) have been widely used in numerous industrial applications. Their toxic properties, persistency and bioaccumulation potential lead to the regulation of their production, emission, and use. After the regulation of PFOS and its salts, fluorochemical manufacturing companies have been developing alternative technologies to replace them. However, PFASs and their alternatives chemicals are still detected widely in water environment and the concern about the PFASs contamination of drinking water is being raised especially, in Korea because major source of drinking water is surface water. Therefore, in this study, the occurrence and distribution patterns of PFASs in drinking water and various types of waste water were investigated to understand the contamination PFASs status and to assess the PFASs discharge from industry to water environment. We found the 2~ 50 times increased PFASs levels in drinking water compared to the results obtained in 2012. Also, some of drinking water samples were even exceed the guideline value of Sweden (90 ng L⁻¹ of 21PFASs) and Australia (70 ng L⁻¹ of PFHxS). Like the case of drinking water, the higher use of PFHxS in industry was found and the detailed discussion regarding PFASs detection characteristics in industrial wastewater and the contribution of their emissions into the environment by each industrial class will be presented at the conference.

MP046 Occurrence of PFAS in Surface Water Following Discharge of Industrial Effluent and Firefighting Foam from a Large Industrial Fire

J.T. Marchiandi, RMIT University / School of Science; D. Szabo, RMIT University / School of Applied Science; T.L. Coggan, RMIT University; B. Clarke, RMIT University / School of Science

Point source pollution of per- and polyfluoroalkyl substance (PFAS)-containing aqueous film-forming foam (AFFF) and industry effluent has led to growing incidences of environmental contamination. The aim of this study was to investigate the occurrence of PFAS contamination in an aquatic ecosystem after AFFF and industry effluent were discharged into surface water following a large industrial pollution event in Footscray, located west of Melbourne, Australia. Sampling campaigns were conducted during and two months post the initial pollution event. PFAS analysis was achieved using a highly sensitive analytical method based on solid-phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS-QqQ). PFASs were detected in 100% of surface water samples indicating ubiquitous dispersal and distribution. The mean sum of forty five PFAS compounds (45PFASs) for all sites investigated ranged from < 26 to 623 ng/L (n = 30) with the highest levels measured at the point source. The compounds displaying the greatest mean in surface water samples were perfluorohexanesulfonate (PFHxS): 153 ng/L (range: < 0.2-1393 ng/L) and 6:2 fluorotelomer sulfoic acid (6:2 FTS): 153 ng/L (range: n.d-1332 ng/L); followed by perfluorooctanesulfonate (PFOS) 94 ng/L (range: < 9-487 ng/L), perfluorohexanoic acid (PFHxA) 28 ng/L (range: < 0.2-165 ng/L) and perfluorooctanoic acid (PFOA) 23 ng/L (range: < 5-45 ng/L). Concentrations of 45PFASs at all sites generally declined post event, with the exception of elevated sulfonamides and fluorotelomer acids which may suggest transformation of PFAS precursor compounds in AFFF and effluent. At all sites during both time periods, PFOS concentrations exceeded the Australian EPA draft guidelines for the ecological protection limit for aquatic ecosystems (0.023 ng/L) by up to three orders of magnitude. Our results suggest legacy and replacement PFAS in effluent from modern industry still present significant environmental implications despite current restrictions of the production and use of long-chain chemistry.

MP047 Grouping PFAS Together for Analysis and Hazard Assessment: Fluoropolymers Don't Fit

B.J. Henry, T. Kennedy, W.L. Gore & Associates, Inc.; H. Fiedler, Orebro University / MTM Research Centre School of Science and Technology

Organic fluorinated compounds have been detected in environmental media and biota. As a result, regulators seek to identify those associated with toxicity, bioaccumulation, mobility and persistence to ban/restrict them lacking clarity around which analytical method(s) should be used to identify and quantify each one. In order to protect public health and the environment, efforts must focus on those compounds that pose the greatest risk, which necessitates an understanding of exposure pathways, physical, chemical and toxicological properties, and valid analytical methods so that sources can be identified and regulated appropriately. Recent legislation (e.g., the CA Safer Consumer Products regulation, WA State's Public Health and Safety Code Title 70 Chapter 70.75A, and "the PFAS Act of 2019", H.R. 535) is defining "PFAS" (per- and polyfluoroalkyl substances) for management actions as anything having at least one -CF₃, which would include the fluoropolymers (e.g., PTFE) satisfying the OECD Polymer of Low Concern (PLC) criteria. By this PFAS definition, drugs, pesticides, and veterinary drugs (e.g., Prozac, Celebrex, Fipronil, Isoflurane, etc.) will all meet the one-fully-fluorinated-carbon definition of a PFAS, like those PFAS listed or recommended for listing in Annex B of the Stockholm Convention Persistent Organic Pollutants (POPs) (e.g., PFOA, PFOS). This one -CF₃ classification is not adequate for grouping for hazard assessment, risk management or regulation, monitoring or analyses. Fluoropolymers have very high molecular weights (>100,000 Da), are not soluble in water and most organic solvents, and represent low inherent hazard. These characteristics are consistent with the definition of a PLC as by OECD 2009, BIO by Deloitte, 2014, and Henry et al., 2018. These PLC fluoropolymers show no degradation under environmental conditions, have little to no water solubility or volatility and therefore pose a low risk of mobility. They would not be expected to be found in water or air or distant from the point of use or disposal nor would they be expected to degrade to lower molecular weight PFAS. Fluoropolymers that are PLCs do not, therefore, demonstrate the toxicity, physical, chemical or thermal properties of the perfluoroalkyl acids (PFAAs), nor do they release or degrade to them. PLC fluoropolymers require analytical and classification approaches differentiating them from other organic fluorine-containing substances.

MP048 Societal Benefits of FluoroTechnology

S. Korzeniowski, FluoroCouncil; E. Laganis, Asahi Glass Co., Ltd.; J. Bowman, FluoroCouncil

FluoroTechnology impacts our daily lives in many advantageous ways by facilitating the safe and reliable operation of a very broad range of products and applications which have become essential to modern living. The unique enabling nature of the carbon-fluorine bond allows FluoroTechnology to provide dynamic properties of strength, durability, heat and chemical-resistance, flexibility and high-performance electrical capabilities. This allows FluoroTechnology to deliver a wide-array of societal benefits, including improved safety, reliability and fuel-efficiency in applications like cars and airplanes, buildings and electronics. It is used in a wide-variety of key markets, e.g. medical, transportation, aerospace, electronics, energy, building and construction, first-responder, chemical processing and consumer products. Thus, FluoroTechnology is beneficial to many major job-creating industries. Globally, the FluoroTechnology Industry was a \$19.7 billion business in 2013. More than \$1.2 trillion of global manufacturing output including FluoroTechnology in either the end-product itself, or in its processing, supporting more than 625,000 jobs in the US and Western Europe. (Updated information will be available in July.)

Exposure

TP001 The Agency for Toxic Substances and Disease Registry's Per- and Polyfluoroalkyl Substances Exposure Assessments

B. Goodwin, ATSDR; R. Rogers, ATSDR / DCHI; M. Karwowski, P. Kowalski, K. Scruton, J. Sautner, C. Gaines, A. Pomaes, H. Tian, C. Reh, CDC/ATSDR

The Agency for Toxic Substances and Disease Registry (ATSDR) will conduct statistically based biomonitoring exposure assessments (EAs) in communities near domestic military installations that have documented exposures to per- and polyfluoroalkyl substances (PFAS) in drinking water. For each site, a statistically based, community sampling design will be used to determine: The distribution of PFAS serum concentrations in communities with recent or past exposures to PFAS in drinking water. PFAS urine concentrations from a subset of participants with recent or past exposures to PFAS in drinking water. PFAS concentrations in indoor dust and tap water samples from a subset of homes of participants in biological sampling. A questionnaire will be administered to all participants to gather information to characterize each individual's exposure. Blood and urine samples from participants will be analyzed to determine the distribution of PFAS levels in each community. Individual and aggregated community serum and urine concentrations will be compared to reference ranges from nationally representative data. Environmental samples will be analyzed to determine PFAS exposure concentrations and, in conjunction with questionnaire data, to provide insight into environmental contributors of biological PFAS concentrations across all included sites. Each exposure assessment will include the following goals: Provide a public health service to the community. The EAs will provide information to community members about their PFAS body burden, including an assessment of how their PFAS concentrations compare to national reference populations. The EAs will also provide information about aggregate serum concentrations and exposure in the community from which participants are selected. Generate information about pathways of exposure in the community. Environmental sampling data will be combined with biological sampling results to generate information about the impact of drinking water and some non-drinking water PFAS exposure pathways on PFAS body burden in each community. Inform future studies to evaluate the impact of PFAS exposure on human health. The results of these EAs will inform the design and implementation of a planned ATSDR-led multi-site health study. We will present an overview of the process used to identify communities as EA sites as well as details of the planned EA work in these communities.

TP002 Total Oxidisable Precursor Assay and Risk Assessment: Assessing Exposure to Precursors

V. Lazenby, Arcadis Australia Pacific Pty. Ltd.

With thousands of per- and poly- fluoroalkyl substances (PFAS) known to exist, there is an increasing focus on ensuring that the total mass present is being appropriately assessed. The standard laboratory methods currently only quantify 20 to 30 of these compounds, leaving a potentially significant data gap when assessing PFAS exposure. Of particular interest is an understanding of how polyfluorinated precursor compounds may contribute to total exposure. Using available empirical data collected from sites internationally, a review was undertaken to assess whether 'standard' PFAS results are appropriate for use in assessing risks to human health and the environment, or whether Total Oxidisable Precursor (TOP) Assay results may need to be considered when assessing exposure to PFAS. This review will compare paired results for 'standard' PFAS and TOP Assay in environmental media, to assess relative mass of precursors relative to that of 'standard' PFAS. An analysis of the available data will demonstrate the relative proportion of precursors present within the total PFAS mass to which receptors may be exposed, under different environmental settings. This will include assessment of different source types (e.g. fire fighting foams, water treatment plants, etc) and assessment of spatial trends in TOP Assay, to gain an understanding of whether precursors contribute similar mass of PFAS at the point of exposure relative to within the source

zone. The review will focus on assessing trends in distribution of poly-fluorinated precursor compounds relative to trends in routinely measured PFAS. These trends will assess whether factors such as source type, distance from source, characteristics of sampled media, or key transport mechanisms (such as bioaccumulation) impact the relative distribution of precursors as a proportion of known PFAS mass. This information can then be considered when undertaking an assessment of exposure to PFAS, to determine whether the mass of non-standard PFAS needs to be incorporated into an assessment of intake, or whether the relative mass of precursors present can be considered to fall within the normal range of uncertainties inherent in contaminant measurement and exposure assessment.

TP003 Prioritization of Eight PFAS by Population Exposure and Reference Dose Uncertainty Ranges

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A methodology for prioritizing per- and polyfluoroalkyl substances (PFAS) based on human health risk is demonstrated for eight PFAS. The methodology is based on the concept that risk is a function of both hazard and exposure, both of which are uncertain and variable. The methodology combines statistical distributions of median U.S. population exposure levels to the eight PFAS, developed using U.S. EPA's SEEM model, with ranges of uncertainty about currently available toxicity reference doses for those PFAS. Resulting distributions of exposure and hazard are compared (in mg/kg/day) for each of the eight PFAS studied. The degree of overlap of these distributions indicates relative risk-based priority. In addition, use of this approach to develop probabilistic characterization of relative population risk as hazard index distributions is explored.

TP004 Bioaccumulation Potential and Cytological Responses of a Short-Chain PFAS, PFBS, in the Sediment Dwelling Worm *Eisenia hortensis* (Annelida)

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The contamination by PFAS of surface, ground- and drinking water and soil, detected in an area covering more than 200 km² in Veneto Region, northern Italy, has become an emergency for the possible consequences on human health, natural populations, agriculture and finally for socio-economic perspectives. Also if the contamination above was historically produced by a local fluorochemical company, the continuous monitoring of PFAS shed light on the fact that other unidentified sources are responsible in the release of these molecules, although at lower levels, in a larger area than previously observed. As a part of the work planned in a EU project, the Life Phoenix project, set up in order to develop a comprehensive study on the PFAS emergency, we report here results on PFAS assumption and effects in a soil organism, a sediment dwelling worm, considered an important bioindicator of this environmental sector. Our attention was in this study devoted to PFBS, a short-chain PFAS, as these molecules are increasingly replacing the long chain PFAS. While long chain PFAS have been largely studied and regulated, the short chain PFAS have been not. However they are highly mobile and persistent in soil and water, so that, once released, short-chain PFAS remain in the environment and permanently interact with living organisms. We have exposed specimens of the worm *Eisenia hortensis* to PFBS in soil microcosms prepared with glass containers. The exposures were lasting up to 42 days, at a measured PFBS soil concentration of 8 ug/g. The PFBS bioaccumulation patterns at 14, 28 and 42 days were measured in the soft tissues and in the coelomic fluid. At the three exposure times the coelomocytes showed a Bioaccumulation Factor (BAF) 8x with respect to the soft tissues and a significantly lower lysosomal membrane stability, with respect to the controls. The comparison with previous PFOA exposure experiments in the same organism

shows greater bioaccumulation factors for PFBS and a higher response by the coelomocytes that are the cells responsible for many immune functions (similar to vertebrate leukocytes). Taking in mind that short-chain PFAS are increasingly used, and that their lives in the environment are as long as those of long chain PFAS, these data strongly suggest the necessity of more studies on their interactions with the environment and the biota.

TP005 Physiological, Ecological and Environmental Determinants of PFAS Uptake in Freshwater Fish: Literature Survey and Project Plan

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An important and ongoing concern is the release of per- and polyfluoroalkyl substances (PFAS) to environmental media as a result of industrial and military uses including use of Aqueous Film Forming Foam (AFFF) for fire suppression or training activities. PFAS are resistant to degradation and readily move within and among different ecosystem components posing a potential risk to human and ecological receptors. Freshwater fish have been used widely in PFAS monitoring studies and are important ecological receptors. However, the field lacks a comprehensive understanding of the factors that explain intra- and inter-species variability in PFAS tissue concentrations in fish from contaminated freshwater systems. Bioaccumulation factors are extremely variable across systems and species and our own studies and analyses show that output from fish bioaccumulation models only sporadically match data from field samples. Moreover, there are a paucity of studies in which biota and environmental samples were co-collected with which to improve our overall understanding and models. What data that are available indicate that bioaccumulation of certain PFAS (mostly longer-chain) can occur via dietary and/or waterborne routes but the extent to which these routes are important and the role of other factors such as differences in species physiology or water quality parameters are generally unknown. This lack of understanding undermines or hinders risk assessment, monitoring and risk communication. Here we present an overview of a project designed to address uncertainties related to bioaccumulation of PFAS in freshwater fish. The goal is to test specific hypotheses that address the role that physiological (e.g. metabolic rate), ecological (e.g., diet composition), and environmental (e.g., water quality parameters) might play in the bioaccumulation of PFAS in three common freshwater fish species. Subsequently, these data will be incorporated into bioaccumulation models which will then be vetted in the field. Resulting data and models will improve our overall understanding of exposure and accumulation of PFAS in fish and will facilitate risk assessments, risk communication and environmental management of PFAS.

TP006 Bioavailability Of Perfluoroalkyl Acids (Pfaa) in Soil and Hydroponically Grown Red Chicory (*Chicorium intybus* L.)

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In 2013, a large-scale contamination with PFAS has been detected in Veneto Region, northern Italy, caused by emissions from local fluorochemical company, effecting surface water, groundwater, drinking water and soil in the area covering more than 200 square kilometers. Being one of the most important agricultural producers in Europe, potential crop uptake and consequential human health concerns are an emerging issue. However, to predict the potential PFAS bioavailability and uptake

in crops, more of mechanistic understanding is needed than currently available in the literature, due to their specific environmental behavior. To assess the influence of soil sorption on bioavailability of 9 PFAA (7 carboxylic and 2 sulfonic acids), typical Veneto crop, red chicory (*radicchio* var. Chioggia) was grown in soil and hydroponic nutrient solution. *Radicchio* cultivated in agricultural soil was exposed to both contaminated irrigation water and pre-contaminated soil in 12 treatments with varying concentrations (clean soil and soil spiked on 100 and 200 ng/g d.w. of each PFAA and 0, 1, 10 and 80 µg/L of each PFAA in irrigation water, respectively), while hydroponic experiment was performed with nutrient solution spiked to 50 µg/L of each PFAA. To assess the sorption of PFAA mixture on soil and to derive soil to water partition coefficients (Kd), set of laboratory batch tests was performed as well. To compare the root uptake, root concentration factors (RCFs) normalized to the pore water (or nutrient solution) concentrations were used, while shoots to roots concentration factors (SRCFs) and transpiration stream concentration factors (TSCFs) were used to assess PFAA uptake to aerial plant parts. Results have shown different patterns of PFAA uptake from soil pore water and hydroponic solution that cannot be explained only by soil sorption as the factor of decreasing bioavailability. They represent a valuable empirical base towards better understanding and modelling of PFAA inter-compartmental plant translocation, being of immense importance for risk assessment.

TP007 Determining Per- and Polyfluoroalkyl Substances (Pfas) Concentrations in Human Placental Tissues and Its Association with Infant Sex

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants that have been found in human serum, breast milk, and umbilical cord blood. Placental transfer of PFAS to developing fetuses is of concern due to an association between PFAS concentration in placenta and low birth weight as well as developmental toxicity of PFAS. We will present a method for extracting and quantifying 11 PFAS chemicals from human placenta by using liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Analysis of a subset of placental samples (n=10) showed PFOS, PFOA, PFHxS, and PFNA were the dominant PFAS in placenta and that maternal and fetal portions of placenta had similar concentrations. Previous work on these placental samples showed differences in brominated flame retardants and thyroid hormone levels between maternal and fetal placenta. Using this developed method, we are currently extracting and quantifying PFAS from nearly 200 whole-thickness placental samples and will determine whether infant sex, birth weight, or gestational age is associated with PFAS concentration in the placenta.

TP008 Use of Bovine Kidney Slices To Predict Renal Reabsorption of Pfaas as Potential Indicator for the Extent of Transfer from Feed to Dairy Products

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Dairy products may be an important source for human exposure to PFASs, especially when cows feed on contaminated feed from hot spots. Significant differences occur in the extent by which PFASs are transferred from feed to milk. In a feeding study by Kowalsczyk et al. (2013)1 with feed containing PFBS, PFHxS, PFOS and PFOA, the plasma concentrations of PFOS and PFHxS became much higher than those of PFOA and PFBS, and especially PFOS retained in the plasma after the exposure was stopped. In addition, PFOS and PFHxS were excreted into milk, whereas this was barely the case for PFOA and PFBS. In the present study we hypothesize that the higher plasma concentrations and milk excretion of PFOS and PFHxS compared with PFOA and PFBS are due to a higher reabsorption of these compounds by the kidneys. To test this hypothesis, experiments with bovine kidney slices (obtained from slaughtered cows) were set up to measure the extent of reabsorption of different PFASs. This was done by comparing the uptake of these compounds in kidney slices at

4°C (representing passive uptake) with 37°C (representing active uptake). Initial results for PFOA revealed higher intracellular concentrations in the slices that were exposed at 37°C compared with 4°C. Further studies will focus on whether this difference in internal PFOA concentration can indeed be attributed to the active transport (reabsorption) and to perform similar experiments with a range of PFASs. The ultimate goal is to use the ex vivo generated reabsorption kinetic data to predict plasma concentrations of PFASs and transfer from feed to milk by integrating the data into physiologically based kinetic (PBK) models. The results will be used to set priorities for the monitoring of milk products on PFASs. Kowalczyk et al., 2013. DOI: 10.1021/jf304680j

TP009 Decline in Serum PFAS Levels Among 3M Decatur Employees and Retirees

G. Olsen, K. Andres, S. Kleinschmidt, 3M Company

The 3M Company's Decatur (Alabama) site was the largest 3M manufacturing site of perfluorooctanesulfonyl (POSF)-related material. Subsequent to the phase-out of long-chain perfluoroalkyl and polyfluoroalkyl substances (PFAS), periodic opportunities have been offered to Decatur employees and retirees to voluntarily have their blood measured for PFOS, PFHxS, and PFOA including the most recent occurrence in 2016. This study was conducted by the 3M Corporate Medical Department and all study participants provided informed consent. After analysis of the collected blood (serum) sample by LC-MS/MS performed at the 3M Environmental Laboratory, participants received their 2016 results via a confidential letter that also presented comparisons to the participants' individual prior PFAS results. Altogether, a total of 271 current employees and 111 retirees participated in this study. Participants self-reported as having work experience in either the chemical plant (n = 98), the film plant (n = 168), or both (n = 116) and whether they were hired before (n = 202) or after (n = 180) the 3M phase-out of PFOS-, PFOA-, and PFHxS-related materials. Among those 66 chemical plant employees (current and retired) hired pre phase-out compared to the 32 hired post phase-out, the 2016 median concentrations, respectively, were PFOS (92.3 ng/mL vs 21.2 ng/mL), PFHxS (51.7 ng/mL vs. 3.6 ng/mL), and PFOA (23.2 ng/mL vs. 9.7 ng/mL). A total of 71 participants (regardless of plant experience) had serum measurements in 2000 that were analyzed just prior to the company's announced phase-out. Comparing these 2000 vs 2016 serum concentrations, median concentrations were PFOS (1150 ng/mL vs 105 ng/mL), PFHxS (245 ng/mL vs 56 ng/mL), and PFOA (1415 ng/mL vs 47 ng/mL). The percent reductions were: PFOS 89%; PFHxS 78%; and PFOA 95%. The results from this study are consistent with serum elimination half-lives that have been reported at this Decatur plant which may have been influenced by some ambient exposures within these confines.

TP010 Perfluorooctane Sulfonate Toxicokinetics at Different Temperatures Using a Rainbow Trout Physiologically-Based Toxicokinetic Model

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This study is part of a project aiming to improve our understanding of the toxicokinetics of perfluoro-alkyl substances (- PFOS, perfluorohexane sulfonate and perfluorononanoate - PFNA) in fish, using the rainbow trout (*Oncorhynchus mykiss*) as model species. The project involves a combination of dietary exposure experiments and physiologically-based toxicokinetic (PBTK) modelling. We developed first a 10-compartment PBTK model to elucidate PFOS kinetics in adult rainbow trout. This PBTK model included various physiological characteristics: blood perfusion to each organ, plasmatic fraction, PFOS free fraction, and growth of individuals. The parameters were optimized using Bayesian inference. This development was based on an experimental dataset, where fish were first fed daily with spiked food, and allowed to depurate for an equivalent duration [1]. We evaluated the relative importance of different absorption as well as elimination routes, and the role of the entero-hepatic cycle. This model was then used to explore the effects of temperature on PFOS

toxicokinetics. The modulation of physiological processes induced by temperature was introduced into the model structure thanks to Arrhenius law. These processes included food absorption, ventilation rate, growth, cardiac output, urinary, biliary and fecal clearances, and relative blood flow distribution to organs. We hypothesized that temperature could also affect PFOS tissue:plasma or tissue:blood partition coefficients. Simulations were performed for water temperatures of 7°C, 11 °C, 15 °C and 19°C and compared to experimental data [1, 2]. The simulation having the lowest Root Mean Squared Error was obtained when all physiological processes were modulated according to temperature. Overall, the model outcomes suggest that PFOS toxicokinetics was more affected by temperature induced changes in partition coefficients than in fish physiology. A sensitivity analysis is ongoing in order to confirm and refine this result. Vidal A, Lafay F, Daniele G, Vulliet E, Rochard E, Garric J, Babut M. in press. Does water temperature influence the distribution and elimination of perfluorinated substances in rainbow trout (*Oncorhynchus mykiss*)? *Environ Sci Pollut Res.* Goeritz I, Falk S, Stahl T, Schafers C, Schlechtriem C. 2013. Biomagnification and tissue distribution of perfluoroalkyl substances (PFASs) in market-size rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 32:2078-2088.

TP011 Surveying the Environmental PFAS Landscape

M.J. Strynar, U.S. Environmental Protection Agency / ORD/NERL/EMMD; J. McCord, U.S. Environmental Protection Agency / ORD

Per- and polyfluoro alkyl substances (PFAS) are environmentally persistent and high interest class of chemical pollutants with a rapidly expanding roster. As manufacturing processes evolve, the number of PFAS in use in commerce has exploded to thousands of analytes, and new and emerging analytical techniques are necessary to identify and monitor the most environmentally relevant chemical pollutants. High resolution mass spectrometry has been a workhorse technique supporting non-targeted approaches to sample analysis, compound discovery, and chemical characterization across a range of media and unique circumstances. The application of the EPA's PFAS lists to HRMS screening has allowed rapid examination of unknown samples for the ever-expanding list of known PFAS chemicals. New chemical species continue to be uncovered and described using a combination of computational tools - formula and fragmentation prediction, homologous series screening (e.g. CF₂, CF₂O, etc.), and geographic/temporal feature correlation. Tentative assignment from the preponderance of chemical evidence (i.e. HRMS isotope/fragment information and spectral predictions) offers a methodology for PFAS analysis while the availability of authenticated, commercially available standards lags the diversity of known species. PFAS screening and discovery approaches have been applied to numerous industrial production/use sites for fluoropolymers and PFAS containing AFFFs throughout the United States and reveal a highly fragmented landscape of emerging PFAS chemicals. In contrast to the prior ubiquity of perfluorinated acids, emerging PFAS are structurally diverse, corresponding to their specialized applications. This presentation details some general approaches for handling the complex chemical space of emerging environmental PFAS research.

TP012 Life Project Phoenix: A New Project for the Management of Water Pollution from Short Chain Perfluoroalkyl Acids in Veneto Region (Italy)

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In 2013 a significant episode of PFAS pollution of surface, ground- and drinking water has been discovered in a large area of the Veneto region, in Northern Italy. The most important source of pollution was identified in a fluorochemical plant, sited in an area of groundwater recharge. The Veneto Region immediately put in place mitigation actions that were more effective for long chain PFAS than for short chain ones. For that reason, within the framework of the Community Life Program, a project on the management of short-chain perfluorinated compounds (PFAS), coordinated by the Department of Health Protection, Food and Veterinary Safety of the Veneto Region, in association with CNR IRSA, ARPAV and University of Padua, has been funded. The LIFE-PHOENIX project, acronym for “Perfluorinated compounds Holistic Environmental Institutional eXperience”, started in September 2017 and will end in March 2021. The approach requires the involvement of regional authorities, developing tools transferable to other geographical contexts characterised by episodes of similar environmental contamination but also proposes mitigation strategies based on innovative technologies. The activities are focused on a real scale case constituted by the PFAS pollution in the provinces of Vicenza, Verona and Padua. The project includes the field monitoring of PFAS in abiotic (water, soil) and biotic (vegetables and animals) matrices. Meanwhile, some innovative technological tools for mitigation of PFAS concentration in water are being validated and compared for irrigation and drinking water purification, using full-scale plant (wetland system) and physico-chemical plants breakdown system (filters). Finally, a typical local crop (red chicory) was cultivated in a greenhouse as model crop, under varying concentrations of pre-contaminated soil and irrigation water. The obtained results are a valuable empirical base for the validation of the existing plant uptake models and can support a mechanistic understanding of contaminants transport from soil to crop. All project activities offer well-timed and innovative environmental tools for the estimation of PFAS distribution and risks based on multidisciplinary datasets aimed to implement policy measures to prevent or limit problems related to the diffusion of persistent mobile organic contaminants.

TP013 The Contribution of Drinking Water to PFAS Blood Serum Levels

D. Andrews, EWG

PFAS contamination of drinking water is an issue of national significance but the relative contribution of water to overall exposure is not well characterized. With the publication of the EPA action plan and numerous state proposals to set drinking water limits there is a strong need to determine the role of different exposure routes. EWG has been publishing a map of known water contamination across the country for the past five years. From 2013-2015 the EPA mandated testing of public water systems for PFAS chemicals but the value of the nationwide sampling for assessing population exposure to PFAS through drinking water was limited due to the high reporting limits of 40 parts per trillion for PFOS and 20ppt for PFOA. Summary level detection frequencies for results down to 2.5 ppt were published in presentations by Eurofins Eaton Analytical. Using this dataset, we have estimated that up to 110 million Americans served by an

estimated 1,500 water systems are providing drinking water with PFAS at levels at or above 2.5ppt. In the Centers for Disease Control’s 2015-2016 national biomonitoring results, the geometric mean concentration of PFOA in blood serum was 1.56 parts per billion (ppb) and the geometric mean concentration for PFOS was 4.72 ppb. Using these most recent blood serum levels, we calculate the expected drinking water concentrations that would account for these serum levels using standard bioconcentration factors. Using the Eurofins Eaton Analytical testing results we have estimated the exposure that is attributable to drinking water contamination. These results provide a rigorous approach to calculating a relative source contribution value that could be used in establishing drinking water regulations.

TP014 Diminishing Returns: Attempting to Reduce PFAS Exposure by Lowering Drinking Water Criteria

T.L. Sorell, Brown and Caldwell / Risk and Toxicology

Concerns about PFAS in drinking water supplies are growing, with many citizens demanding increasingly low standards, and agencies pushing the drinking water and groundwater action levels well below the United States Environmental Protection Agency (EPA) Drinking Water Advisory (70 nanograms per liter [ng/L] for combined PFOA/ PFOS). Examples are California, which has notification levels of 13 and 14 ng/L for PFOA and PFOS, respectively, and New Jersey, which recently established Interim Groundwater Quality Criteria of 10 ng/L for both compounds. However, these actions ignore the widespread exposure to PFAS from other sources, notably food and consumer products. Many studies have attempted to quantify overall PFAS intake in human populations, but the number of variables makes it challenging. There is a large body of literature describing exposure via diet, food packaging, and consumer products, which this presentation will draw on to quantitatively address exposure reduction. For example, using the default EPA drinking water intake of 2.5 L per day, daily intake at the New Jersey PFOS criterion of 10 ng/L would be 25 ng. This is less than one quarter of a published estimate of dietary PFOS intake of 110 ng per day. It is thus questionable whether increasingly stringent standards are the best way to reduce overall exposure. PFAS removal from drinking water supplies requires advanced water treatment, which can be prohibitively expensive for municipalities that purvey drinking water. Based on EPA’s estimate of unit treatment costs, a medium-sized city could spend millions of dollars per year to treat PFAS. Other proposed measures, such as New Jersey’s pending requirement for well testing associated with every sale or lease, could impose substantial financial burdens across economic sectors, impair markets, and create unnecessary alarm. While treatment of contaminated water supplies associated with identified releases will continue to be important, requiring widespread testing and treatment associated with trace concentrations in water supplies may not be a sound public health investment. The development of PFAS action levels should be evaluated in the context of overall exposures, which may best be reduced through a combination of measures such as manufacturing advances to reduce PFAS content of goods and consumer education.

TP015 Comparative Analysis on Selected U.S. States’ Response and Regulation of Per-and Polyfluoroalkyl Substances (PFAS)

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The United States has seen a great increase in drinking water contamination due to Per-and Polyfluoroalkyl Substances (PFAS). The lack of federal regulation regarding PFAS has forced states to act by themselves when a PFAS pollution site is found. By looking at where PFAS has currently been found and the potential future contamination sites, and reviewing online resources including government documents, several states are leading the charge in testing, regulating, and studying PFAS in their communities, especially in regards to drinking water and its sources. However, other states face political and industry-led challenges to action, and the comparative analysis offered in this research can provide a better idea of what has worked well and not so well in the selected states. From this analysis, key instruments for success found include water quality

testing and strong state regulations. Ultimately, though, action needs to come from the federal level, and it is the hope that these actions on the state and local level can push the federal agencies to incorporate PFAS into existing environmental and public health regulations. In the end, strong and targeted regulations at all levels can provide a framework that monitors PFAS contamination, mitigates polluted sites, and reduces the use of PFAS so that the country's public health may be protected.

TP016 Pfas Levels in Zooplankton Collected in Italian Deep Lakes

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Zooplankton is composed by a heterogenous group of organisms which differ in body size, metabolic rates and ecological roles but are fundamental for the transfer of matter and energy from the base to the upper levels of food webs. Also pollutants could be transferred along food chains and zooplankton is undoubtedly a key route for POPs biomagnification. Despite its importance zooplankton is often neglected and there are very few ecotoxicological studies that analysed in depth the variation of contaminants in this cluster. The deep lakes Maggiore, Como and Iseo are among the largest and deepest Italian lakes. They are located within the River Po basin in the pre-alpine area. They constitute an important water source for potable, agricultural and industrial purposes in Northern Italy and are a resource for fishery and recreational-touristic use. In this work we i) determined the concentrations of 12 perfluoroalkyl substances (PFAS) in crustacean communities of sub-alpine lakes Maggiore, Como, Iseo; ii) compared the pattern of PFAS congeners among lakes; iii) evaluated the influence of size and seasonality on PFAS concentrations. We sampled every season for 2 years (2016-2018) with nylon nets with increasing mesh (200, 450 and 800 um) from surface layer to 20 m depth. Biomass was calculated for every samples with length-weight regression equations. Collected zooplankton included Copepoda (Cyclopoida and Calanoida) and Cladocera (*Daphnia longispina* group, *Eubosmina coregoni*, *Diaphanosoma brachyurum*, *Leptodora kindtii* and *Bythotrephes longimanus*). Considering the sum of all compounds analysed, zooplankton of Lake Maggiore and Como was the most contaminated, with mean concentrations of 7.5 ± 5 ng/g ww and 4.7 ± 2.6 ng/g ww, respectively. PFOS was the most predominant compound in all lakes, ranging from 30% in Lake Iseo to 67% in Lake Maggiore. Despite we could recognise a seasonal trend, with concentrations higher in cold seasons and lower in spring and summer, PFAS levels didn't showed significant variation in relation to seasonality and, then, biomass. Also, PFAS concentrations didn't vary with the increase of body size.

TP017 Overview of Fish Contamination by a Range of Perfluoroalkyl Chemicals in European Deep Lakes

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Within continental waters, large lakes present a special interest for monitoring persistent contaminants, because of their physical characteristics, particularly a prolonged residence time. Moreover, professional fisheries take place in these large lakes, entailing human exposure to chemicals bioaccumulated. For these reasons it has long been acknowledged that monitoring persistent contaminants in fish is an essential component of environmental and health risk assessment in such large lakes. We report data on fish contamination by a range of perfluoroalkyl substances for

seven European deep lakes located in populated areas, namely Lake Geneva, Lake Lugano and Lake Maggiore (all three being trans-national lakes), and Lake Iseo, Lake Como, Lake Garda and Lake Mergozzo in Italy. In addition, fish from two high altitude alpine lakes in Switzerland (>2000 m) were also sampled. These high altitude lakes were assumed to be references sites, as only atmospheric deposition should bring PFAS to them. All the other lakes are located in highly urbanized areas but without known industrial PFAS sources. A range of perfluorocarboxylic and perfluorosulfonic acids were analyzed in fillets from seven fish species occupying different habitats. Furthermore, in order to be able to assess wildlife as well as human exposure, different body fractions were analyzed separately in selected cases. Along with a summary of the results (in terms of concentration ranges and descriptive statistics), we will provide insights on the effect of seasons or type of PFAS source, and discuss about the relationships between concentrations in fillets and whole-fish.

TP018 Monitoring of PFAS in Edible Crops of an Highly Contaminated Area

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After the discovery of PerFluoro Alkyl Substances (PFAS) pollution of surface, ground and drinking water in a large area of the Veneto region (Northern Italy), mitigation actions have been immediately put in place. In particular, the Department of Health Protection, Food and Veterinary Safety of the Veneto Region, in association with CNR IRSA, ARPAV and University of Padua, proposed the "Perfluorinated compounds Holistic Environmental Institutional eXperience" (LIFE-PHOENIX) project, within the framework of the Community Life Program. The main goal is to organize and integrate the information of already existing data from several institutional database providing information on environment, agriculture and health to carry out cross analyses and propose effective mitigation and prevention solutions to several chemical contaminations by compounds with similar characteristics of persistence and mobility (i.e. other PMOCs). For that reason, the project envisages an extensive monitoring program to assess the distribution of selected pollutants in different environmental matrices (water, soil, plants and animals). The sample collection and analysis follow the most suitable Standard Operating Procedures (SOPs) developed for the analytical determination of PFAS in the all environmental matrices involved. The on-field monitoring activity planned during all project has been carrying out in the selected sampling stations, representative of the framed area with different levels of PFAS pressures. These sites are all characterized by the presence of a water body and enough soil to characterise the area of plants growth, moreover the selected stations are all featured by the presence of one ubiquitous aquatic vegetal species (*Zea mais*, *Lactuca sativa*, *Cichorium intybus*, *Allium cepa*, *Phragmites australis*) and of two ubiquitous animal species, one aquatic (*Lymnaea stagnalis*) and one terrestrial (*Eisenia hortensis*). Water, soil and biological samples have been collected in 10 areas, including uncompromised site. The monitoring activity started in May 2018 and will last in March 2021.

TP019 Open Questions on Perfluorooctane Sulfonic Acid and Perfluorooctanoic Acid Occurrence Data in German Food

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With a reevaluation of the risks posed by the intake of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), the European Food Safety Authority (EFSA) decided in 2018 to substantially lower the corresponding tolerable weekly intake (TWI). Using PFOS/PFOA occurrence data in food and water (averaged from different European countries) together with national dietary intake data allowed EFSA to estimate the PFOS/PFOA intake for individual EU countries. As outcome, the intake of PFOS/PFOA for certain age groups exceeds the TWI which implies a risk. Therefore, the German Federal Institute of Risk Assessment (BfR) decided to investigate German PFOS/PFOA occurrence data. While analysing the available data, the following uncertainties were identified: firstly, the used analytical methods have rather high limits of quantification (LOQ), leading to large differences between the lower and upper bound approach. Secondly, there are doubts whether the samples for the occurrence data represent the overall German situation. The samples are very unevenly distributed among the federal states. Furthermore, it became apparent that many samples seemed to concentrate around areas with distinctive elevated concentrations (hotspots) of PFOS/PFOA in the environment (e.g. due to industry emissions or use of contaminated clearing sludge as fertilizer on fields). Given the small-scale characteristic of many of these hotspots, it is extremely challenging to distinguish the samples of hotspot areas from the rest. Therefore, the representativeness of the samples for the general population is further undermined. Thirdly, the sample size within important food groups like pork meat were very small and further complicated by the large ratio of non-quantifiable samples, thus contributing to the overall uncertainty. To reduce all of these uncertainties, it is necessary to improve the sampling strategy as well as to employ more sensitive methods to measure PFOS/PFOA in food. The BfR is improving the information on concentration of perfluorinated compounds in foods by considering this substance group in the first German Total Diet Study (BfR MEAL Study). Overall 16 perfluorinated sulfonic acids and carboxylic acids are analysed in about 590 representative pooled samples of which several foods are sampled in four different regions in Germany and analysed separately to meet regional differences. Results will be available in 2020.

TP020 A Meta-Analysis of Perfluorooctane Sulfonate (PFOS) Concentrations in Birds Around the World

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Perfluorooctane sulfonate (PFOS) is an emerging contaminant that is restricted under Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs). PFOS has been shown to bioaccumulate and biomagnify in marine food webs due to its proteophilic properties, yet no comprehensive analysis exists of temporal, geographic and taxonomic variation in its concentrations. This meta-analysis explores variation in the concentration of PFOS across bird species, which are reliable sentinels for monitoring the concentrations and risk associated with organic contaminants. We collected more than 500 estimates of PFOS concentrations in eggs, liver, and blood from more than 100 different bird species across all continents, to explore taxonomic, geographic, and temporal variation in PFOS concentrations. Our results demonstrate a > 3.5-fold increase in PFOS concentrations in bird eggs from the 1970s (47.4 ng/g ww, N = 12) to the 2010s (180.9 ng/g ww, N = 138), with the largest temporal increase occurring in the temperate region. In general, the temperate region had higher average concentrations than the polar region for both egg and liver samples. Birds from temperate freshwater and marine environments had higher mean concentrations than temperate terrestrial and polar marine ones. These results are consistent with most anthropogenic sources being at temperate latitudes, including industrial waste and the use of aqueous

fire-fighting foams. Piscivorous and opportunistic species had the highest concentrations among all feeding guilds, while herbivores had the lowest concentrations. This result confirms that species-specific diet is an important predictor of PFOS concentration and its associated risk among birds. It is also consistent with PFOS biomagnification in marine and terrestrial food webs. Overall, this study shows a global, widespread increase in the concentrations of PFOS in birds' tissues, reaching egg and liver concentrations that exceed values associated with adverse effects. This work will help identify wildlife groups most at risk, and can help direct future studies of the toxicity of PFOS to wildlife.

TP021 Tissue-Specific Distribution of Legacy and Emerging Per- and Polyfluoroalkyl Substances in Seabirds from Atlantic Offshore and Coastal Environments

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Long-chain per- and polyfluoroalkyl substances (PFASs) demonstrate remarkable environmental persistence, bioaccumulative capacity, and have been found globally in surface water and biota, including birds from diverse habitats. Public health concerns and regulatory attention have caused a shift in PFAS production, causing industry to move away from longer-chain perfluorinated structures towards short-chain analogues or structurally diverse compounds with variable functional groups or fluorination patterns. Seabirds are ideal sentinels to assess the occurrence of both legacy and emerging PFASs in food webs, as their upper trophic level position allows them to assimilate resources and related biological, physical, and chemical conditions across multiple ecosystems and temporal scales. Here, PFASs were measured in multiple tissues from juvenile Atlantic seabirds collected in 2017 and 2018. Species sampled included deceased immature or juvenile herring gulls, great shearwaters, terns, and pelicans from Narragansett Bay in Rhode Island, Massachusetts Bay off the coast of Massachusetts, and the Cape Fear River Estuary (CFRE) in southeastern North Carolina. Samples were analyzed for legacy and emerging PFASs using liquid chromatography/high resolution mass spectrometry, employing both targeted and suspect screening methods. Tissues screened included heart, brain, kidney, lungs, uropygial gland, adipose fat, liver, muscle, blood, and feces. PFOS dominated all liver samples across all individuals and habitats. Emerging compounds previously found in the CFRE system were variably detected in tissues from CFRE chicks, with high inter-individual variability. Emerging compound Nafion byproduct-2 was detected at concentrations similar to or exceeding legacy PFOS in brain and muscular tissues, driven largely by low PFOS concentrations in these matrices in conjunction with significant Nafion byproduct-2 concentrations. Concentrations of emerging compounds decreased significantly in chicks collected from the CFRE system in 2018, suggesting a rapid food web response to cessation of upstream sources.

Ecological Toxicity

TP022 Avian Toxicity of PFAS Compounds and Mixtures

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Aqueous film forming foam (AFFF) has been used by the Department of Defense for over 40 years for fire-training and emergency response activities. As a result of these activities, ground water, surface water and biota in the vicinity of relevant military installations have become contaminated with perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and other poly- and perfluoroalkyl substances (PFASs). As part of an effort to develop avian ecotoxicity information for compounds associated with AFFF, the effects of dietary exposure to acute and chronic levels of PFOS, PFOA separately and in combination. Studies were

performed on Japanese quail (*Coturnix japonica*), as a surrogate avian species. Ten-day-old Japanese quail were administered PFOS at nominal dietary concentrations of 0, 70, 141, 281, 562, 843, 1125, 1687, or 2250 $\mu\text{g/g}$ feed or PFOA at 0, 200, 350, 500, 625, 750, 1000, 1250, or 1500 $\mu\text{g/g}$ or combined PFOS+PFOA at comparable concentrations, or 3M AFFF at levels resulting in similar PFOS concentrations, or Ansul AFFF at levels to produce a comparable concentration of 6:2 fluorotelomer thioamido sulfonate. Quail were fed for five days and then fed untreated feed for an additional 18 days. LC50 values will be presented. Chronic studies involved day-old Japanese quail being administered PFOS at analyzed dietary concentrations of 0, 2.1, 4.0, 8.6, 14 or 18 $\mu\text{g/g}$ feed for a total of 140 days. At four weeks of age, 16 male/female pairs were assigned to each treatment group. At 10 weeks of age, females began laying eggs. For the next 10 weeks, eggs were collected daily, set weekly and incubated for 18 days. On the day of hatch, hatchlings were counted, weighed and placed in a brooder battery on clean feed for two weeks. Unhatched eggs were opened to determine the stage of development. Chronic endpoints were egg laying, egg hatchability, chick survivability and weight. Liver and serum PFAS concentrations for all treatments were performed.

TP023 Development of Toxicity Reference Values for Per- and Polyfluoroalkyl Substances in White-Footed Mice

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Per- and polyfluoroalkyl substances (PFAS) are compounds manufactured for use in paints, cleaning agents, fire suppressants, non-stick cookware and food containers, and water-resistant products. Concerns about PFAS stem from their ubiquitous presence in the environment, widespread reports of general toxicity, and the resistance of these compounds to degradation. Initial experiments sought to determine the rate at which steady state concentrations were reached for six PFAS [perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexanesulfonate (PFHxS), perfluorobutanesulfonate (PFBS), 6:2 fluorotelomer sulfonate (6:2 FtS), and perfluorononanoic acid (PFNA)] in the white-footed mouse (*Peromyscus leucopus*) following 28 consecutive days of oral exposure. Adverse effects observed in these studies included: reduction in thymus weights (6:2 FtS), increased liver and decreased ovary weights (PFNA), and increased liver, kidney, testes and decreased uterus and ovary weights (PFOS). No adverse effects were observed from exposure to PFOA, PFHxS, and PFBS. Subsequent reproductive/developmental toxicity tests will serve to derive wildlife Toxicity Reference Values (TRVs) to assist in the development of appropriate site-specific risk assessment and decisions related to mitigation of exposures and/or future cleanup. To date, only one reproductive/developmental toxicity test has been completed (PFOS). Although analysis of data from the study is still pending, F1 pup survivability was reduced (0 at 5 mg/kg-d and ~50% at 1 mg/kg-d).

TP024 Determination of Biomagnification Potentials for Per/ Polyfluoroalkyl Substances in Terrestrial Food-webs

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Per- and polyfluoroalkyl substances (PFASs) have been used globally for several decades to the extent that they have become ubiquitous in the environment. PFASs are persistent in the environment, which can lead to bioaccumulation and biomagnification of these substances in terrestrial systems. The main objective of the ongoing research is to develop empirical data for the uptake and elimination kinetics of PFASs in terrestrial organisms at different trophic levels in order to determine food-web biomagnification potentials for the common PFASs, including those that demonstrated greatest biomagnification risks. We have initiated this research to investigate the uptake and bioaccumulation of PFASs at different trophic levels in two key terrestrial trophic-chains: soil -> terrestrial plant -> herbivore mammal and soil -> soil invertebrate -> predatory

amphibian. Studies will be conducted with ecologically relevant species using a natural soil that supports high bioavailability of PFASs. The uptake, accumulation and elimination kinetics, and biomagnification of PFASs in the food-web organisms will be assessed using PFAS mixtures in order to assess the potential impacts of competitive uptake and selective bioaccumulation of PFASs. Our goal is to determine biomagnification of PFASs in terrestrial food-webs from lower trophic level organisms (primary consumers) to higher trophic level organisms (secondary consumers) using environmentally relevant mixtures. Ecotoxicological data developed in this research will fill the knowledge gaps regarding biomagnification potential for PFASs released into the terrestrial environment.

TP025 Adverse Outcome Pathway and Transcriptional Point of Departure Assessment of the Effects of Perfluorooctanesulfonic Acid on Zebrafish Embryos

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Perfluoroalkyl substances (PFAS) such as perfluorooctanesulfonic acid (PFOA) have been widely used in industrial and consumer products which has led to wide spread contamination of the environment. We examined the effects of PFOA on developing zebrafish embryos to better understand potential mechanisms and adverse outcome pathways by which PFOA can cause developmental toxicity. We exposed zebrafish embryos from 6 hours post fertilization to 96 hours post fertilization to four different concentrations of PFOS. We then used transcriptional and morphological effects to identify potential adverse outcome pathways (AOP) and effect thresholds for PFOS. Morphological effects observed at high doses included swim bladder malformation, cranial malformations, spinal curvature, and embryo orientation. Functional analysis of gene expression indicated that PFOS caused oxidative stress in embryos with antioxidant genes TXN, TXNRD1 and GSS up regulated monotonically leading to cell cycle arrest with downregulation of genes involved in DNA synthesis, DNA damage repair, telomere extension, and the cell cycle. Additionally, there was strong down regulation of genes involved in extracellular matrix production including collagen production and lysyl oxidase function indicating a reduction in extracellular matrix. Over all, at high doses, affected gene functions point to an overall senescence response. Using genes with monotonic dose-responsive expression and observed apical effects, we developed an AOP network where PFOS causes oxidative stress, leading to autophagy and degradation of the extracellular matrix, altering embryo notochord development and ultimately leading to malformations and mortality. The AOP no observable adverse effect level (NOAEL) was below the lowest concentration measured (7.367 mg/L PFOS). Extrapolation of the NOAEL to account for uncertainties (10X intra-human, 10X toxicodynamic, 10X subchronic-to-chronic and 3X database) results in a human drinking water RfC of 0.25ppb, suitable for an emergency response if no other information were available. This approach appears promising in identifying plausible AOPs underlying the effects of PFASs and developing AOP based concentration thresholds.

TP026 Effects of PFAS Mixtures Reflective of AFFF Sites on Amphibian Development: Is Perfluorooctane Sulfonate (PFOS) the Primary Driver?

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Per- and poly-fluorinated alkyl substances (PFAS) are of emerging concern because they are frequently detected in drinking water and natural waterways, are slow to degrade, accumulate in animals, and exert toxicity via a range of mechanisms. Contaminated surface waters contain complex mixtures of PFAS, so understanding mixture toxicity is critical. Among components of mixtures found in surface waters at sites contaminated

with aqueous film forming foam (AFFF), an important source of environmental PFAS, PFOS is the most frequently detected and at the highest concentrations. Further, it is known to be more bioaccumulative and more toxic than most other frequently detected PFAS contaminants. If PFOS alone is the primary driver of the toxicity of PFAS mixtures representative of AFFF sites, then risk assessments could be simplified and remediation efforts could prioritize removal of this compound from contaminated sites. We designed a mesocosm study to examine effects of PFAS mixtures reflective of AFFF contaminated surface waters to amphibians, which are likely to be exposed and good models given the role of thyroid hormones in regulation of metamorphosis and the suspected thyroid disrupting effects of PFAS. We will test the hypothesis that PFOS is the primary driver of toxicity in these mixtures and that removing PFOS from mixtures reduces effects, even when accounting for total PFAS loads. Northern leopard frog (*Lithobates pipiens*) tadpoles will be exposed to no PFAS, PFOS at 4 ppb, PFOS at 10 ppb, an environmentally relevant mixture with PFOS (4 ppb PFOS, 1.25 ppb PFOA, 3 ppb PFHxS, 1.25 ppb PFHxA, and 0.5 ppb PFPeA; total PFAS load = 10 ppb), the same mixture but with PFOS removed (1.25 ppb PFOA, 3 ppb PFHxS, 1.25 ppb PFHxA, and 0.5 ppb PFPeA; total PFAS load = 6 ppb), and a mixture with PFOS removed but with other compounds proportionally increased to 10 ppb total PFAS (2.1 ppb PFOA, 5 ppb PFHxS, 2.1 ppb PFHxA, and 0.8 ppb PFPeA). We will report effects of treatments on size and developmental stage 10 days post-exposure, as well size and time to metamorphosis (Gosner stage 42) and size and time to tail absorption (Gosner stage 46). We will also highlight our approach to examine thyroid disruption, including planned hormonal, histological, and transcriptomic analyses. Our data will elucidate the importance of PFOS relative to other common mixture components among AFFF sites and provide a screen for evidence of thyroid disruption in an amphibian.

TP027 Summary of Aquatic Toxicity and Bioaccumulation Data for Per- and Polyfluoroalkyl Substances

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Per- and polyfluoroalkyl substances (PFAS) are an emerging contaminant of environmental concern due to their persistence within the environment and in organisms, fate and transport potential in aqueous systems (e.g., groundwater), historically ubiquitous use in commercial products, and potential health and ecological effects at low levels of exposure. Currently, there are limited aquatic criteria/guidelines that have been developed internationally for PFAS in surface water, and even less for sediment. There are various toxicity information and toxicity reference values (TRVs) for PFAS in the open literature with most information limited primarily to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). The majority of toxicity data reviewed are available for aquatic organisms, but some data are available for birds, mammals, and aquatic plants. A review of the available literature, and a summary of surface water, sediment, and tissue screening levels and toxicity data for birds, mammals, and aquatic organisms was compiled. In addition, available literature on ambient background concentrations of PFAS in various abiotic and biotic media in the United States and in other countries was assembled and summarized. Finally, data on the bioaccumulation potential of PFAS in aquatic organisms is presented.

TP028 Acute and Chronic Effects of Perfluoroalkyl Substance (PFAS) Mixtures in Larval Bullfrogs (*Lithobates catesbeianus*)

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The effects of perfluoroalkyl substances (PFAS) are known largely from acute toxicity studies of single PFAS compounds in model organisms, while little is understood concerning effects of mixtures on native species. To address this gap, we examined the acute and chronic effects of two of

the most common PFAS (perfluorooctanesulfonic acid [PFOS] and perfluorooctanoic acid [PFOA]) and their mixtures on the survival, growth, and development of American bullfrog (*Lithobates catesbeianus*) tadpoles. In 96 h acute toxicity tests, PFOS was 10X more toxic than PFOA and the effects of the two chemicals in combination followed expectations of additivity. We also examined chronic effects by exposing tadpoles to combinations of PFOS and PFOA at sublethal concentrations for 73 d. The independent and combined effects of PFOS and PFOA varied by the endpoint under consideration. The negative effects of PFAS on tadpole mass and developmental stage were largely driven by PFOS and there was no evidence of interactions between the chemicals suggesting deviations from additivity. However, there was a significant interaction between chemicals on snout-vent length, such that observed reductions in length in mixture treatments were greater than expected based on the effects of the two chemicals independently. Further, effects on snout-vent length in single chemical exposures were only observed with PFOA. PFOS, even at the highest concentration, had no significant effect on snout-vent length. Our results highlight the importance of assessing the combined effects of chemicals commonly co-occurring in the environment and suggest caution in extrapolating from acute toxicity studies to more environmentally relevant exposure scenarios. While acute toxicity of mixtures appears additive, the sublethal effects of mixtures are more complex and provide evidence of greater than additive effects. Future studies examining the effects of environmentally relevant mixtures on native wildlife will be essential for effective environmental risk assessment and management.

TP029 Assessing the Trophic Dynamics of Per- and Polyfluoroalkyl Substances in the Food Web of a Southeastern USA Atlantic Slope River

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Understanding the sources, transport, and fate of new classes of contaminants is essential to characterize ecological exposure and risk. Per- and polyfluoroalkyl substances (PFASs) have attracted scientific and regulatory attention due to their persistence, bioaccumulative potential, toxicity, and global distribution. We assessed the accumulation and trophic transfer of 14 PFASs within the food web of the Yadkin-Pee Dee River of North Carolina and South Carolina, USA. Food web components and pathways were determined by stable isotope analyses of producers, consumers, and organic matter. Analyses of water, sediment, organic matter, and aquatic biota revealed that PFASs were prevalent in all food web compartments, with most detections and greatest concentrations in aquatic insects. All 14 PFASs were detected in aquatic insect samples (range, below detection limit [BDL] - 1,670 ng/g wet weight [WW]) and fish tissues (range, BDL - 797 ng/g WW). Perfluorooctane sulfonate (PFOS) was the dominant PFAS among all samples (64%). The ova of an imperiled fish, the Robust Redhorse (*Moxostoma robustum*), had concentrations above detection limits for 10 PFASs (range, BDL - 483 ng/g WW) and PFOS concentration was exceptionally high (483 ng/g WW), indicating likely maternal transfer. Our findings demonstrate the prevalence of PFASs in a freshwater food web with potential implications for ecological and human health.

TP030 Chronic Effects of a Low Per- and Polyfluoroalkyl Substance Mixture on Northern Leopard Frog (*Lithobates pipiens*) Development

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In the environment, per and polyfluoroalkyl substances (PFAS) are generally found as mixtures of multiple contaminants, especially at Aqueous

Film Forming Foam (AFFF) sites. Due to their persistence and frequent detection in aquatic systems, bioaccumulative potential in wildlife, and varied mechanisms of toxicity, PFAS are considered emerging pollutants of environmental concern. Among the PFAS, perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) are usually the most common and found at the highest concentrations. Furthermore, considering that the sulfonates are more bioaccumulative and more toxic than the carboxylates, and given the suspected endocrine disrupting effects of PFAS on thyroid signaling, we designed a low dose laboratory experiment that mirrored environmentally relevant concentrations of PFOS and PFHxS at AFFF sites. Low single doses of 0.5 and 1 ppb, and a binary mixture of 0.5 + 0.5 ppb of PFOS and PFHxS were used to expose larval *Lithobates pipiens* in the water column. Northern leopard frogs were exposed at the free-swimming stage (Gosner 26-28) and reared through tail absorption (Gosner 46) under normal laboratory conditions, and sampled at three time periods (Gosner 31, 42, and 46) to monitor the developmental effects of PFAS. In particular, the study will report effects on survival, body length, body mass, developmental stage, and thyroid gland histopathology, followed by mechanistic endpoints including gene expression. Results of the present study will be useful for the development of improved environmental quality criteria for two of the most commonly detected PFAS (PFOS and PFHxS).

TP031 Developmental Exposure to Perfluorooctane Sulfonate and Perfluorooctanoic Acid Selectively Decreases Brain Dopamine Levels in Northern Leopard Frogs

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Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds that are a major public health concern due to widespread use, long environmental and biological half-lives, detection in most human plasma samples, and links to multiple adverse health outcomes. The literature suggests that some PFAS may be neurotoxic. However, there are major gaps in the literature with respect to how environmentally-relevant doses during development may influence the nervous system. To address this gap, we utilized a sentinel species, Northern leopard frogs (*Lithobates pipiens*), to determine the effects of developmental exposure to environmentally relevant perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) on major neurotransmitter systems. Frog larvae at Gosner stage 25 were exposed to 10, 100, or 1000 ppb PFOS or PFOA for 30 days before neurochemical analysis. High performance liquid chromatography (HPLC) with electrochemical detection or a fluorescent detection assay was used to measure neurotransmitter levels, which were normalized to protein levels in each sample. Dopamine decreased significantly in the brains of frogs treated with PFOA (1000 ppb) and PFOS (100 and 1000 ppb). Significant increases in dopamine turnover also resulted from PFOA and PFOS treatment. Neither PFOS, nor PFOA produced detectable alterations in serotonin or its metabolite, norepinephrine, gamma-amino butyric acid (GABA), glutamate, or acetylcholine. PFAS body burdens showed that PFOS accumulated relative to dose, while PFOA did not. These data suggest that dopaminergic neurotransmission is selectively affected in developmentally exposed amphibians and that PFAS should be evaluated for a potential role in diseases that target the DA system. Further, these data have prompted us to consider the contributions of both time (longer exposures) and PFAS mixtures [i.e. 10ppb PFAS comprised of 4ppb PFOS, 3ppb perfluorohexanesulfonic acid (PFHxS), 1.25ppb PFOA, 1.25ppb perfluorohexanoic acid (PFHxA), and 0.5ppb perfluoropentanoic acid (PFPeA)]. Taken together, completed and ongoing studies aim to determine how environmentally relevant PFAS doses may contribute to developmental origins of long-term neurodegeneration.

TP032 Toxicological Response of *Chironomus dilutus* to Six Perfluoroalkyl Compounds

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A multi-faceted bioassay study was conducted on six different perfluoroalkyl substances with the intent of obtaining data to evaluate relative or proportional toxicity between the substances. The six substances were selected from the US Environmental Protection Agency (EPA) Third Unregulated Contaminant Monitoring Rule (UCMR3) list, the Health Canada (January 2017) Drinking Water Screening Values, and available Environment and Climate Change Canada guidance documents. Two of the substances, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are well studied. The other four substances (Perfluorononanoic Acid [PFNA], Perfluorobutanesulfonic Acid [PFBS], Perfluorohexanesulfonic Acid [PFHxS], and Perfluoroheptanoic Acid [PFHpA]) are commonly detected in groundwater, particularly near aqueous film-forming foam releases (AFFF), but far less is known about their toxicological potential. 10-day acute range finding tests and 20-day chronic definitive renewal bioassays were run on all six compounds using the freshwater midge, *Chironomus dilutus*. Exposure doses were established above and below environmentally relevant concentrations. Measurement endpoints included: larval survival; larval growth; and number of larvae, pupae, and emergent adults at test termination. Results from the 10-day acute survival tests were used to inform dosing for the 20-day chronic growth tests. Statistical analyses were performed with R statistical software. Results are in concert with previous work showing PFOS to be the most toxic per- or poly-fluoroalkyl substance (PFAS). There is evidence of a concentration response relationship for PFOS and PFOA in both survival and growth and log-logistic models were fit to the data. Concentration responses were not apparent for single chemical exposures of PFNA, PFBS, PFHxS, and PFHpA suggesting limited toxicity, particularly at environmentally relevant concentrations. Based upon analysis of 219 surface water samples from various DoD installations that used AFFF, 81% of sampling sites included PFOS and PFHxS as the dominant PFAS. A second round of chronic testing will be performed with PFOS and PFHxS to investigate potential additive and synergistic toxicity of the most dominant PFAS in surface waters next to AFFF sites.

TP033 A Comparison of Different Routes of Exposure (Aquatic, Oral, and Dermal) on Amphibian Per- and Polyfluoroalkyl Substance Bioaccumulation Factors

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Per- and polyfluoroalkyl substances (PFAS) are persistent, bioaccumulative and potentially toxic. Exposure to PFAS occurs in the form of mixtures that vary in the number of halogenated carbons and functional groups, which directly impacts half-lives: longer chain (>C8) and sulfonate PFAS are more bioaccumulative compared to shorter chain (C6) and carboxylated forms. Due to their unique physiology and life history, amphibians are at high risk for both bioaccumulation and toxicity following environmental exposure to these chemicals; however, data on bioaccumulation factors for this taxonomic group are limited. We determined bioaccumulation factors in American Toads (*Anaxyrus americanus*), Eastern Tiger Salamanders (*Ambystoma tigrinum*) and Northern Leopard Frogs (*Lithobates pipiens*) after aqueous (larvae) and dermal (juveniles) exposures. Dietary exposure through contaminated crickets was also conducted with salamander juveniles. Our treatments included perfluorooctanoic acid (PFOA), perfluorooctane sulfonate

(PFOS), perfluorohexane sulfonate (PFHxS), and 6:2 fluorotelomer sulfonate (6:2 FTS) at concentrations of 10, 100, and 1000 parts per billion, and a control treatment with exposures lasting 10 to 40 d. Bioaccumulation factors were highest for PFOS regardless of species and route of exposure. Aqueous exposures resulted in higher bioaccumulation factors, followed by oral and dermal exposures. In addition, steady-state was reached in < 96 h in larvae. Body burdens for 6:2 FTS decreased over time suggesting metabolism by amphibians. Determination of bioaccumulation factors is essential for risk assessment at the hundreds of PFAS-contaminated sites across the United States.

TP034 Ecotoxicity, Exposure and Ecological Risk of Per- and Polyfluoroalkyl Substances to Terrestrial Reptiles

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Per- and polyfluoroalkyl substances (PFAS) are important environmental contaminants because they are relatively common and are sometimes at high concentrations. With regard to ecological receptors, PFAS exposure has been convincingly confirmed by measurable tissue concentrations. Their occurrence in environmental and biological samples has led to concern over potential effects on ecological receptors. There are considerable uncertainties, however, for other terrestrial taxa such as reptiles. To address this uncertainty we have reviewed the literature, developed allometric exposure models, and conducted laboratory exposure and toxicity studies to better understand PFAS risk to reptilian receptors. There are multiple examples in the literature that confirm exposure to reptilian species; these mostly focus on long-lived and large reptiles such as sea turtles and crocodylians. Nonetheless, it is clear that terrestrial reptiles can be exposed and can accumulate PFAS. Moreover, exposure modeling also compares field observations indicating that reptiles can be exposed to relatively high levels of certain PFAS. In the laboratory, we have developed the brown anole, *Anolis sagrei*, as a model species for reptile ecotoxicity testing. We have completed PFOS toxicity studies and found significant effects on growth over a 35-day exposure period to environmentally relevant concentrations of PFOS. We then completed a toxicity study on both male and female brown anoles and found apparent reproductive effects. We also conducted a study to determine if dermal exposure to PFOS was a relevant exposure pathway for reptiles and, preliminarily, results suggest that dermal exposures may occur. Among the most surprising results is that the toxicity threshold for brown anoles exposed to PFOS is comparable to avian species. Our preliminary analysis of the risk potential for PFOS to reptilian receptors based on a dataset of PFAS soil concentrations suggest that some reptiles may be exposed to concentrations of PFOS that could exceed current (but limited) toxicity thresholds. Collectively, our analyses, suggest that reptiles are relevant ecological receptors based on confirmed exposures in the field and existing toxicity data. The goal of ongoing and future studies is to refine our understanding of PFAS exposure and risk to reptiles which can then translate to efficient and accurate risk estimation.

TP035 A Comparison of Different Routes Of Exposure (Aquatic, Oral, and Dermal) to Per- And Polyperfluoroalkyl Substance on Amphibian Growth and Development

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Per- and polyfluoroalkyl substances (PFAS) are persistent, bioaccumulative and potentially toxic. While PFAS toxicity has been examined in a variety of taxa (e.g., birds, mammals, fish), research on amphibians is limited. Because PFAS are both water soluble and bind to sediments, the risk of PFAS exposure is high for amphibians. Moreover, PFAS can act as potential thyroid disruptors, which can affect amphibian physiology. We

determined the effects of aqueous (larvae) and dermal (metamorph) PFAS exposures on the development and growth of American Toads (*Anaxyrus americanus*), Eastern Tiger Salamanders (*Ambystoma tigrinum*) and Northern Leopard Frogs (*Lithobates pipiens*). Dietary exposure through contaminated crickets was also conducted with salamander metamorphs. Our treatments included perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and 6:2 fluorotelomer sulfonate (6:2 FTS) at concentrations of 10, 100, and 1000 parts per billion, and a control treatment with exposures lasting 30 to 40 d. Although aqueous exposures resulted in similar body burdens regardless of species, delayed development and decreased scaled mass index (SMI, the predicted body mass of an individual at a specific snout-vent length) were observed in frogs (starting at 10 ppb) and to a lesser extent salamanders, with no effects observed in toads. Dermal exposures resulted in an increased SMI in all three species whereas oral exposure caused a decrease in SMI in salamanders. Overall, no clear dose-response or chemical-specific effects were observed for any of the endpoints measured. We conclude that developmental and growth effects appear to be species specific. Although we detected observable effects at the lowest concentration tested in some instances, the patterns were inconsistent. Additional studies are underway testing effects of low concentrations of PFAS mixtures.

TP036 Effects of PFAS on Sensitive Life-Stages of the Fathead Minnow

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There is widespread and growing concern regarding the environmental effects of Per- and polyfluoroalkyl substances (PFASs). Among the most commonly detected and studied PFAS are perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonic acid (PFHxS). To date, the consensus is that PFOS is likely the most toxic PFAS and generally dominates environmental samples. Importantly, these two PFAS infrequently occur by themselves; there are often a multitude of "other" PFAS that co-occur with PFOS and PFHxS although for these PFAS and mixtures, there are considerably less data. Indeed, even for PFOS more toxicity data over longer-term durations and critical life-stages would be useful in fine tuning toxicity and threshold estimates. Hence, an important research objective moving forward in environmental assessment and management of PFAS is to better define the ecotoxicity of relevant PFAS and PFAS mixtures. We have conducted three of eight planned toxicity studies that encompass sensitive life-stages critical to reproduction and development on a representative freshwater fish, the fathead minnow (*Pimephales promelas*). These are 42-day studies where exposures occur over spawning and then continue for 21-days of the F1 generation. Preliminary results show that PFOS, a predominant PFAS, significantly decreases survival of the spawning adults at 500 ug PFOS/L; moreover, there is a very clear threshold for developmental effects between 250 and 500 ug PFOS/L where survival significantly decreases from 92% to 10%, respectively. There were no effects seen in the fish exposed to PFHxS alone; however, the combination of PFOS-PFHxS resulted in decreased survival of the juvenile (F1) fish at 200:132 ug PFOS:PFHxS / L. Importantly, this indicates that additional PFAS (here PFHxS) may increase the sensitivity of fish to PFOS beyond that of PFOS alone exposures. This research demonstrates the need to continue evaluating the toxicity of PFAS mixtures to sensitive life-stages and in longer durations to ecological receptors as additive or synergistic effects are relevant scenarios.

TP037 Development of Adverse Outcome Pathways Relevant to the Ecological Effects of Per- and Polyfluoroalkyl Substances (PFAS)

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The adverse outcome pathway (AOP) framework depicts causal linkages between biological responses across levels of organization, starting with a molecular initiating event, proceeding through intermediate key events, and culminating in apical effects on endpoints meaningful to risk assessment (i.e., survival, growth, reproduction). AOPs organize and synthesize complex biological knowledge and empirical data in a manner that facilitates prediction of chemical effects across different chemical structures, target species, and biological endpoints. AOP development can also highlight key data gaps. Accordingly, AOPs are a logical conceptual construct for helping assess the potential ecological effects of PFAS that have received little or no testing in non-mammalian taxa. This presentation outlines a strategic approach to the development of AOPs relevant for assessing ecological effects of PFAS. The approach uses a diversity of knowledge/data streams and tools, including (a) the ECOTOX Knowledgebase; (b) the AOP Wiki; (c) high-throughput in vitro assays; (d) conventional mammalian toxicological literature; (e) comparative protein sequence evaluation based on data from NCBI analyzed with the Sequence Alignment to Predict Across Species Susceptibility tool; and (e) fundamental biological/physiological knowledge. Examples of AOPs relevant to the effects of PFAS on non-mammalian species are presented, including those capturing perturbation of processes associated with the hypothalamic-pituitary-gonadal and thyroidal axes and peroxisome proliferator-activated receptors. The contents of this presentation do not reflect USEPA policy.

TP038 Identification and Mapping Evidence of Ecological Toxicity of Per- and Polyfluoroalkyl Substances (PFAS) with ECOTOXicology Knowledgebase Protocols

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Given the persistence and wide distribution of some per- and polyfluoroalkyl substances (PFAS) in the environment, there is a recognized need to characterize potential ecological impacts and risks of these substances. Instead of exhaustive whole animal toxicity testing, chemical assessments must rely on existing empirical data, computational models, and predictive tools with limited targeted testing. Our goal was to comprehensively and systematically assemble and describe available evidence for ecological effects of PFAS using the well-established curation protocols of the ECOTOXicology Knowledgebase (ECOTOX, <http://cfpub.epa.gov/ecotox/>). ECOTOX is a publicly available web-based database providing curated single-chemical toxicity data for aquatic life, terrestrial plants, and wildlife. For over 30 years, ECOTOX has curated ecologically-relevant toxicity data to meet the systematic review and data transparency needs of the U.S. EPA for risk assessments and regulatory decisions. A significant effort was recently undertaken to update ECOTOX with data for PFAS through a comprehensive literature search for over 250 PFAS names and CASRNs. This search resulted in a list of over 8,000 references, from which title and abstract screening identified ~350 papers that met ECOTOX applicability and initial acceptability criteria (e.g., ecologically-relevant species, verifiable CASRN, endpoint and control reported). Full review and extraction of pertinent information (species, chemicals, test methods, toxicity results) are underway to add results from these references to the almost 200 PFAS references already in ECOTOX.

Characterization of the extent, distribution, and types of evidence of the curated dataset to-date indicate that most data were from studies on PFOS and PFOA in a handful of species (e.g., zebrafish, earthworm, chicken). Traditional growth/reproduction/mortality toxicity endpoints represented about 25% of reported effects, while 34% were biochemical or genetic effects. This effort identified, and subsequently curated, ecological toxicity data for PFAS which are potentially suitable for ecological risk assessments and other applications, and also highlighted knowledge gaps for biological species and classes of PFAS. In addition, this large set of empirical data for ecologically-relevant species can inform computational, translational, and species extrapolation models and tools. The contents of this presentation do not reflect USEPA policy.

Human Toxicity

TP039 Public Health Evaluation of PFAS Exposures and Breastfeeding: Methodology Used for a Systematic Literature Review

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The Agency for Toxic Substances and Disease Registry (ATSDR) is working with community members exposed to per- and polyfluoroalkyl substances (PFAS) in drinking water. Nursing mothers and health professionals have raised concerns about infant PFAS exposures through breastmilk. Fetuses and infants may be exposed to PFAS through transplacental transfer and breastmilk, respectively. Longer breastfeeding duration is associated with greater levels of most PFAS in infant serum. Based on the American Academy of Pediatrics and CDC guidelines that the risks of exposure to environmental pollutants are unlikely to outweigh the well documented health benefits of breastfeeding for both mothers and infants, ATSDR recommends that mothers who have been exposed to PFAS contaminated drinking water continue to breastfeed their infants. However, a formal review of evidence documenting any health effects associated with infant exposure to PFAS through breastmilk was not available to ATSDR. ATSDR is conducting a systematic review of observational human and experimental animal literature published through February 2018 to answer the following questions: (1) What is the evidence that exposure to PFAS through breastfeeding leads to a health outcome in humans or animals? and (2) What are the circumstances, if any, when a clinician would recommend that a mother consider not breastfeeding her child? ATSDR followed the National Toxicology Program's Office of Health Assessment and Translation protocol to identify key questions using a PECO (population, exposure, comparator, outcome) framework and formulate criteria for inclusion and exclusion of published studies. ATSDR identified 4, 297 unique records from 7 databases; 87 records for additional title/abstract screening; and 39 articles for full-text review. Two articles were excluded after risk of bias evaluation. ATSDR is now conducting data extraction and data visualization of the remaining 37 articles (3 human, 34 animal). Only a few studies were identified where the contribution of exposure of the infant through milk could be isolated from exposure of the fetus in utero. This review will inform ATSDR's recommendations to nursing mothers using state-of-the-science evidence of the contribute of exposure to PFAS in breastmilk to health outcomes in infants and children. The findings in this presentation have not been formally disseminated by the ATSDR and should not be construed to represent any agency determination or policy.

TP040 Analysis of Health Effects Hazards and Causation with Environmental Exposure to PFAS

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Many human health hazards have been associated with PFAS environmental exposures but causation has not yet been established. This lack of clarity hampers the assessment of potential risk to human health. To determine if these associated hazards have a biological basis for causation, we

conducted a systematic review of the available workplace, epidemiology and animal studies. We focused on the hazards associated with perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) as representatives of the broader PFAS category of environmental contaminants. When available, hazards were related to measures of exposure such as serum concentrations of PFPA and PFOS. For each hazard we applied elements of the Bradford Hill criteria for causation. We evaluated the consistency in observations across studies in humans. We also considered the plausible explanation for the mechanism of action (MOA), to determine if the noted effects had a reasonable biological basis. Finally, we evaluated the consistency between the observations from human studies and animal studies to determine if there was coherence across species. Using this approach, we found that only a small number of hazards associated with environmental exposure to PFAS have a biological basis for causality in humans. This type of analysis has a direct bearing on hazard endpoints used to set health-effects criteria.

TP041 Data Derived Extrapolation Factors for Developmental Toxicity: A Preliminary Research Case Study with Perfluorooctanoate (PFOA)

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Guidelines of the United States Environmental Protection Agency (EPA, 1991) and the International Programme on Chemical Safety (IPCS, 2005) suggest two different default positions for dosimetric extrapolation from experimental animals to humans when the dosimetry of the critical effect is not known. The default position of EPA (1991) for developmental toxicity is to use peak concentration (or Cmax) for this dosimetric extrapolation. In contrast, IPCS (2005) states its default position for dosimetric choice in the absence of data is to use the area under the curve (or AUC). The choice of the appropriate dosimeter is important in the development of either a Chemical Specific Adjustment Factor (CSAF) of IPCS (2005) or a Data Derived Extrapolation Factor (DDEF) of EPA (2014). Our research shows the development of a DDEF for developmental toxicity for perfluorooctanoate (PFOA), a chemical of current interest. Here, we attempted to identify the appropriate dosimetric adjustment from a review of developmental effects identified by EPA (2016). Some of these effects appear to be related to Cmax, few if any related to AUC, and most related to the average concentration during the exposure window of concern for a particular effect. We then compared kinetic data from PFOA exposure during pregnancy in mice with newly available clinical data in humans after up to 25 weeks of PFOA exposure. The resulting DDEF was 14. Although current population exposures to PFOA are generally much lower than both the experimental animal data and the clinical human study, the development of this DDEF is consistent with current guidelines of both EPA (2014) and IPCS (2005).

TP042 Comparative Developmental Toxicity of Perfluorooctanoic Acid (PFOA) and Shorter Chain Perfluorocarboxylic Acids Using the Zebrafish Model System

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Perfluoroalkyl substances (PFAS) are an emerging class of environmental chemical contaminants that are associated with various adverse human health outcomes. Developing organisms are particularly sensitive to toxicant exposure and there is currently limited information concerning developmental toxicity of most PFAS. Perfluorooctanoic acid (PFOA) was one of the first PFAS ubiquitously detected in the environment as well as the general human population. PFOA was voluntarily eliminated with shorter chain derivatives being used as replacement compounds due to evidence of decreased acute toxicity. However, there is limited

developmental toxicity information for these shorter perfluorocarboxylic acids (PFCAs), such as perfluorohexanoic acid (PFHxA) and perfluorobutanoic acid (PFBA), that are becoming more predominant in the environment. The objective of this study was to compare the developmental toxicity of PFOA to the shorter chain derivatives (PFHxA and PFBA) including transcriptomic, morphometric, and behavioral analyses. We hypothesized that an embryonic exposure to PFCAs will cause dissimilar alterations to gene expression and biological pathways and decreased morphological and behavioral alterations in the zebrafish vertebrate model system. Zebrafish were exposed to 0, 4, 40, or 400 ppb (ug/L) of the PFCAs throughout embryonic development and transcriptomic, morphology, and behavioral endpoints for each PFCA assessed. A few disease and disorders were similar among the PFCAs including organismal injury and abnormalities and gastrointestinal disease, but most were different including cancer as the top disease only for PFOA. Morphological and behavioral alterations were also chemical-specific including hyperactivity for PFOA and hypoactivity for PFBA. These findings further the comparison between the PFCAs and provide guidance on additional molecular and disease pathways to target for a more thorough understanding of the potential adverse health outcomes of an embryonic exposure to PFOA, PFHxA, or PFBA.

TP043 In Utero Exposure to hexafluoropropylene Oxide-Dimer Acid (GenX) Produces Low Birth Weight and Neonatal Mortality

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Hexafluoropropylene oxide dimer acid (HFPO-DA, aka GenX) is a member of the per- and polyfluoroalkyl substances (PFAS) chemical class. More specifically, HFPO-DA is a perfluoroalkyl ether carboxylic acid (PFECA) which, along with perfluoroalkyl ether sulfonic acids (PFESA), have recently been used as industrial replacements for straight-chain PFAS, presumably due to favorable toxicity profiles. Previously, we dosed Sprague-Dawley rat dams daily via oral gavage with 1-500 mg HFPO-DA/kg/d from gestation day (GD) 14-18 and reported significant dose-responsive increases in maternal liver weight (≥ 62.5 mg/kg), reduced maternal serum thyroid hormone and lipid profiles (≥ 30 mg/kg), and highly upregulated expression of genes related to PPAR signaling pathways in maternal and fetal livers (≥ 1 mg/kg). Here, we dosed dams with 1-125 mg/kg/d (n=4 litters per dose) from GD16-20 and with 10-250 mg/kg/d (n=5 litters per dose) from GD8 - postnatal day (PND) 3. Results analyzed thus far from GD16-20 dosing have not been distinct from GD14-18 dosing. Dosing from GD8-PND3 resulted in a significant dose-responsive increase in neonatal mortality (≥ 62.5 mg/kg), which occurred shortly after birth (< 24 hrs). There was a significant decrease in pup body weight at birth, PND1, and PND2 (≥ 30 mg/kg) and increased relative liver weight at necropsy (PND2) in all dose groups (≥ 10 mg/kg). The only histopathological finding from PND0 pup livers was a dose-responsive decrease in glycogen stores. Dam weight gain during gestation was significantly reduced at ≥ 125 mg/kg and liver weight (measured on PND2) was increased at all doses (≥ 10 mg/kg). Dam serum HFPO-DA concentrations were ~3-fold greater than fetal serum concentrations at a given maternal dose; however, liver concentrations were identical between mother and fetus. Further, fetal livers contained ~10-fold greater HFPO-DA concentrations than PND2 pup livers, indicating a lack of appreciable lactational transfer and rapid clearance. Overall, HFPO-DA exposure produced multiple adverse effects similar to PFOS and PFOA; however, at higher oral doses. The specific mechanism by which these effects occur is still unknown. Studies have begun with other PFAS with known human exposure, including perfluoromethoxy acetic acid (PFMOAA) and nafion byproduct 2. To date PFMOAA appears less toxic than HFPO-DA. The views presented here do not necessarily reflect the views or policy of the USEPA.

TP044 Perfluorooctanoic Acid or Its Replacement GenX Targets Placental Trophoblasts in CD-1 Mice and Human-Derived JEG-3 Cells

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Perfluorooctanoic acid (PFOA) is a perfluoroalkyl substance (PFAS) associated with adverse maternal-fetal health outcomes including increased gestational weight gain (GWG) and fetal growth restriction (FGR). PFOA is one of the most well-studied PFAS, however the mechanism through which it induces FGR is not known but it is hypothesized that the placenta is a target. PFOA has been phased out of production in the US and replaced with new compounds, including GenX, but remains an exposure concern due to its environmental and biological persistence. Its replacement, GenX, was designed as a safer alternative to PFOA, but existing animal data are limited. To determine a potential mechanism of action of FGR for PFOA and evaluate whether its replacement, GenX, has similar effects on FGR and GWG, pregnant CD-1 mice were exposed daily to PFOA (1 and 5 mg/kg) or GenX (2 and 10 mg/kg) from embryonic day (E) 1.5 to E17.5. At E17.5, placental weight was increased in mice exposed to 5 mg/kg PFOA and 10 mg/kg GenX. At E17.5, placental weight was significantly increased in 5 mg/kg/day PFOA and 10 mg/kg/day GenX treated mice relative to controls (~21 mg and ~15.5 mg increase in placental weight relative to controls, respectively). Fetal:placental weight ratios were significantly reduced relative to controls in 5 mg/kg PFOA and 10 mg/kg GenX treated mice at E17.5. Histopathological analysis of placentas revealed two main lesions affecting the trophoblast cells in PFOA and GenX exposed mice, labyrinth atrophy and labyrinth congestion. The degree to which individual litters had ≥ 1 abnormal placenta at E17.5 was 10 mg/kg GenX (100%), 2 mg/kg GenX (80%), 5 mg/kg PFOA (60%), 1 mg/kg PFOA (40%), and vehicle control (0%). A panel of 43 unique PFAS structures were evaluated in a high-throughput screen using human-derived JEG-3 placental trophoblasts. Analysis of differential expression of a suite of candidate genes involved in nutrient signaling, fetal growth, oxidative stress, and inflammation in both mouse placenta and JEG-3 trophoblasts suggests that GenX and PFOA exert adverse effects on the placenta via distinct pathways. These findings suggest GenX has potential to adversely affect maternal-fetal health in the human population, and that it exerts placental toxicity through a mechanism unique to that of its predecessor, PFOA.

TP045 Assessing the Effect of PFAS on Placental Epigenetic Machinery

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Per- and poly-fluoroalkyl substances (PFAS), also known as fluorosurfactants, are a group of chemicals that have been widely used in stain repellents, paints, polishes, fire fighting foams, and protective coatings. While some of these chemicals, including perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been discontinued in the United States, their persistence in the environment and continued use internationally make them a toxicological concern. Moreover, GenX, a modern fluorosurfactant replacement for PFOA, has recently contaminated drinking water in the Cape Fear River in the Wilmington, NC area, warranting immediate analysis of the risks associated. The EPA acknowledges that certain PFAS can cause reproductive, developmental, liver, kidney, and immunological toxicity and may even be carcinogenic. While prior reports have shown that PFAS can cross the placenta, there is little understanding of how this class of compounds can impact placental epigenetic machinery. We therefore developed an mRNA biomarker panel designed to comprehensively examine potential epigenetic machinery changes in the placenta. This panel utilizes 90 well-established mRNA markers of epigenetic machinery including genes tied to histone phosphorylation, ubiquitination, methylation, deacetylation, acetyltransferases, and methyltransferases in tandem with the Fluidigm Biomark HD platform for high-throughput qPCR analysis. JEG-3 cells, an in vitro trophoblast cell line, and ex vivo explants isolated from human

term placentas were treated with PFOA, PFOS, and GenX (10-1000 ng/mL) for 24 hours. Subsequently, mRNA expression was quantified using fluidigm platform, and protein expression was analyzed by western blot. Results have shown changes in the expression a variety of histone altering proteins including but not limited to HDACs, PRMTs, and AURKs suggesting multiple mechanisms of epigenetic regulation and reorganization of chromatin structure that may lead to altered cell signaling and ultimately altered cell growth and migration.

TP046 Effects of Short-Chain Fluorotelomer Alcohols and Perfluorocarboxylic Acids on Oxidative Stress in *Caenorhabditis elegans*

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Per- and poly-fluoroalkyl substances (PFAS) have high commercial value, but given their persistence, bioaccumulation, and toxicity, there is need for new information on the fate of PFAS in biological systems, including perfluorinated carboxylic acids (PFCAs) and associated precursors and transformation products. The current study connects the biotransformation of short chain PFAS with toxicity and modes of action. Using subcellular mammalian fractions and the nematode *Caenorhabditis elegans* (*C. elegans*), we examined the involvement of the enzyme cytochrome P450 2E1 (CYP2E1) in metabolizing the 6:2 fluorotelomer alcohol (FTOH). A selective enzyme inhibitor, diallyl sulfide, inhibited 6:2 FTOH metabolism, indicating that CYP2E1 plays a role in 6:2 FTOH metabolism. While metabolism is primarily considered a detoxification pathway, leading to more polar products that are readily eliminated, FTOH metabolism forms highly persistent PFCAs and reactive fluorinated aldehydes that have potential to exert oxidative stress by sequestering anti-oxidant proteins and peptides. To examine the comparative oxidative stress of FTOHs, PFCAs, and associated PFAS, we used *C. elegans* mutant strains expressing the anti-oxidant glutathione S-transferase attached to green fluorescent protein. Changes in glutathione-S-transferase levels were measured using Western blot and fluorescence microscopy. The 6:2 FTOH promoted glutathione-S-transferase expression whereas other PFAS tested, including the PFCAs PFHxA and PFOA, had little effect. These results suggest that, in this model organism, 6:2 FTOH and/or its reactive metabolites are leading to greater cell stress and toxicity than those PFAS phased from production. We are further developing these *in vitro* metabolic and *in vivo* oxidative stress models as screening tools to assess metabolism and toxicity of other classes of emerging PFAS.

TP047 A Pathology Review of the Lower GI Tract in Rats and *Cynomolgus* Macaques Treated with Ammonium Perfluorooctanoate: No Evidence of Ulcerative Colitis

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Among the numerous short-term, subchronic, and chronic toxicology studies with ammonium perfluorooctanoate (PFOA), the gastrointestinal tract has not been identified as a target organ for PFOA-related toxicity in laboratory animals where the corresponding serum PFOA concentrations typically approach several orders of magnitude higher than the general human population. These lack of gastrointestinal tract-related findings were in direct contrast to an epidemiologic observation where a positive trend was observed for an idiopathic chronic inflammatory condition of the gut, ulcerative colitis, by quartile of estimated PFOA exposure in a Mid-Ohio River community whose drinking water contained higher levels of PFOA. This study was taken to perform a histologic re-evaluation of large intestine sections in laboratory animals from two long-term toxicological studies: one was with Sprague Dawley rats that received ammonium PFOA in their diet for two years and the other one was with cynomolgus macaques that received daily capsules of ammonium PFOA for 6 months. In both studies there was a lack of histologic evidence of treatment-related inflammatory lesions that was suggestive of the occurrence of ulcerative colitis in these laboratory animals even under the most rigorous treatment schedules. These findings do not offer support for the biological plausibility of the epidemiological associations reported.

TP048 Evaluation of the Combined Effects and Toxicological Interactions of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) Mixtures in HepG2 Cells

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There is a paucity of information about the potential combined toxicity and interactions of perfluoroalkyl and polyfluoroalkyl substances (PFASs), even though they co-exist in the same environment. In this study, the individual and combined toxicity of PFASs were evaluated in HepG2 cells after 24 h exposure using MTS assay. The combination Index (CI)-isobologram equation method was used to evaluate the toxicological interactions of PFASs in binary, ternary and multicomponent mixtures. The results of individual compounds indicated that the inhibitory effect on HepG2 cells increased with increasing carbon chain lengths and is higher in sulfonate compared to carboxylates. The respective cytotoxicity of PFASs is in the order of PFDA > PFOS > PFNA > PFOA > PFHxS > PFHpA. The toxicological interaction of PFOS and PFOA with other PFASs clearly showed a different pattern of combined toxicity in HepG2 Cells. The binary and ternary combinations of PFOS with PFOA, PFNA, PFDA, PFHxS, and PFHpA displayed synergistic interactions for almost all inhibitory effect (cytotoxicity) levels tested, whereas, either synergistic or antagonistic effect was observed in mixtures with PFOA. The pattern of interactions of PFAS mixtures is dominated by synergism, especially at low to medium effect levels; the exceptions to this were the antagonistic interactions found in the mixtures of PFOA, PFHxS and PFHpA. These cytotoxicity results may have an implication on the health risk assessment of PFAS mixtures. Other biological end-points are being investigated.

TP049 PFOA Induces Liver and Serum Dyslipidemia in a Humanized PPAR α Mouse Model Fed an American Diet

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Americans are exposed daily to mixtures of perfluoroalkylated substances (PFAS) in their drinking water, food, air, dust in their homes, and direct use of consumer products. Increased concentrations of serum total cholesterol and low density lipoprotein cholesterol (LDL-C) are among the best supported endpoints by epidemiology studies. Dyslipidemia (elevated triglycerides, decreased high density lipoprotein cholesterol) and increased serum LDL-C are major contributors to cardiovascular disease, the leading cause of mortality in the US. Despite evidence that human PFAS exposure is associated with dyslipidemia, these effects of PFAS have received little toxicological research focus, largely because there is no suitable animal model. As a result, the European Food Safety Authority has proposed Tolerable Daily Intakes for PFAS based on epidemiological data. Mechanisms by which PFAS cause lipid-disrupting metabolic effects are not well understood, but given their structural similarity to fatty acids, interaction with human peroxisome proliferator activated receptor α (PPAR α) is a logical molecular initiating event. PPAR α is an essential mediator of cholesterol and lipid homeostasis. Moreover, polymorphisms in human PPAR α are associated with changes in serum cholesterol. Thus, PPAR activation is relevant and important as a likely PFAS mechanism of action. Our data show that PFAS activate human PPAR α transcriptional activity. Mice expressing human PPAR α and fed a diet based on the "What we eat in America" analysis provide a new animal model needed for PFAS-induced dyslipidemia research. Perfluorooctanoic acid (PFOA) exposure in drinking water (8 μ M) for 6 weeks resulted in a serum PFOA concentration of 78.8 ± 5.4 μ g/ml, a level approximately 4-fold higher than serum levels found in fluorochemical workers. PFOA exposure increased liver mass, increased lipid content, perturbed the lipidome and induced PPAR α target gene expression. PFOA increased serum triglycerides and total cholesterol, while also perturbing the serum lipidome. Sex-dependent effects were evident. A literature-based analysis of effects of PFOA on serum triglyceride and cholesterol across multiple mouse models revealed a non-monotonic dose response.

The humanized PPAR α mouse model fed an American diet is an important new model for examining PFAS-induced dyslipidemia and defining the mechanism of action.

TP050 In Vitro Hepatic Clearance of Per- and Polyfluoroalkyl Substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals that have been used in a variety of industries throughout the world. The two most studied PFAS compounds are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) which have both been shown to be persistent in both the environment and in humans as neither is broken down in the environment or metabolized in humans. To better understand the significance to public health a group of 75 PFAS compounds was selected to cover different classes of PFAS. The goal of this work was to determine whether or not these compounds are hepatically cleared via metabolism using primary human hepatocytes (PHHs). Allowing for the potential assessment of the persistence of these chemicals in humans. In total, 27 of the 75 PFAS were amenable to liquid chromatography mass spectrometry (LCMS) using ESI. These 27 PFAS compounds were subjected to an initial screen for hepatic clearance where 10-donor pooled primary human hepatocytes (PHHs) were incubated with 10 μ M of PFAS for 0 and 4 hours at 37 celsius. Heat inactivated PHHs were also incubated with 10 μ M of PFAS for 0 and 4 hours at 37 celsius as a control. Comparison of peak area ratios (4 hours/0 hours) for the active and heat inactivated PHHs was completed and a t-test was used to determine whether or not there was a significant difference between active and inactive PHHs. In total, 23 of the 27 PFAS compounds studied were shown to have statistically significant hepatic clearance. To further investigate in vitro hepatic clearance, those PFAS compounds found to be cleared in the initial screen are being evaluated in more detail via incubations with 50-donor pooled primary human hepatocytes at 0, 15, 30, 60, 90, 120, and 240 minutes. Thus, allowing for the calculation of a hepatic clearance rate to better understand the kinetics of the clearance of PFAS compounds. Finally, metabolites for each parent PFAS compounds were predicted using ADMET Predictor/MedChem Studio. This information will be used to assist in the metabolite profiling of the cleared PFAS compounds to identify, at this time, unknown PFAS metabolites.

Risk Assessment and Characterization

WP001 From Long-Chain to Short-Chain PFASs - Better or Worse? A REACH Perspective

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In order to minimize emissions voluntary industry measures as well as regulatory measures are in force or underway for so-called long-chain PFASs. Under the European Chemicals Regulation REACH (EC 1907/2006), several long-chain PFCAs are listed as Substances of Very High Concern (SVHC) on the REACH Candidate List due to their persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) properties. A Europe-wide restriction of PFOA, its salts and related substances, which might degrade to PFOA, will come into force in 2020. Furthermore, a listing of these substances under the Stockholm Convention is currently under discussion. A Europe-wide restriction of perfluorocarboxylic acids containing 9 to 14 fully fluorinated carbon atoms (C9-C14 PFCAs), their salts and related substances is on its way. In consequence, short-chain PFASs are increasingly used as substitutes for the long-chain homologues. They are considered not bioaccumulative. However, apart from their persistence, short-chain PFASs have other properties which also give rise to concern. This is for

instance shown by the case of Rastatt (Germany). Here the environmental contamination with short-chain PFASs is causing substantial problems with the supply of drinking water due to the high water solubility, low sorption potential and thus high mobility. Furthermore short-chain PFASs enrich in plants. In Rastatt crop could thus not be used as food. The pollution caused by the use of contaminated sludge has been discovered in 2013 but problems remain up to date. Common remediation techniques are not effective. Once emitted to the environment, short-chain PFASs cause irreversible contaminations thus causing high socio-economic costs and threats to men and environment. Though the Rastatt case describes a case on local scale short chained PFASs are ubiquitously found in the environment and can be transported over long distances and occur in remote regions. The described concerns are not “classical” in the sense of PBT or vPvB and are not explicitly addressed in REACH. This makes it challenging to implement regulatory measures and to justify their equivalent concern. The concerns as well the challenge in taking regulatory steps will be elaborated in detail in the presentation using PFHxA as an example. In Europe, also for PFHxA, its salts and related substances a restriction under REACH is currently in preparation.

WP002 Status of PFAS Knowledge in New Zealand

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A wide range of PFAS are highly mobile and persistent in the environment. There are few New Zealand investigations, but they confirm that these compounds can be found at elevated concentrations in surface and groundwater samples. An All of Government National PFAS Programme was established to manage and coordinate the government response to PFAS contamination in New Zealand, and to provide confidence and assurance to the public. The group has representatives from Ministries, Defence Force, Fire and Emergency, Crown Law Office, and Local Government. The group aims to develop interim drinking water criteria and draft soil and water sampling guidelines. All freshwater fish species sampled near contaminated sites accumulated PFOS and PFHxS to some degree. There were some cases where PFOS residues measured in aquatic organisms were at levels above food safety limits. The results were highly variable between sites and bio-accumulation factors could not be estimated. Some clear trends are apparent: 1) PFOS is the most commonly detected of the 28 PFAS compounds analysed; 2) perfluorosulphonic acids bioaccumulate more strongly than the perfluorocarboxylic acids and 3) long chain PFAS bioaccumulate more than the shorter chain compounds. These are consistent with overseas studies. Testing of roe from trout collected from an impacted waterway found much higher concentrations of PFOS within the fish eggs than the muscle tissue. Results so far show that some marine organisms accumulate PFAS far more readily than other species. For instance, hornshell accumulate more PFOS than mud crab. However, no detectable PFOS (or any other PFAS compounds) was found in the edible tissue of green lipped mussel collected near contaminated sites. There is limited data on New Zealand marine mammals but low levels of PFOS and PFOSA were detected in dolphin muscle tissue. There is limited information on PFAS within New Zealand ecosystems. There is also a dearth of information on the impacts of PFAS on our unique native species. There are initiatives to identify knowledge gaps to address and ensure that we connect with international research groups working on PFAS risk assessment and management.

WP003 Per- and Poly- Fluoroalkyl Substances (PFAS): Challenges to Assessing Risk in United Kingdom Surface Waters

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Per- and Poly- Fluoroalkyl Substances (PFAS) are extremely persistent in the environment, where they also accumulate in biota. It is therefore

inevitable that people and wildlife will be subjected to long-term exposure to these substances. PFAS have been detected in air, surface waters, groundwater, soils and food commodities on a global scale. The United Kingdom (UK) was the first European country to introduce a national ban on perfluorooctane sulfonic acid (PFOS), which has subsequently been controlled under the Stockholm Convention on persistent organic pollutants. Data presented here will focus on PFOS in the UK environment. However, further work is being performed monitoring numerous PFAS at a national level. PFOS has been monitored for Water Framework Directive (WFD; 2000/60/EC)/Ground Water Daughter Directive (GWDD; 2006/118/EC) purposes in order to characterise or identify potential risk by comparison with Environment Quality Standards (EQS). The Environment Agency routinely monitors water samples from surface and ground- waters, as part of the UK national monitoring network. Recognising the tendency for certain PFAS to bioaccumulate, biota samples (fish and molluscs) have also been analysed since 2015. These data have been used to assess risks on a national scale and to help identify use patterns, sources and pathways, and trends in occurrence. Such substances pose a number of technical challenges because of the restricted spatial scale of biota monitoring, and difficulties in translating between residues measured in water to those in biota, Our poster explains how we have approached these challenges in the UK to help paint a national picture of the risks posed by this group of substances.

WP004 Problem Formulation in the Risk Assessment of Long- and Short-Chain Per- and Polyfluoroalkyl Substances (PFAS)

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The framework for scientific and regulatory communities to refine the measurement, characterization of fate and transport, toxicology, and risk and remediation of emerging environmental contaminants has evolved for decades. Per- and Polyfluoroalkyl substances (PFAS) involve a new set of environmental risk and remedial engineering challenges to the life cycle of ‘typical’ emerging contaminants. These unique challenges arise from the compounds’ prevalence and persistence in the environment, potential for long-range migration, and potential toxicity at environmentally relevant concentrations. Decades of experience addressing emerging contaminants and public perception still apply, but the unique characteristics of PFAS provide an equally unique set of issues and approaches that must be addressed so that risk input to remedial decision making is appropriately calibrated and effective. A further complicating factor is the differences in fate and transport and toxicity of long-chain compared to short-chain PFAS, and how these differences dictate problem formulation and environmental risk assessment where these compounds are present. The current study presents and discusses the properties of a simplistic conceptual model with respect to long- and short-chain PFAS in terms of uncertainties and challenges in environmental risk assessment. Each step of the process will be explored, including identification of constituents of chemical concern, hazard and exposure assessment, and risk characterization and management. The literature-based study includes delineation of simple food webs, conceptual site models, and different approaches to risk assessment; it further discusses how the framework and results may differ based on PFAS chain length. Study findings will provide a problem formulation framework as a basis for long- and short-chain PFAS environmental risk assessment. It will also identify areas where additional study is needed to advance risk assessment outcomes and efficacy informing remedial decision making.

WP005 Prioritising Key Issues for Human Health Risk Assessment and Risk Management Approaches at PFAS Impacted Cattle and Sheep Farms

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While PFAS were not manufactured in Australia, they were imported and used in high volumes in fire fighting foams, amongst other uses. In Victoria, PFAS contamination in agricultural areas is largely associated with historic fire training activities. In the last 5 years, over 100 cattle and 70 sheep have had serum tested for PFAS during environmental investigations. Despite a range of PFAS reported in stockwater, relatively few were measured in serum. Cattle reported detection rates for PFBS(1%), PFHpS(66%), PFHxS(100%), PFNA(16%) and PFOS(100%), whilst sheep reported detection rates for PFBS(6%), PFHpA(2%), PFHpS(68%), PFHxA(2%), PFHxS(70%), PFNA(12%) and PFOS(66%). Grass and soil concentrations were not material. EPA Victoria's data shows that farm practices and proportion of stockwater impacted play a significant role. Cattle whose sole water source contained 4 - 8 ug/L PFOS reported up to 1800 ng/mL in serum. Conversely farms with multiple water sources reported serum PFOS concentration at least an order of magnitude lower. Deterministic models often employed to predict PFAS uptake from environmental media were not easily validated. This further highlights the importance of understanding the dynamic nature of livestock exposure patterns and pharmacokinetic behaviour of PFAS in livestock. From 2017, EPA Victoria, Agriculture Victoria, the Department of Health and Human Services and ToxConsult Pty Ltd and have funded toxicokinetic studies to increase our understanding of PFAS distribution to edible tissue and elimination half lives in cattle and sheep (Drew, R et al 2017, 2019 a,b). This research, coupled with analysis of EPA Victoria data, has informed our risk management framework and direction of future research investment, both of which will be described in more detail. References: Drew R, Hagen T, Champness D and Ryan J (2017). PFAS serum half-life in sheep of different ages and gender. Clean Up Conference Proceedings, 10 - 15 September 2017. Melbourne. Abstract 8A.1. p433-434. Drew R, Hagen T, Champness D, and Sellier A (2019a). PFAS Tissue Distribution in cattle. *The Toxicologist*. pg. 42-43 Poster #1179. American Society of Toxicology Annual Scientific Meeting 2019, Baltimore. Hagen T, Drew R, Champness D, and Sellier A (2019b). PFAS Clearance in cattle. *The Toxicologist* pg44. Poster#1185. American Society of Toxicology Annual Scientific Meeting 2019, Baltimore.

WP006 An In-Situ Toxicity Identification Evaluation (iTIE) System for Determining Which Chemicals Drive Impairments at Contaminated Sites

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Human-dominated waterways contain thousands of chemicals. Determining which chemical and biological stressor is most important; therefore, is very challenging. The U.S. EPA's Toxicity identification evaluation (TIE) procedure is a biological-chemical fractionation approach attempting to identify causes of toxicity uses a series of manipulations to fractionate chemicals then expose organisms. While this may provide useful information, it lacks ecological realism as it is subject to laboratory-related artifacts and is resource intensive. The in-situ TIE (iTIE) technology was developed to improve this approach and has undergone a number of modifications over the past several years. The 2019 prototype consists of an array of iTIE ambient water fractionation units. Each unit consists of an initial chamber with differing resin absorbents to fractionate chemical types from porewater, surface water or wastewater effluents. After resin sorption, sample waters pass into a chamber

containing various types of toxicity test organisms. Waters then pass into a sample collection bottle for confirmatory chemical analysis. A secondary containment unit houses peristaltic pumps and electronic controller providing flow through conditions for 24 to 48 hrs. The iTIE system has been deployed to a depth of 3 meters and evaluated in streams and marine harbors. Chemical analyses of water and iTIE chemical sorptive resins confirmed lethal to sublethal responses by differing classes of chemicals. This latest iTIE prototype provides a robust technology that improves stressor-causality linkages. The system assists in "weight-of-evidence" studies to assess stream and harbor biological impairments by determining which chemicals are driving site toxicity.

WP007 Toxicity of Emerging Perfluoroether Carboxylic Acids in the Zebrafish Embryo Model

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Perfluoroalkyl substances (PFASs) are persistent organic contaminants that have been detected in wildlife, humans and the environment. They have been associated with negative health effects including cancers, neurotoxicity, thyroid malfunction, reproductive problems, immunotoxicity, lung toxicity, and hepatotoxicity. Studies have shown that the toxicity of PFAS is determined by the carbon chain length as well as the attached functional group. Toward phasing-out PFASs (e.g., perfluorooctanoic acid [PFOA]) with known adverse health effects, these long-chain PFASs have been replaced with short-chain perfluoro ether carboxylic acids (PFECAs) and other fluorinated derivatives. Except for a few studies, mostly from manufacturers, however, there is no detailed information regarding the toxicity, pharmacokinetic behavior, and release of these alternative PFASs. In the present study, the zebrafish embryo was employed, as a toxicological model, to evaluate seven of these emerging PFECAs, along with PFOA, through 7 days post-fertilization (dpf), including assessment of lethality, developmental toxicity and neurobehavioral effects. Rapid onset of dose-dependent toxicity including significant lethality, and neurobehavioral and morphological abnormalities, was observed at 24 hours post-fertilization. Toxicity was time-dependent (over 7 dpf), and quantitatively correlated with PFECA chain length. To our knowledge, this is the first toxicological study of the PFECAs, as emerging replacements for PFASs, to show significant toxicity in the zebrafish embryo model.

WP008 Toxicokinetic Models for PFAS Transfer into Pork, Wild Boars and Cows' Milk

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Toxicokinetic models are mathematical representations of the absorption, distribution, metabolism and excretion of toxic substances in humans and animals. We have developed a series of models based on experimental and computational work enabling the simulation of the transfer of poly- and perfluoroalkyl substances (PFAS) from animal feed and drinking water into food of animal origin. Pigs: The transfer of a mixture of seven perfluoroalkyl acids (PFAAs) from contaminated feed into the edible tissues of 24 fattening pigs was investigated. Unexcreted PFAAs accumulated in plasma (up to 51% of total non-excreted), fat, and muscle tissues (collectively, meat 40-49%), liver (under 7%), and kidney (under 2%) for most substances. An exception was perfluorooctanesulfonic acid (PFOS), with lower affinity for plasma (23%) and higher for liver (35%). A model was developed to quantify the toxicokinetics of PFAAs and to calculate elimination half-lives. Perfluorohexanoic acid (PFHxA) had the shortest half-life at 4.1 days. PFASs were eliminated more slowly (e.g., half-life of 634 days for PFOS). PFAAs in pigs exhibit longer elimination half-lives than in most organisms reported in the literature, but still shorter than in humans. Wild boars: A biomonitoring program in three German Federal States showed liver concentrations at similar level among different regions. We observed an average ratio of PFOS:PFOA concentration in liver of 20.5:1. In order to backtrack the ratio of PFOS:PFOA in the wild boars' diet, the above toxicokinetic model for pigs was used. According to

our model, the PFOS exposure is only 2.2 times that of PFOA due to the slower elimination kinetics of PFOS and higher affinity for the liver than PFOA. Cow's milk: A toxicokinetic model was developed that describes the uptake of PFOS from contaminated feed by cows and its subsequent excretion through the milk. Parameter values of the model were estimated by fitting to experimental data from our group. The calculations show that almost all ingested PFOS is excreted with the milk. At the same time, the excretion was slow with an estimated half-life of 56 days. The derived models can be used to estimate the transfer of PFAS in the food chain. Beyond the published model, easy to use tools with a graphical interface have been programmed and made available as RITOPS and PERCOW.

WP009 Development of an Oral Reference Dose for GenX Using the Latest Toxicological and Risk Assessment Methodologies

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Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (GenX) is one of several chemistries developed as an alternative to long-chain per- and poly-fluoroalkyl substances (PFAS), which tend to have long clearance half-lives and are environmentally persistent. Recently there has been increased interest in developing health-protective toxicity values for drinking water exposures to PFAS. Therefore, an oral reference dose (RfD) was developed based on available studies which includes a chronic bioassay in rats and subchronic studies in mice. While mice appear to be more sensitive, an evaluation of these studies indicates that the mode of action (MOA) for effects in mice is possibly dependent on sensitive PPAR-alpha (PPARα)-related responses. To assess the involvement of PPARα, RNA sequencing was conducted on fixed liver sections from male and female mice exposed to 75 mg/kg GenX via oral gavage for 90 days. Significant pathway enrichment for PPARα activation was evident in males and females exposed to 0.5 and 5 mg/kg GenX, with no enrichment at 0.1 mg/kg in either sex. Other gene sets related to PPAR signaling, including fatty acid metabolism and non-specific peroxisome-related processes, were among the most significantly enriched across all gene sets queried. Consistent with these analyses, livers from treated mice exhibited increased weight and hypertrophy. Liver sections from mice exposed to GenX that were previously diagnosed as exhibiting 'hepatocyte single cell necrosis' were reevaluated using the most current diagnostic criteria, and the lesions were concluded to exhibit characteristics of apoptosis as opposed to necrosis. Immunostaining for activated caspase-3 confirmed the updated diagnoses. In vitro PPARα receptor activation assays showed activation of mouse PPARα. Together, these analyses indicate that liver effects in mice are PPARα-mediated and that the absence of liver necrosis, fibrosis, and inflammation indicate that the mouse liver lesions have limited relevance for risk assessment. Therefore, numerous lesions in the chronic rat bioassay were modeled using both frequentist and Bayesian benchmark dose (BMD) models, and subsequently used to derive deterministic and probabilistic RfDs for GenX.

WP010 Physical Properties and Toxicity Assessment of Short-Chain (C6) Fluorosurfactants

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Aqueous Film Forming Foams (AFFF) are the most effective firefighting agents currently available to control and extinguish hydrocarbon fuel fires. As aqueous film formers, AFFFs act as fuel vapor suppressors thereby augmenting the fire-extinguishing efficiency of the foam and preventing the ignition or re-ignition of the hydrocarbon fuel vapors. Fluorinated surfactants are a key ingredient in AFFF in that they provide the required low surface tension (15 to 17 dynes/cm) and positive spreading coefficient necessary to produce the film formation of water on top of lighter fuels. Older generations of AFFF foam concentrates were C8-based and contained fluorosurfactants that may be precursors to PFOA when they break down in the environment. Due to concerns related to the potential impact of PFOA and other long-chain (C8) fluorinated surfactants on human

health and the environment, the US EPA launched the PFOA Stewardship Program in 2006 with the goal of eliminating PFOA and PFOA-related chemicals in industrial products. Short-chain (C6) fluorosurfactants have shown to be suitable replacements for C8 surfactants historically used in firefighting foam concentrates. For this reason, many AFFFs were reformulated with C6 fluorosurfactants that do not breakdown to yield PFOA and are arguably less toxic and less bioaccumulative in wildlife and humans. In order to effectively communicate risk and provide regulatory guidance, regulatory agencies need an adequate understanding of the chemical's toxicology as well as fate of the substance and its effects on the environment. To date, data available for evaluation of the C6 fluorosurfactants is extremely limited. Critical evaluation of health and environmental impact of C6 fluorosurfactants requires a wide range of tests including characterization of the physical and chemical properties of the substance as well as ecotoxicology and acute toxicology testing. Here we present a collection of unbiased data generated by two C6 fluorosurfactants (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctane-1-thiol (CAS# 34451-26-8); sodium 2-methyl-2-((3-((3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)thio)propanoyl)amino)propane-1-sulfonate (CAS# 62880-93-7)). Data was generated by several different Contract Research Organizations that specialize in Toxicological, chemical, and environmental testing. All data was generated following GLP practices and OECD, European Commission (EC), and EPA OPPTS guidelines.

WP011 Generating In Vitro Toxicokinetic Data on Per and Polyfluoroalkyl Substances (PFAS) for use in In Vitro-In Vivo Extrapolation (IVIVE) Modeling

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Concern over exposure and potential health effects of per- and polyfluoroalkyl substances (PFAS) continues to increase as more is learned about their environmental persistence and human bioaccumulative potential. With estimates of unique PFAS currently exceeding 4600 structures, the limited toxicologic data currently available across a small subset is inadequate to provide an understanding of the potential exposures, toxicokinetics and toxicities across this diverse domain. New approach methodologies (NAMs) that use in vitro high-throughput screening (HTS) assays to assess potential effects require incorporation of in vitro toxicokinetic data to translate bioactive in vitro assay concentrations out to an administered dose equivalent necessary in chemical risk evaluations. To address this need, experimental in vitro measures of human plasma protein binding and hepatic clearance are being generated across a panel of PFAS for use in in vitro-in vivo extrapolation (IVIVE) models to predict systemic concentrations given a certain external exposure. Plasma protein binding (PPB) measures using ultracentrifugation showed very high binding rates (i.e., >99.5% bound) across over half of the chemicals assessed to date. The lowest percent binding was ~80% for two compounds, perfluorobutanoic acid and 4H-perfluorobutanoic acid. Overall, this trend toward high PPB rates is distinct from the distribution of values previously observed in studies assessing the commercial chemical space covered under Toxic Substances Control Act (TSCA). Hepatic clearance rates are being derived in cryopreserved primary human hepatocytes (mixed-donor pools) in a tiered fashion, using an initial high-throughput screen followed by a more comprehensive time course to quantitate intrinsic clearance (Clint) rates for those PFAS shown to be cleared. Interestingly, only four of the PFAS screened to date were shown to not undergo hepatic clearance. Follow-up time-course assessments are underway, as are efforts to generate additional TK data across a broader range of PFAS. Once the hepatic clearance and PPB data along with renal clearance estimates are incorporated into the IVIVE model, predicted internal concentrations can

then be combined with HTS bioactivity data to provide a risk context for a more informed assessment. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

WP012 PFAS in Victorian Waterfowl: An Investigation of the Presence of PFAS in 19 Wetlands in Victoria, Australia

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In 2018, EPA measured PFAS concentrations in waterfowl from 19 wetlands around the state of Victoria, Australia. This study aimed to identify potential risks to human health associated with the consumption of waterfowl from popular recreational hunting sites. A total of 166 waterfowl comprising of four species were harvested from the 19 wetlands for analysis. Chemical analysis was conducted on 41 composite samples (either breast or liver tissue within a species and wetland), as well as on water, sediment and soil samples from the wetlands. Thirteen common PFAS compounds were analysed in all sample types, including perfluoroalkyl sulfonic acids such as PFOS (perfluorooctane sulfonate) and PFHxS (perfluorohexane sulfonic acid) and perfluoroalkyl carboxylic acids such as perfluorooctanoic acid (PFOA). PFAS concentrations were compared with guidelines for human health and ecological protection, which were available for only three compounds, PFOA, PFOS and PFHxS. A human health risk assessment was conducted where concentrations exceeded guideline values. PFAS compounds were detected in 18 of the 19 wetlands. All sediment and soil concentrations were below the ecological guidelines and human health based investigation levels for soil. In waters and sediments, PFOS was the most frequent compound detected. Breast and liver samples contained variable concentrations and detection frequencies (FOD) of PFAS. PFOS (median 0.9 ug/kg, max 33 ug/kg, FOD 68%) and PFHxS (< 0.5 ug/kg, 21 ug/kg, 20%) were the most frequently detected in breast; PFOS (9.5 ug/kg, 340 ug/kg, 95%), perfluoro nonanoic acid (PFNA, < 0.5 ug/kg, 13 ug/kg, 49%), perfluorodecanoic acid (PFDA, < 0.5 ug/kg, 27 ug/kg, 41%) and PFHxS (0.5 ug/kg, 7 ug/kg, 39%) were the most commonly detected in liver. Concentrations of PFOS+PFHxS in waterfowl from 9 wetlands exceeded Food Standards Australia and New Zealand trigger points for investigation. A comparison of PFOS + PFHxS concentrations with a tolerable daily intake (TDI) based on consumption scenarios resulted in health advisories being issued for three wetlands. All the affected wetlands were close to urban centres or received runoff from a known PFAS contaminated site. The widespread dispersal ability of waterfowl, the proximity of some contaminated areas to other wetlands and unknown depuration rates poses further uncertainty about safe consumption advice.

WP013 Toxicokinetic Model of Laying Hen Biotransformation of PFAS Including PFAA-Precursors in Eggs

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Metabolization of perfluoroalkyl acid (PFAA) precursors has been revealed in vitro in rat hepatocytes and in vivo in e.g. Great Lakes herring gulls, a bird species. Here, we present a toxicokinetic model for the transfer of poly- and perfluoroalkyl substances (PFAS) including PFAA-precursors in laying hens and their eggs. The experiment was performed with 12 laying hens (Lohmann brown) fed a controlled PFAS contaminated diet for 25 days (exposure period) to quantify the PFAS transfer into edible tissues and eggs. After the exposure period, 4 hens were

slaughtered, while the other 8 were fed non-contaminated feed for 42 days (depuration period). After slaughter, blood, liver, kidneys and muscle tissue were analyzed. Eggs were sampled every 2nd day during the experimental period pooling whites and yolk separately. To also quantify the presence of unknown precursors, a Total Oxidizable Precursor (TOP) assay was applied to yolk and feed. Targeted analysis was performed for several PFAAs as well as other PFAS (known precursors) in animal tissues and eggs. Up to 8 PFAAs of different chain lengths (C4-C8) were detected in feed and egg yolk, with only minor amounts in egg white. At the end of this study, PFOS and PFHxS were still measurable in yolk, indicating that 42 days of elimination were insufficient for complete elimination in eggs. Table 1: The central column shows the proportion of PFAAs excreted with egg yolk to the PFAAs ingested with the feed. For some substances, apparently more than 100% is excreted, which has led us to postulate the hypothesis that the animal feed contained not only PFAAs but PFAA-precursors. The 3rd column shows apparent yolk excretion half-lives, very short and uniform across substances compared to other species. Subst. / Apparent feed-yolk transfer rate / Apparent half-life PFHpS >81% 5.2d PFOS 182% 4.5d PFHxS 111% 7.8d PFOA 39% 4.6d PFNA >21% N/APFBS 4.8% 1.7d PFHxA 2.6% N/APFHpA 1.7% N/A The analytical results before and after the TOP assay (sample precursor balance) will be used as mathematical lower and upper bounds to calculate the biological precursor balance, which will in turn be used to fit a toxicokinetic model. The model can be used to predict PFAS transfer from feed and animal drinking water into eggs of laying hens in the context of consumer risk assessment. In an accompanying poster, details on the TOP and targeted analytics will be presented.

WP014 Statewide Screening Study of Perfluorinated Compounds in New Jersey Fish, Surface Water, and Sediment

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Some per- and polyfluoroalkyl substances (PFAS) bioaccumulate in fish tissue, and consumption of PFAS-contaminated fish can result in human health risks. The NJ Dept. of Environmental Protection conducted a statewide screening survey of 13 PFAS, all perfluorinated, in fish tissue (n=94), surface water (n=14), and sediments (n=14) at 11 waterways across NJ. Sites were selected for their proximity to potential PFAS sources and likelihood of recreational and fishing activity. At least 4 PFAS were detected in all surface water samples, with the highest total concentration in a lake near a military installation where aqueous film forming foam was used. Consistent with the known greater partitioning of longer chain PFAS to sediment and shorter chain PFAS to the water column, only PFAS with a carbon chain length of ≤ 9 were detected in surface water. Between one and eight PFAS, with carbon chain length of ≥ 6 , were detected in 10 of the 14 sediment samples. Sediment concentrations were highest in the lake with the highest PFAS in surface water; 88% of total sediment PFAS in this lake was perfluorooctane sulfonate (PFOS). At Echo Lake, often used as a NJ "background" site, no PFAS were detected in the sediment. One to three individual fish from two to four species were collected and analyzed at each site. The average detectable tissue concentrations (non-detects were not included in the averaging of the tissue concentrations) showed that all species at all sites were impacted by one or more PFAS compounds. These contaminants bind to proteins in blood, liver and muscle tissue; they do not bioaccumulate in fatty tissue like other persistent organic pollutants frequently found in fish (e.g. PCBs, dioxins). In general, PFAS levels in fish were higher at sites with identified PFAS sources and detectable PFAS levels in surface water and sediment. Fish consumption triggers for perfluorononanoic acid (PFNA), perfluorooctanoic acid (PFOA) and PFOS were developed based on NJ Reference Doses for these compounds, and an assumed 227 g (8 oz.) meal size and 70 kg body weight. Fish consumption advisories for PFOS ranging from "once per week" to "once per year" were issued for the general population for one to three species at most study sites. For the high risk population

(infants, children, pregnant and nursing women, women of childbearing age), “do not eat” advisories were issued for some species at some sites. A second study of additional NJ sites is ongoing.

WP015 Monitoring PFAS in Aquatic Environment in Norway

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NIVA is a major provider in Norway of analyses of PFAS in aquatic environment in Norway. The first samples were analysed in 1993 but were analysed on a regular basis since 2004. The number of PFAS analysed has increased and currently >100 different compounds are monitored on a regular basis in the aquatic environment. We will present data from monitoring of the Norwegian coast, large lakes, urban fjords, terrestrial city environments, pristine rivers and polluted rivers, in addition to aquatic environments nearby an industrial site with known release of PFAS to water. PFAS were analysed by LC/MS-MS, and since 2014 a high resolution TOF instrument was employed, enabling a suspect screening of previously analysed samples. The number of analytes during the years was >60 000, constituting roughly 3 000 individual samples. The matrices analysed were in most cases biota (>80%), but also sediment (13%), water (3%), particles, soil, sludge and waste water have been analysed. The biota was in most cases fish (liver and/or filet), but also birds (blood or eggs). To a smaller extent, aquatic organisms such as blue mussel, zooplankton and worms were also analysed. Using the categorisation of OECD, most groups of PFAS analysed were acids, but also sulfates, prePFOS and telomers were included in the analytical repertoire. The following results could be made on the basis of samples with one or more positive detection of PFAS: The detection frequency of PFOS was 90%, PFOSA 60% and acids of chain length 10-14 20-45%. The mean and median of number of PFAS detected in samples were 4.3 and 3 respectively. EU’s Water Framework Directive has a regulatory limit (environmental quality standard, EQS) of 9.1 ng/g in biota samples for PFOS. The EQS was exceeded in many fish and birds, but only in a few instances in aquatic organisms. In fish, the PFOS levels were highest in the lake which had a known pollution of PFAS, but also one of the main rivers of Oslo city, with no known point sources of PFAS, had quite high levels in fish liver. The concentration of PFOS in liver reached >600 ng/g, while levels in filet reached 40 ng/g. Even though the frequency of exceedance of the EQS were lower in monitoring programs targeting reference and non-polluted environments, all had examples of fish exceeding the EQS, even in the supposedly pristine rivers.

WP016 New Jersey Drinking Water Maximum Contaminant Levels for PFOA, PFOS, and PFNA

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Long-chain perfluoroalkyl acids (PFAAs) such as perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorononanoic acid (PFNA) are of concern in drinking water because of their widespread occurrence, extreme environmental persistence, long human half-lives, multiple toxicological effects, and associations of low exposures with human health effects. Unlike other well-known persistent, bioaccumulative, and toxic (PBT) contaminants (e.g. dioxins and PCBs), drinking water is an important exposure route for these PFAAs; low levels in drinking water can overwhelm exposures from other sources (e.g. food, consumer products) prevalent in the general population. The New Jersey Department of Environmental Protection has adopted a drinking water standard (Maximum Contaminant Level, MCL) for PFNA of 13 ng/L, and it has proposed MCLs for PFOA of 14 ng/L and PFOS of 13 ng/L. In the national USEPA Unregulated Contaminant Monitoring Rule 3 study, these three PFAAs occurred more frequently in NJ public water systems than in the U.S. as a whole. The quantitative basis of the risk assessments (i.e. Health-based MCLs) is animal toxicology data, with associations of human health effects with low exposures providing support for a protective approach. Both non-cancer and cancer effects were evaluated, and a

detailed mode of action analysis was performed to evaluate human relevance of toxicological effects in animals. Interspecies comparisons were based on internal dose (blood serum PFAA levels) to account for the much longer half-lives of these PFAAs in humans versus laboratory animals. For PFOA, a stringent Health-based MCL of 0.77 ng/L for delayed mammary gland (MG) development in mice was considered to be scientifically valid but was not proposed, primarily because of lack of precedent for use of this effect as the primary basis for risk assessment or health-based standards. The Health-based MCL of 14 ng/L for PFOA for increased liver weight in mice incorporates a database uncertainty factor of 10 for more sensitive effects such as delayed MG development. The Health-based MCL for PFNA is also based on increased liver weight in mice, and the Health-based MCL for PFOS is based on decreased immune response in mice. Analytical considerations (Practical Quantitation Levels) and availability of treatment removal technologies did not limit achievement of the Health-based MCLs. Therefore, the MCLs are identical to the Health-based MCLs.

WP017 Framework for Human Health Risk Assessment of Federal Sites in Canada Impacted with Per- and Polyfluoroalkylated Substances

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Background and Challenges: Federal contaminated sites in Canada are located on land owned or leased by the federal government, or on land where the federal government has accepted responsibility for the contamination. Health Canada is an Expert Support Department that provides scientific advice on human health risks associated with contamination on federal sites. In recent years, per- and polyfluoroalkylated substances (PFAS) have been the focus of much public attention. A common source of PFAS contamination at federal contaminated sites in Canada is past and/or current use of aqueous film forming foams associated with fire-fighting and/or training activities. To help navigate the evolving science on PFAS, Health Canada has developed a framework to support federal departments in their assessment of PFAS contamination and evaluation of associated human health risks. Approach: As the science and our understanding of PFAS, and associated class of substances is growing rapidly, Health Canada’s Contaminated Sites Division has developed a framework to help guide the undertaking of human health risk assessments (HHRAs) at Canadian federal sites impacted with PFAS. The poster will outline Health Canada’s recommended HHRA methodology, and will provide key aspects of the framework such as: site characterization including off-site considerations, contaminant identification including consideration of precursors like fluorotelomer sulfonates that degrade into terminal PFAS products, identification of people that may be exposed and how they may be exposed, and assessment of toxicity and risk. The poster will also include a summary of Health Canada’s most up to date human health soil screening values, and drinking water guidelines and screening values for various PFAS.

WP018 An Approach for Developing Risk-Based Screening Criteria for Consumption of Food Crops Impacted by Poly and Perfluoroalkyl Substances

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Poly and perfluoroalkyl substances (PFAS) have been identified as chemicals of concern in agricultural soils, biosolids-amended soil, and irrigation water due to their toxicity, persistence, bioaccumulation potential and occurrence in these media. The agricultural use of PFAS-contaminated water, municipal and industrial biosolids, and municipal compost can all contribute to the exposure of food crops to PFAS. Irrigation water containing variable levels of PFASs may contaminate soil over time resulting

in additional exposure pathways to food crops. The uptake and accumulation of PFAS in food crops is an important and continuing concern for protecting human health. There is an urgent need for regulatory agencies to assess the accumulation of PFAS in the edible portion of food crops as consumers often eat these foods fresh or with minimal processing. Recognition of PFAS in food as a potentially important contributor to human exposure, as well as the identification of PFAS-impacted irrigation water and soils in areas with agricultural activities, has resulted in several studies on the uptake of PFAS into crops. Using available crop-specific transfer factors (TFs) from lettuce bioaccumulation studies, exposure data, and consumption rates for homegrown produce, exposure intakes are estimated for a range of concentrations for different population subgroups using Monte Carlo Simulation in a tiered stochastic modeling approach. For PFASs with oral toxicity reference doses, risk-based concentrations (RBCs) for selected PFAS in contaminated soil and irrigation water were determined for adults and children. The RBCs are compared to available health advisories or criteria and the influence of other exposure pathways is evaluated. Lastly, we explore the utility and challenges of implementing a relative potency factor approach to assess risk from a mixture of PFAS. Prediction outcomes from a hypothetical farm illustrate the model application to a real-world scenario.

WP019 Mixture Exposure to PFAS: A Relative Potency Factor Approach

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PFAS often occur together as contamination in soil, groundwater or drinking water. To assess the risks of the mixture of PFAS, the RIVM has investigated the possibility to express the potency of a number of PFAS as compared to PFOA. It was concluded that this can be done by using so-called Relative Potency Factors (RPFs). The use of the RPF method has an important condition, namely that a (minimal) set of comparable toxicity data for individual PFAS is available. For the relevant health effect (on the liver of laboratory animals), such information was available for 13 perfluoroalkyl acids. Dose-response analysis was applied to derive the relative potencies of three perfluoroalkyl sulfonic acids (PFBS, PFHxS, PFOS), nine perfluoroalkyl carboxylic acids (PFBA, PFHxA, PFOA, PFNA, PFUA, PFDoA, PFTeDA, PFHxDA, PFODa) and one perfluoroalkyl ether carboxylic acid (GenX) compared to PFOA. In addition, the RPFs of seven other perfluoroalkyl acids could be estimated based on their carbon-chain length. In general, it is concluded that perfluoroalkyl acids with 7 to 12 perfluorinated carbon atoms are equally potent to, or more potent than PFOA. Shorter and longer chain perfluoroalkyl acids are less potent. Here the exposure to a PFAS mixture is expressed in PFOA equivalents. Measured PFAS quantities are expressed in PFOA units, so that they can be compared with established external PFOA limits. This method can be applied when evaluating human exposure to PFASs from various matrices, e.g., contaminated soil, groundwater or drinking water.

WP020 Estimates and Perspectives on Risk of PFOS to Aquatic and Terrestrial Receptors

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Per- and polyfluoroalkyl substances (PFAS) have been detected in a wide variety of terrestrial and aquatic environments. While the paucity of data on PFAS toxic effects remains an issue, in general, data suggest

that perfluorooctane sulfonate (PFOS) is the majority contributor to exposure and that effects of PFOS exposure is relatively well described in both aquatic and terrestrial systems. Specifically, species sensitivity distributions have been used to estimate an HC5 for aquatic systems and bioaccumulation/bioconcentration models have been used to estimate uptake in avian systems which can be compared to a toxicity reference value (TRV). Using data from USAF installations, we were able to demonstrate that exposure and effects are relatively likely but the magnitude was organism- and location-specific. In aquatic systems, minimum rate of site-specific exceedance of HC5 was 26% and the maximum site-specific exceedance rate of lower confidence level (LCL) of the HC5 was 65%. These two scenarios represent extremes (low risk and high risk, respectively) but importantly, demonstrate that many aquatic sites within USAF installations may exceed existing toxicity thresholds. Further yet, site-specific data within installations showed that many sites can be either "hot-spots" or potential refugia, indicating that PFOS risk is location-specific in aquatic systems. In terrestrial systems, we used two example taxa with differing life histories--American woodcock (AMWO) and northern bobwhite (NOBO); a transient insectivore and a sedentary granivore, respectively. Time-on-site and life history have large effects on TRV exceedance--AMWO were in exceedance in greater than 5% of sites at any level above 1% time-on-site while NOBO were in exceedance above 5% of sites only when time-on-site was greater than 32%. Additionally, NOBO only reached a maximum exceedance rate of ~10% with 100% time-on-site. Clearly, while time-on-site matters, diet and PFAS-specific BSAF/BCF of diet items was the majority contributor to overall exceedance patterns. In summary, it appears that aquatic and terrestrial receptors on USAF installations may potentially be exposed to levels of PFOS that exceed existing benchmarks but risk clearly depends on their life-history and their specific location.

WP021 Guidance for Assessing the Ecological Risks of Threatened and Endangered Species at Aqueous Film Forming Foam (AFFF)-Impacted Sites

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Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) have been widely used in numerous applications since the 1950s, including aqueous film forming foams (AFFFs) used for fire suppression at airports, firefighting training facilities, and other industrial locations. PFASs are routinely-detected in a wide variety of environmental media impacted by AFFF, and have prompted regulatory focus on exposures and risks. Many AFFF sites host ecological habitat or, due to the offsite transport potential for PFASs, PFAS-impacted AFFF sites may affect nearby and downgradient habitats. As part of a Strategic Environmental Research and Development Program project, a guidance document was developed to provide a current state-of-the-practice overview of methods, practices, and key data gaps for assessing the potential for risks from exposure to PFAS for threatened and endangered (T&E) species at AFFF-impacted sites. The guidance is intended to provide clear guidance to quantitatively evaluate ecological risks and enable site managers to make defensible, risk-based management decisions using the best available information and approaches. The key objectives are: 1) to provide a framework for the evaluation of T&E species during ecological risk assessments (ERAs) at AFFF-impacted sites; 2) to provide the reader with an understanding of the specific T&E species or general feeding guilds typically expected to be considered most at risk at AFFF-impacted DoD sites; 3) to provide the reader with recommended parameters (exposure factors, toxicity reference values [TRVs], uptake factors) to perform a food web model-based ERA for wildlife T&E species; and 5) to provide the reader with an understanding of key data gaps and uncertainties when evaluating T&E species at AFFF-impacted sites. Several hundred toxicity values and over 1000 bioaccumulation parameters were assessed in the guidance review effort, resulting in 150 recommended values for use in ERAs. In addition to providing a review

of the available literature and recommendations for ERA parameters, the guidance reaches the following key conclusions: 1) ERAs for AFFF sites are feasible; 2) off-site habitats are most at risk; 3) aquatic habitats are critical to address; 4) terrestrial habitats may be important at some sites; 5) risks from mixtures is uncertain; and 6) the exposures and effects of many PFAS-constituents in AFFF are unknown.

WP022 Approaches for Perfluoroalkyl Acid Grouping and Assessment of Mixture Toxicity

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Per- and polyfluoroalkyl substances (PFAS) are often found in the environment as complex mixtures. Given that perfluoroalkyl acids (PFAAs) are a subclass of PFAS that may exist due to direct manufacture and use or from degradation of “precursors,” this subclass is a useful starting place for addressing complex mixtures and potential additivity of effects. PFAAs can exhibit a wide range of properties and approaches to evaluate the uncertainty in PFAA mixture toxicity are needed. Based on well-established toxicology principals used for other complex environmental mixtures, such as dioxin- like compounds, it is reasonable to expect that carbon chain length and functional groups may continue to serve as indicators of relative potency, critical effect endpoints, and bioaccumulation potential. For example, perfluorooctanoic acid (PFOA; C8) and perfluorohexanoic acid (PFHxA; C6) differ in chain length by two carbons; however, human elimination half-lives (2.3-years (PFOA) vs. 32-days (PFHxA)), chronic oral toxicity values (0.00002 (PFOA) vs. 0.25 (PFHxA) mg/kg-day), and critical effects (development (PFOA) vs. kidney (PFHxA)) of these PFAAs differ considerably. Thus, a crucial first step in assessing PFAA mixture toxicity is to develop a classification scheme for evaluating discrete subgroups of PFAAs that can each be assessed for toxicity individually, and science-based criteria by which to evaluate mixtures quantitatively. Using available data, we propose four PFAA subgroups that can be used to assess PFAA mixture toxicity: 1) long-chain perfluorocarboxylic acids (PFCAs) (>=8C in length); 2) short-chain PFCAs (=6C in length); and 4) short-chain PFSAs (

WP023 Human Health Risk Assessment of Per- and Polyfluoroalkyl Substances (PFAS) Using a Toxic Equivalency Factor (TEF) Approach for a Site in New Jersey

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Most exposures to PFAS in the environment involve concurrent exposure to a mixture of multiple contaminants. Currently, the New Jersey Department of Environmental Protection (NJDEP) has developed risk-based standards for only three PFAS analytes: Perfluorononanoic acid (PFNA), Perfluorooctanoic acid (PFOA), and Perfluorooctanesulfonic Acid (PFOS). Some regulatory agencies have proposed the use of a scaling factor, based on liver toxicity, to characterize the non-cancer health hazards associated with PFAS exposure to a larger group of PFAS analytes. To understand the potential implications of enacting such a policy in the future in New Jersey, Langan completed a human health risk assessment to study PFAS contamination in groundwater using PFOA as the index chemical. Groundwater samples were collected from nine groundwater monitoring wells to conduct a baseline characterization of water quality. The samples were analyzed by a modified United States Environmental Protection Agency (EPA) method 537M with isotope dilution for 23 PFAS analytes. The associated reporting limits (RL) were approximately 4 nanograms per liter. Twenty individual PFAS analytes were detected above the RL in groundwater samples. Initially, a comparison of analytical data to the NJDEP groundwater quality standards resulted in the identification of PFOS and PFOA as contaminants of potential concern (COPC). A screening-level risk assessment was performed to derive a hazard index (HI) for the 3 analytes with published, risk-based standards. Groundwater concentrations for individual PFAS were then converted to PFOA equivalents by adjusting each PFAS chemical concentration by its specific TEF, selected from a hierarchy of published sources. The resulting index chemical equivalent concentrations were

summed to estimate the PFOA toxic equivalence and subsequently used to derive a HI associated with exposure to the larger chemical mixture. The resulting non-cancer HI was significantly greater than the screening-level HI calculated using NJDEP standards. In addition, three additional risk-driving COPCs (Perfluorodecanoic Acid, Perfluorohexane Sulfonate, and Perfluorononanoic Acid) were identified. The results of this study indicate that the absence of criteria or standards for PFAS analytes without promulgated reference doses for liver toxicity will likely underestimate the potential health effects associated with exposure to the mixture.

WP024 Developing an Approach for Assessing Per- and Polyfluoroalkyl Substances Risk to Threatened and Endangered Species

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Contamination of soils and waters by per- and polyfluoroalkyl substances (PFAS) is a widespread problem, including at Department of Defense (DoD) installations where use of aqueous film forming foam (AFFF) was common. Evaluating chemical risks to threatened and endangered (T&E) species associated with AFFF use is a required component for Superfund ecological risk assessments; however, limited information exists on the uptake and toxicity of PFAS to these receptors. The goal of this project is to develop a risk-based approach and tools (i.e., risk-based screening levels [RBSLs]) for assessing potential PFAS risk to T&E species, considering the known fate and transport of these chemicals and the receptors most likely to be impacted by AFFF use at military installations. RBSLs for several commonly encountered PFAS were developed for wildlife receptors (birds and mammals), aquatic life (fish and aquatic invertebrates), terrestrial invertebrates, and plants. To develop RBSLs for wildlife, representative T&E species and surrogate receptors were selected to represent different habitat types, feeding guilds, and trophic levels. Exposure factors were compiled from existing literature. High-quality uptake and toxicity data from the literature were combined with the exposure assumptions to derive RBSLs for terrestrial and aquatic birds and mammals for several PFASs, including PFOA, PFAS, PFHxS, PFHxA, and PFBS. For PFAS lacking adequate toxicity and uptake data to develop a PFAS-specific RBSL, RBSLs based on surrogate PFAS or classes of PFAS were considered. RBSLs for aquatic life were developed following the Great Lakes Initiative (GLI) methodology. Published literature reviews and peer-reviewed aquatic toxicity studies were employed in the GLI methodology to develop aquatic life criteria for 23 PFAS. Development of RBSLs allow for a strategic approach for addressing potential risk to T&E species that could be applied to all DoD facilities and will result in significant efficiencies and cost savings over the course of time.

WP025 Investigating Potential Risk to Threatened and Endangered Species from PFAS on Department of Defense Sites

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Per- and polyfluoroalkyl substances (PFAS) are emerging contaminants of concern on many DoD sites. As such, there is growing interest regarding the potential effects of PFAS to public and environmental health, as well as concern from the DoD regarding liability under federal statutes such as the Endangered Species Act (ESA). Section 7(a)(2) of the ESA states “each federal agency shall, in consultation with the Secretary, insure that any action authorized, funded, or carried out by such agency is not likely to jeopardize the continued existence of threatened and endangered species or destroy or result in the destruction or adverse modification of habitat of such species which is determined by the Secretary...” Given the lack of information concerning the potential risk of PFAS to T&E species on DoD lands, we have developed a framework to conduct screening-level risk assessment for listed species on installations. Using

Joint Base McGuire-Dix-Lakehurst as a model installation, percent of PFAS impacted habitat per species was estimated using spatial analysis by overlaying the areas of impacted soil/surface water with species-specific habitat (landscape layer(s)). Additionally, to determine if there are specific taxa at risk based on dietary preferences we overlaid the extrapolated PFAS GIS layer with taxa-diet landscape layers and found that insectivorous avian and amphibian species may have the greatest exposure to contaminated habitat compared to other avian and reptilian taxa with different dietary preferences. Listed species that are obligate aquatic organisms or have a biphasic lifecycle tend to have greater proportions of their habitat impacted by PFAS as surface water is the terminal environmental resource. Currently, we have preliminary data for the screening-level risk assessment of the Northern Pine Snake (state listed in NJ) at JB MDL which indicates snakes may be exposed to 0.03 - 2.15 mg PFOS/kg/d. The working Toxicity Reference Value (TRV) for reptiles is 2.0 mg/kg/d based on a growth endpoint. Taken together, the high exposure estimate of 2.15 mg/kg/d at a former fire training area on the installation would likely pose a risk to the Pine Snakes.

WP026 Per and Polyfluoroalkyl Substances and Superfund

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Intentional and unintentional releases to environmental media during use, waste disposal and spills have led to PFAS concentrations in sites that may fall under Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly known as “Superfund”, authority. While PFAS are not currently listed as hazardous substances under CERCLA, analysis of soil, water, and other media at current and potential Superfund sites is increasingly identifying PFAS substances as potential contaminants of concern. PFAS have been detected at greater than 100 sites on the National Priorities List. Many, perhaps hundreds, of additional sites may be contaminated with these chemicals and may be eligible for remediation under CERCLA authority. Current tools for dealing with fluorochemicals at Superfund sites are limited. EPA is taking a number of actions to help increase our ability to manage potential risks posed by PFAS. These include setting groundwater screening and clean-up goals for PFOA and PFOS; evaluating designation of some PFAS as hazardous substances to clarify listing and cleanup decisions and facilitate cost recovery; developing and validating analytical methods and sampling methods for quantification in different environmental media; collecting information on the number, locations and characteristics of PFAS contaminated sites; and evaluating cleanup technologies and approaches. This presentation will give an overview of current EPA activities related to PFAS under Superfund including current and ongoing efforts at characterization, regulatory activities, and cleanup. The views expressed are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

WP027 Superfund Research Program PFAS Activities: Identifying Exposures, Hazards, and Solutions

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The NIEHS Superfund Research Program (SRP) funds university and small business multidisciplinary research on human health and environmental issues related to hazardous substances, including per- and poly- fluorinated alkyl substances (PFAS). The central goal of the SRP is to understand and break the link between chemical exposure and disease; hence, the SRP portfolio includes broad research areas to advance our understanding of PFAS exposures, hazards, and potential solutions. This presentation will give an overview of currently funded PFAS research, as well as some of their preliminary findings. The University of Rhode Island has initiated “Sources, Transport, Exposure and Effects of PFASs (STEEP),” a Center entirely focused on PFAS. Their projects and cores are assessing the impact of PFAS exposures on immune dysfunction and metabolic abnormalities; tracing unique PFAS chemical fingerprints at a contaminated groundwater site; developing and validating novel passive

sampling tools to measure time weighted average concentrations for PFAS and their volatile precursors; and engaging communities on ways to effectively reduce human exposure to PFAS. Several SRP Centers, including STEEP, work closely with Federal and State officials to translate scientifically defensible findings to guide best practices for PFAS monitoring and management—including several outreach efforts within regions impacted by PFAS. For example, the Brown University Superfund Research Center has developed Geographical Information Systems (GIS)-based databases for identifying municipalities at risk for PFAS exposure based on past land use data. The SRP also supports novel, cost-effective remediation strategies for water and soil. Research at the University of Arizona develops groundwater modeling tools to predict how PFAS move in the subsurface, helping to understand where to target remediation approaches. University of California-Berkeley, Michigan State University, and Texas A&M University Centers are developing strategies to remediate PFAS via biological and chemical treatment options. In addition, several small business grants are validating novel extraction, removal, and destruction technologies for PFAS. Collectively, these ongoing activities will improve our understanding of the impact of these emerging contaminants, which will lead to lower environmental cleanup costs, reduced risk of exposure, and improvements in human health.

WP028 A Framework for Assessing Bioaccumulation and Exposure Risks of Per- and Polyfluoroalkyl Substances in Threatened and Endangered Species

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For several decades, the U.S. Department of Defense (DoD) widely used aqueous film forming foams (AFFF) formulations for training and operations related to fire suppression. These AFFF formulations contained relative high quantities of perfluorooctane sulfonate (PFOS), as well a range of other of per- and poly-fluoroalkyl substances (PFASs). Currently, there is a critical need to assess PFAS bioaccumulation and exposure risks in threatened and endangered (T&E) species at AFFF-impacted DoD sites. The objective of the present study is to develop a framework for conducting scientifically sound risk assessments for PFASs in T&E species at these sites. We propose that a robust, data-driven approach should be the foundation for a PFAS risk assessments involving T&E species. We propose utilizing a combination of field-based measurements and mechanistic bioaccumulation models to determine exposure (i.e., external and internal dose) in T&E species. Toxicity reference values (TRVs) are derived from conventional toxicity assay data for surrogate test species, as well as available in vitro assay data (e.g., ToxCast assays). We also propose utilizing a chemical activity-based approach for exposure and effects characterization. In this approach, chemical activity (a , unitless) in a given medium is related to the observed or predicted concentration (C , mol/m³) and the corresponding solubility (S , mol/m³) of the chemical in the studied media (i.e., $a = C/S$). We identified several DoD installations with known AFFF contamination for demonstration of the proposed PFAS risk assessment framework. Based on the U.S. Fish and Wildlife Service Endangered Species database, we identified the various T&E species listed in counties of the selected DoD installations. We applied the framework to assess PFAS exposure risks in several T&E species at these sites, including Bog Turtle (*Clemmys muhlenbergii*), Northern Long-Eared Bat (*Myotis septentrionalis*), Red Knot (*Calidris canutus rufa*), Red-cockaded Woodpecker (*Picoides borealis*) and Eastern Massasauga Rattlesnake (*Sistrurus catenatus*). While PFAS concentrations at these sites can greatly exceed water quality guideline values, estimated exposure values for T&E species were generally well-below TRVs. The study will help guide future research efforts and risk assessment initiatives regarding ecological risk assessment of legacy PFASs at AFFF-impacted sites.

WP029 Developing Ecological Screening Levels for PFAS in Soils and Surface Waters

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Argonne National Laboratory is developing PFAS ecological screening levels (Eco SLs) to support the Air Force Civil Engineer Center (AFCEC) with anticipated remediation efforts. Working in collaboration with the Tri-Services Environmental Risk Assessment Working Group, and U.S. EPA's Ecological Risk Assessment Forum, our objective is develop scientifically defensible Eco SLs following relevant guidance and using toxicity data from the published literature that meet specific study and data quality criteria. The richness of the published toxicity data will determine both the methods used to develop the Eco SLs as well as their usefulness in screening ecological risk assessments. AFCEC has nominally selected eight PFAS as an initial study set, composed of the perfluorinated C4, C6, C8, C9 and C10 linear carboxylates and the C4, C6, and C8 linear sulfonates. We are initially developing the Eco SLs for marine and fresh waters and for soil, with sediments potentially addressed later. Derivation of Eco SLs will consider a number of methods, described in EPA's 2005 Guidance for Developing Ecological Soil Screening Levels and 1985 Guidelines for Developing Numerical Water Quality Criteria, and the Army Public Health Center's 2000 Standard Practice for Wildlife Toxicity Values. These methods include NOAEL-LOAEL species summary plots, species-sensitivity distribution plots, benchmark dose development, as well as approximation approaches for deriving Toxicity Reference Values (TRVs). In terrestrial systems, the Eco SLs will be soil concentrations derived from TRVs, which are protective of exposed vegetation and wildlife. In aquatic systems, Eco SLs will be surface water concentrations protective of the most sensitive native aquatic genera, and of aquatic food chain-dependent birds and mammals.

WP030 Framework for Assessing Risks to Threatened and Endangered Aquatic Life at PFAS Impacted Sites

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The United States Department of Defense is one of the primary stewards of Threatened and Endangered (T&E) species on Federal lands. As part of a Strategic Environmental Research and Development Program project, guidance on the current methods for assessing risks to T&E aquatic life from exposure to per and polyfluoroalkyl substances (PFAS) was developed. To evaluate risks to aquatic T&E species, over 50 laboratory aquatic toxicity studies on PFAS were reviewed. To reflect a high level of conservatism for T&E species, no observed effect concentrations (NOEC) or 10% effective concentrations (EC10) were compiled for U.S. resident species and reviewed for inclusion in a Species Sensitivity Distribution (SSD) for PFOS and PFOA (no other PFAS met minimum data requirements for SSDs). A total of 82 NOECs for freshwater aquatic species and 14 NOECs for marine aquatic species were included in SSDs for PFOS and PFOA. One percent and five percent hazardous concentrations (HC1 and HC5) were calculated from the SSD following USEPA guidance. HC1 values for PFOS were 0.56 ug/L and 2.57 ug/L for freshwater and marine species, respectively. HC5 values for PFOS were 5.8 and 7.7 ug/L for freshwater and marine species, respectively. The HC1 and HC5 value for PFOA were 537 ug/L and 1,110 ug/L for freshwater species, respectively (data were insufficient for marine species). HC5 values are typically accepted as protective thresholds for aquatic life. Based on the inclusion of NOEC values as the basis for the SSD, the HC5 values reported here are expected to represent conservative thresholds for evaluating risks to aquatic T&E species and aquatic life.

WP031 Towards a Chemical-Safe Environment: Novel Tools for Assessing Environmental and Human Health Impacts of PFAS and Other Persistent and Mobile Compounds

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Highly persistent and mobile organic chemicals (PMOCs) are a class of widely-used contaminants, triggering emerging concern because of a lack of basic understanding of their environmental fate and effects and consequent societal impacts. Therefore, there is an urgent need to develop insight into the potential human and environmental risk of (mixtures of) these compounds. Products from the GenX technology and the additional >4,500 perfluorinated compounds (PFAS), but also several modern pesticides and industrial chemicals are considered as PMOCs. Innovative tools are needed for assessing their actual risk at existing cases of environmental pollution, like the emission of PFAS from GenX technology by Chemours in Dordrecht, Netherlands, and cases of contamination with PFAS on sites where fire-fighting foams are regularly applied (e.g. airports, fire brigade training centers). Current analytical techniques for detecting PMOCs are insufficient, methods for their removal from contaminated sites are lacking, and risk assessment of PMOCs is still underdeveloped. This clearly hampers adequate actions, for instance, in the disposal of contaminated soils and sediments. Because of the combination of high persistence and mobility, methods for predicting their environmental fate and their effects on human and environmental health from chemical properties are still lacking. For the short term, developing approaches for managing, mitigating and communicating the risks of PMOCs are urgently requested by stakeholders like drinking water companies, water authorities and soil managers. For the long term, possibilities to develop suitable but safe alternatives (safe-by-design) need to be explored. These considerations triggered us to start working on an ambitious research proposal that should find solutions for many of these issues. Our research proposal aims at generating the required fundamental understanding of the factors determining the fate and effects of PMOCs, and developing novel tools for assessing their environmental impacts and for remediating contaminated media. The newly developed tools and modeling approaches will be validated using case studies and demonstrator labs on PFAS. The tools will be used to create a framework for practical applications for policy makers and environmental managers. The proposed project will also explicitly address risk communication and risk perception issues related to PMOC exposure in general and PFAS in particular.

WP032 Guidelines for PFOS and PFOA: Scientific Learnings Using New Methodologies in Australia and New Zealand

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PFOS and PFOA present challenges for risk assessment and derivation of aquatic ecosystem guidelines. Standard duration ecotoxicity tests and endpoints of ecological relevance (growth, survival and reproduction) may not be sufficient to measure the range of effects of these persistent, bioaccumulative and biomagnifying toxicants. A substantial volume of scientific literature for PFOS indicate effects at very low concentrations in long-term, partial-, full- and multi-life cycle studies. Many of the measured effects include developmental, reproductive and, notably, endocrine-based effects. Some of these effects may be due to bioaccumulation and maternal transfer of PFOS. For PFOA, there are fewer of these types of studies, however, the studies available indicate similar types of effects at similar low concentrations. In addition, dietary uptake of PFOS and PFOA is an important pathway of exposure for aquatic organisms that may not be included in standard ecotoxicity tests. Consideration of these effects in guideline development is necessary to ensure adequate species protection. In Australia and New Zealand, the preferred method

for deriving water quality guidelines is to use species sensitivity distributions, which result in guidelines based on percent species protection levels (e.g., 90%, 95%, 99%). For compounds such as PFOS and PFOA which bioaccumulate and biomagnify, an increased percent species protection is required. This approach has no biological basis, but is made as a precaution against potential effects due to bioaccumulation which standard ecotoxicity tests do not typically measure. Under the Australian method, permissible endpoints require demonstrated ecological relevance. Consequently, for toxicants such as PFOS and PFOA, many of the development and reproductive effect endpoints in long-term studies are not permissible, which may result in under-protection. The following is suggested for PFAS aquatic ecosystems guideline development: 1) inclusions of dietary exposure studies; 2) long-term partial-, full-, and multi-life cycle studies; measures of bioaccumulation in long-term ecotoxicity tests; and expansion of acceptable endpoints to include reproductive, developmental and endocrine effects.

WP033 Developing Hazard Rating Calculation Methodologies for Per- and Poly-fluoroalkyl Substances (PFAS)

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Historical and present-day use of per- and poly-fluoroalkyl substances (PFAS), particularly at sites such as military bases and airports, has resulted in environmental and human health impacts that prove challenging to address. Therefore, prioritization of PFAS based on observed or predicted toxicological and environmental fate is critical in the development of effective risk management practices. Hazard rating calculations are used for assessing the threat risk posed by current and future land use activities involving PFAS by providing a method for determining qualitative hazard rankings of PFAS from a range of quantitative data. Historically, hazard ratings have been calculated for other contaminants of concern, but less so for emerging contaminants such as PFAS. Hazard rating calculation methodologies were thus developed for various PFAS at Department of National Defense (DND) bases across Canada. The purpose of developing the hazard rating calculations was to assess the threat risk posed to activities involving PFAS, including firefighting training areas (FFTAs), storage and handling of PFAS-containing chemicals, and application of PFAS-containing compounds during emergency situations. The methodology is based largely on risk and hazard calculations derived by agencies (e.g., Ontario Ministry of Environment, Conservation and Parks), providing assurance of regulatory acceptance. In total, nine PFAS were evaluated based on prevalence or use in aqueous film-forming foams (AFFF), and the availability of reference toxicity data. The hazard rating was based on four specific factors: toxicity (T), environmental fate (F), deleterious quantity (Q), and release impact modifier (RIM). Each factor was given a score between 0 and 10, depending on its significance. Perfluorooctanoate (PFOA) and perfluoropentanoate (PFPeA) received the strongest hazard ratings with values between 8 and 9 for the various uses. Development of a hazard rating system for PFAS is beneficial for risk management of these substances. One particular benefit of this hazard rating system is the ability to explore the prioritization of short-chain PFAS to help categorize their toxicity, bioaccumulation, and/or persistence compared to their longer-chain counterparts. Overall, this hazard rating framework can be integrated into future regulatory framework and contaminant prioritization.

WP034 Predicting Ecotoxicity of Per- and Polyfluoroalkyl Substances (PFAS): Using In Vitro and In Silico Information

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Available ecotoxicity data for per- and poly-fluoroalkyl substances (PFAS) are generally limited to a few specific compounds, including perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorobutanesulfonate (PFBS). Studies on developmental, reproductive, and other effects using laboratory mammals and birds have also

focused on few key compounds. Ecotoxicity benchmarks for wide ranging PFAS are critical in assessing ecological risk attributable to mixtures of these emerging contaminants. An approach was developed to determine relative ecotoxicity of PFAS by coupling in silico approaches and in vitro data. Relatively well-established ecotoxicity benchmarks for key compounds were then extrapolated for a range of ecologically relevant PFAS. Protein-based bioaccumulation of PFAS represents intra-cellular uptake and bioavailability. In silico approaches using quantitative structure activity relationships (QSARs) have been developed for predicting bioaccumulation potential for wide ranging PFAS. A range of PFAS has also been tested in vitro for their relative binding affinity (RBA) to sub-cellular receptors for mechanistic understanding of certain health effects. Examples of the latter include competitive binding of PFAS and thyroxine (T4, the transport form of thyroid hormone) to the human thyroid hormone transport protein transthyretin (TTR, e.g., transthyretin) and PFAS and 17 β -estradiol (E2) to the estrogen receptor (ER, e.g., isolated from rainbow trout). These RBAs are expected to reasonably represent the inherent toxicity of individual PFAS, via the tested sub-cellular receptor-associated pathways. Bioavailability (based on bioaccumulation) and toxicity (based on RBAs) were coupled to establish relative ecotoxicity for a range of PFAS; relatively well-established ecotoxicity benchmarks for key PFAS were then extrapolated for the remaining PFAS. The approach and the results will be compared to existing approaches and methods for evaluating mixtures, including but not limited to endpoint-specific ecotoxicity data and toxicity equivalency or relative potency factors (TEFs or RPFs). Overall utility of the resulting benchmarks and associated uncertainties will be evaluated for various applications, including prioritization of PFAS for toxicity testing and ecological risk assessments with particular focus on birds and mammals.

WP035 The Ecological Risk and Transport of Poly- and Perfluoroalkyl Substances Throughout a Trophic System of the Tidal Freshwater Potomac River

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Per- and polyfluoroalkyl substances (PFAS), are thought to be ubiquitous in aquatic environments in spatial proximity to nonpoint and point sources such as storm water runoff and wastewater treatment plants. The tidal freshwater Potomac River, a major tributary of the Chesapeake Bay, is serviced by several treatment plants including Alexandria Renew Enterprises (Alexandria, Virginia) and Noman M. Cole, Jr. (Lorton, Virginia). Trophic interactions can provide insight to the potential sinks and transport pathways of degradation-resistant contaminants once they have entered an aquatic ecosystem. Previous isotopic analyses identified established trophic connections of a common freshwater fish species, banded killifish (*Fundulus diaphanus*), at the target embayments. The current study examines the presence of at least 25 PFAS in surface water, sediment, zooplankton, benthic invertebrates, submersed aquatic vegetation (SAV), and *F. diaphanus*. The preferred habitat of *F. diaphanus*, littoral SAV beds, are conducive to slowed water flow and particulate deposition. These deposition zones are hypothesized to have increased instances of PFAS bioaccumulation and probable ecological risk. Extracts will be examined using a triple quadrupole liquid chromatograph mass spectrometer. Hazard quotients will be determined by calculating the predicted no effect concentration for each sample type.

Treatment and Remediation

WP036 PFAS and Waste – What to Do with It Now?

H. Behzadi, SGS

PFAS are a class of synthetic fluorinated chemicals used in many industrial and consumer products, including defense-related applications. They are persistent - found at low levels in the environment - and

bio-accumulate. Landfills are the final resting place for many products containing per- and polyfluoroalkyl substances (PFAS) and their presence have been reported in landfill leachates. The EPA is concerned, under current management practices, of the potential for eventual PFAS discharge to the broader environment through landfill leachates. Therefore, landfilling of PFAS-impacted solid wastes is not currently considered to be a suitable waste management solution. Thermal treatment and incineration are considered another option for PFAS impacted waste, however, this technology has its own drawbacks and issues. In this presentation, we discuss environmental issues that arise using both these technologies. In addition, we present two case studies. The first examines the environmental impact of PFAS in the carpet industry. Once released to the environment during product manufacture, use, or disposal, PFAS becomes part of a virtually closed cycle leading to chronic, lifelong human and ecological exposures. In the other case study, we discuss the release of PFAS to the environment by wastewater treatment plants (WWTP). WWTPs have been recognized as a significant source of PFAS release into the environment and for their accumulation into biosolids, particularly when industrial water releases are processed. In addition to removing nutrients and pathogens, many wastewater treatment plant processes often result in destruction of hazardous substances. However, perfluorinated compounds are resistant to biodegradation. As a result, potential of their release to the environment are significant due to wastewater discharge and biosolids application to the farm industry.

WP037 Waste Incineration of Polytetrafluoroethylene (PTFE) to Evaluate Potential Formation of Per- and Polyfluorinated Substances (PFAS) in Flue Gas

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In recent years, concerns over some per- and polyfluorinated alkyl substances (PFAS) have grown steadily. PFAS are a large group of chemical substances with widely differing properties. While one class of PFAS, fluoropolymers, have been demonstrated to meet the OECD criteria for polymers of low concern during the in use phase of their lifecycle, questions remain regarding waste handling at the end of useful life for products containing fluoropolymers. To show that polytetrafluoroethylene (PTFE) can be almost fully transformed into fluorine (F) (as hydrofluoric acid (HF)) and to study the possible generation of low molecular weight per- and polyfluorinated alkyl substances (PFAS), PTFE combustion under typical waste incineration conditions at the BRENDA (German acronym for “Brennkammer mit Dampfkessel”) pilot plant at Karlsruhe Institute of Technology (KIT) was investigated. Results indicate that, within procedural quantitation limits, no statistically significant evidence was found that the PFAS studied were created during the incineration of PTFE. Therefore, municipal incineration of PTFE using best available waste treatment technologies (BAT) is not a significant source of the studied PFAS and should be considered an acceptable form of waste treatment.

WP038 Evaluation of the Removal of Amphoteric Foaming Agents Present in an AFFF Product from Groundwater using Granular Activated Carbon

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Aqueous film-forming foams (AFFFs) are synthetic Class-B fire extinguishing agents critical for extinguishing hydrocarbon fuel fires. AFFFs utilize fluoroaliphatic surfactants to create a stable foam for fire extinguishment and to form an aqueous-surfactant film layer on the surface of the fuel that acts as a vapor barrier to prevent flashbacks. Some AFFF products manufactured by 3M contained perfluorooctane sulfonate (PFOS) film forming agent and a separate fluoroaliphatic amphoteric foaming agent. Rather than a distinct single compound, the foaming agent was a complex mixture of several chemicals requiring careful

characterization to enable quantitative analysis of the foaming agent in environmental matrices. Here we present the results of the chemical characterization of a foaming agent used in a 3M Lightwater™ FC-203CF product as well as an evaluation of the ability of granular activated carbon (GAC) to remove such foaming chemicals from groundwater.

WP039 Effects of Carbon-Chain Length, Functional Group, and Common Groundwater Matrix on PFAAs Reduction by NiFe0-AC

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Perfluoroalkyl acids (PFAAs) are recalcitrant to several degradation processes and recognized as widespread in the environment. In this study, reduction by nNiFe0-AC is shown to be effective in the degradation of PFAAs in deionized water. Thus, the effects of chain length (C3-C8) and functional head group (-SO3- and -CO2-) on PFAAs degradation by nNiFe0-AC are presented here as well as nNiFe0-AC effectiveness to degrade a mixture of PFAAs in a PFAA-contaminated groundwater sample collected near a former fire-training area in Massachusetts. Similar degradation alkyl chain length trends were observed for perfluoroalkyl carboxylic (PFCAs) and sulfonic acids (PFSAs). Like PFOS, PFOA degradation plateaued by 1 d with a ~50% transformed, whereas shorter chains required longer time likely due to their lower sorption to reactive surfaces. F- and SO32- generation confirmed for both PFCA or PFSA degradation. However, measured F mass balance was higher for PFOS and PFOA (> 90% F) compared to short-chain PFAAs (750-70% F). Organic products detected for PFCAs included per- and polyfluoroalkyl carboxylates and alcohols with single and double bonds. While for PFSAs, single and double-bonded polyfluoroalkyl sulfonates, perfluoroalkyl carboxylates, and paraffins were generated. PFAAs present in AFFF-contaminated groundwater were degraded using nNiFe0-AC, but appeared lower than in single-solute deionized water.

WP040 Assessing the Effectiveness of Point-of-Use Residential Drinking Water Filters for Perfluoroalkyl Substances (PFAS)

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Poly- and Perfluoroalkyl substances (PFAS) have come under increased scrutiny due to concerns about their potential toxicity and prevalence in the environment, particularly drinking water. Studies examining full scale water treatment systems using granulated activated carbon (GAC) filters have been shown to have poor overall removal, especially with short chain PFASs, while reverse osmosis (RO) systems have been shown to generally remove all PFASs to below detection limits. Here we evaluated the effectiveness of point of use (POU) residential filters in removing 11 PFAS compounds in 61 homes in central North Carolina. All reverse osmosis (RO) and two-stage filters we tested showed near complete removal for all PFAS compounds we evaluated. The activated carbon (AC) based filters were inconsistent, with only 46% showing significant removal. We found no statistically significant relationship between the age of the filter and the removal efficiency for any class of AC filters or any of our target compounds. A significant and positive correlation was observed between PFAS removal efficiency and chain length in AC filters and on average PFSAs were removed better than their PFCA counterparts of equal chain length. This is important to note because short-chain PFAS compounds are currently used as replacements for legacy long-chain PFAS compounds and accounted for 70% of the PFAS in our study. While RO filters were shown to be effective in removing all PFAS compounds tested here, they are not always an economically viable solution, and could lead to further disparities in exposure based on socioeconomic status. One community in our study experienced significantly higher levels in tap water (geometric mean = 95 ng/L) than the other utilities in our study and had a peak concentration of 729 ng/L. After sampling the source water

for this utility we found no statistically significant difference (p -value > 0.05) between PFAS levels in the drinking water supply and the source water. Notably, both the source water and drinking water levels correlated with streamflow.

WP041 Occurrence and Treatment of Per- and Polyfluoroalkyl Ether Acids in North Carolina Surface Water

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Recent concerns over health implications associated with exposure to long-chain per- and polyfluoroalkyl substances (PFAS) has led to a shift towards the production and use of shorter-chain PFAS and per- and polyfluoroalkyl ether acids (PFEA). Occurrence of short-chain PFAS and PFEA in water supplies is a concern because conventional and many advanced treatments (e.g. ozonation, biofiltration, and disinfection by ultraviolet light) are not effective for their removal. Our research aimed to (1) determine occurrence of PFAS, including PFEA, in North Carolina (NC) drinking water sources, (2) assess the effectiveness of granular activated carbon (GAC) for short-chain PFAS and PFEA removal, and (3) identify predictors of PFAS adsorbability by GAC. Sampling campaigns conducted in the Cape Fear River basin of NC demonstrated that traditionally studied perfluoroalkyl acids impact many surface water supplies throughout the watershed. In addition, recently identified PFEA impact both private wells and surface water in the vicinity of a fluorochemical manufacturer and pose a risk to downstream surface water supplies. PFEA were introduced into the environment through air emissions and wastewater discharges, and fluorochemical fingerprinting could determine whether a site was impacted by wastewater discharge or air emissions. To reduce PFAS exposure, GAC adsorption is a scalable treatment option that is being considered for private well users and public water systems. However, little is known about the effectiveness of GAC for PFEA removal. In bench-scale column tests, PFEA began to break through after treating ~5,000 bed volumes of coagulated Cape Fear River water. PFEA characteristics such as chain-length, presence of ether oxygen, and branching affected adsorbability. Compared to coagulated surface water, GAC effectiveness for PFAS removal was substantially greater in groundwater because groundwater typically contains lower concentrations of naturally occurring organic matter that competes with PFAS for adsorption sites.

WP042 Effect of Soil Compost-Amendment on Mitigation of PFAS Uptake into Lettuce

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Food crops uptake of PFAS from contaminated soil due to reuse practices (e.g., reclaimed water irrigation and biosolids amendment) or uncontrolled releases have raised concerns about human exposure through dietary intake, with potential health implications. However, the strategies to mitigate the plant uptake of these chemicals from soil have not yet been well established. Compost is rich in organic carbon and other nutrients, which has been proven as a great fertilizer facilitating the food crops growth. Organic carbon has shown an excellent capacity for sorption of hydrophobic PFAS, thereby reducing their bioavailability. In this study, soil was amended with commercialized compost at three rates (0, 5, 10 and 20%) to evaluate the effects on the mitigation the mixture of nine commonly detected PFAS (e.g., PFMOAA, GenX, PFBS, PFHxS, PFOS, 6:2 FtS, 4:2 FtS, 8:2 FtS, and 10:2 FtS) uptake by lettuce. The pots uptake experiment was conducted in pots in a greenhouse; the mixture of PFAS chemicals was spiked into the soil at two concentration levels, i.e., 10 ng/g and 100 ng/g. The pots were irrigated daily with deionized water to maintain soil water content at 60% by monitoring the pot weights. All experimental pots were prepared in triplicate, including the PFAS-free control. After 50 days of exposure, the PFAS concentrations in lettuce roots and shoots grown in the soil with different rates of compost will be comparative

analyzed. In addition, the biomass of lettuces will also be measured to examine if the compost amendment could reduce the lettuce uptake of PFAS and at the same time increase the vegetable biomass.

WP043 Sediment Management Implications at Sites with Perfluoroalkyl Acids and Perfluoroalkyl Acid Precursors

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Per- and polyfluoroalkyl substances (PFAS) encompass a wide range of chemicals with highly disparate properties and behavior in the environment. Regulatory criteria or guidelines have been established in various jurisdictions for a subset of PFAS, typically selected perfluoroalkyl acids (PFAAs), such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). Unlike typical chemicals that drive sediment remediation, e.g., mercury and polychlorinated biphenyls, PFAAs are expected to depurate relatively rapidly from sediments following source control (due to low sorption) and not constitute a long-term sediment concern. However, the presence of certain PFAS ("precursors"), which transform to PFAAs and may be more tightly bound to sediments, indicates a need for modeling to better understand chemical longevity in the sediments. Evaluating the duration of possible PFAA exposure is complicated by differing transformation rates in anaerobic sediments and the aerobic water column. The objective of this study is to provide insights to whether there exist certain conditions under which PFAS may constitute a sediment management concern due to the formation of PFAAs from precursors. A 1-D chemical fate and transport sediment bed model was used to simulate potential scenarios to evaluate how combinations of precursor transformation rates, partitioning, diffusion, and groundwater flux affect PFAA migration from sediments to the water column. Similarly, the overlying water column was evaluated to identify combinations of transformation rates and advective transport that may lead to elevated concentrations of PFAAs. Modeling results indicate combinations of precursor transformation rates (in anaerobic and aerobic environments), partition coefficients, groundwater transport, and surface water transport that may lead to a buildup of PFAAs in either sediment porewater or the water column. After the combinations of conditions that may result in buildup of PFAAs are understood, an evaluation using known properties of selected precursors and PFAA end products is presented to provide context to the results. These results suggest conditions under which dredging, capping, and other sediment management strategies may or may not be necessary following source control.

WP044 Modeling of Potential PFAS Leaching from Land Applied Biosolids to Groundwater

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Several screening level modeling efforts have been conducted in New York and New England in the attempt to quantify potential concentrations of PFAS in shallow groundwater resulting from land application of biosolids. In order to connect a maximum contaminant level of PFAS in biosolids to a residential tapwater screening level concentration endpoint, these efforts have implemented EPA Regional Screening Level (RSL) calculation methods in combination with the SESOIL/AT123D leaching to groundwater model. Assumptions must be made concerning chemical adsorption to soil, chemical degradation rates in the soils, hydrologic conditions (weather, surface runoff, and infiltration processes), and biological effects on the chemical (e.g., plant uptake). The models used, their assumptions, and model inputs have a significant impact on the resulting groundwater concentrations and biosolid residue concentrations estimated to result in concentration endpoints of potential concern. A review of these modeling efforts conducted in Maine is presented, with a focus on the appropriateness of the conceptual models to the biosolids application source of potential PFAS exposure in groundwater. As these screening level models appear to be less physically rigorous in determining PFAS soil and biosolids concentration limits compared to other

existing tools developed by EPA, the EPA's Pesticide Root Zone Model (PRZM) is here considered. This model is used in both screening level and refined pesticide exposure risk assessments and is a physically based model that accounts for agronomic practices and hydrologic processes specific to local climate, soil, and groundwater conditions. The chemical fate processes in the model include runoff and erosion losses, sorption, volatilization, biological and chemical degradation, and plant uptake. An evaluation of the EPA's PRZM model for use in predicting PFOA and PFOS leaching from biosolids, along with a comparison to the previously conducted modeling approaches will be presented and provide insight as to whether alternative tools with more explicit accounting of near-surface fate and transport processes can provide estimates of PFOA/PFOS leaching that better fit accepted conceptual model understanding and available monitoring data.

WP045 6:2 Fluorotelomer sulfonate (6:2 FtS) Biodegradation: Desulfonation and defluorination

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6:2 Fluorotelomer sulfonate (6:2 FtS) is a major transformation product from aqueous film-forming foams (AFFFs). The occurrence of 6:2 FtS in humans, biota, and the environment are increasing. Exposure to 6:2 FtS has been linked to liver weight increase, inflammation, and necrosis of mice. Elevated concentrations of 6:2 FtS are widely detected in AFFF-impacted soils and groundwater. Little or very slow biodegradation of 6:2 FtS was observed in anaerobic sediment or anaerobic sludge. Under aerobic conditions, biodegradation of 6:2 FtS has been observed; however, the degradation was slow and factors controlling the biodegradation remained unclear. This study reported biodegradation of 6:2 FtS by three different bacterial strains under sulfur(S)-limiting conditions. These bacteria are able to grow in S-free media with 6:2 FtS, suggesting that 6:2 FtS was used as a sole S source for the microbial growth. Fluoride release was detected after one-day of incubation, indicating that 6:2 FtS biotransformation occurred after desulfonation. Enzymes responsible for desulfonation of 6:2 FtS have been identified and their expression under S-limiting conditions has been confirmed. Types of carbon sources play an important role in defluorination of 6:2 FtS. The results of our study offer a new approach for effective biodegradation of 6:2 FtS in 6:2 FtS-contaminated soils and water.

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