32nd Annual Meeting of the Rocky Mountain Chapter of the Society of Environmental Toxicology and Chemistry

April 25-26, 2019

Colorado Division of Public Health and Environment (CDPHE)
4300 Cherry Creek Drive South
Denver, CO 80246

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Colorado Division of Public Health and Environment (CDPHE)
4300 Cherry Creek Drive South
Denver, CO 80246

Our meeting will be in **Building A on the Main Campus**.

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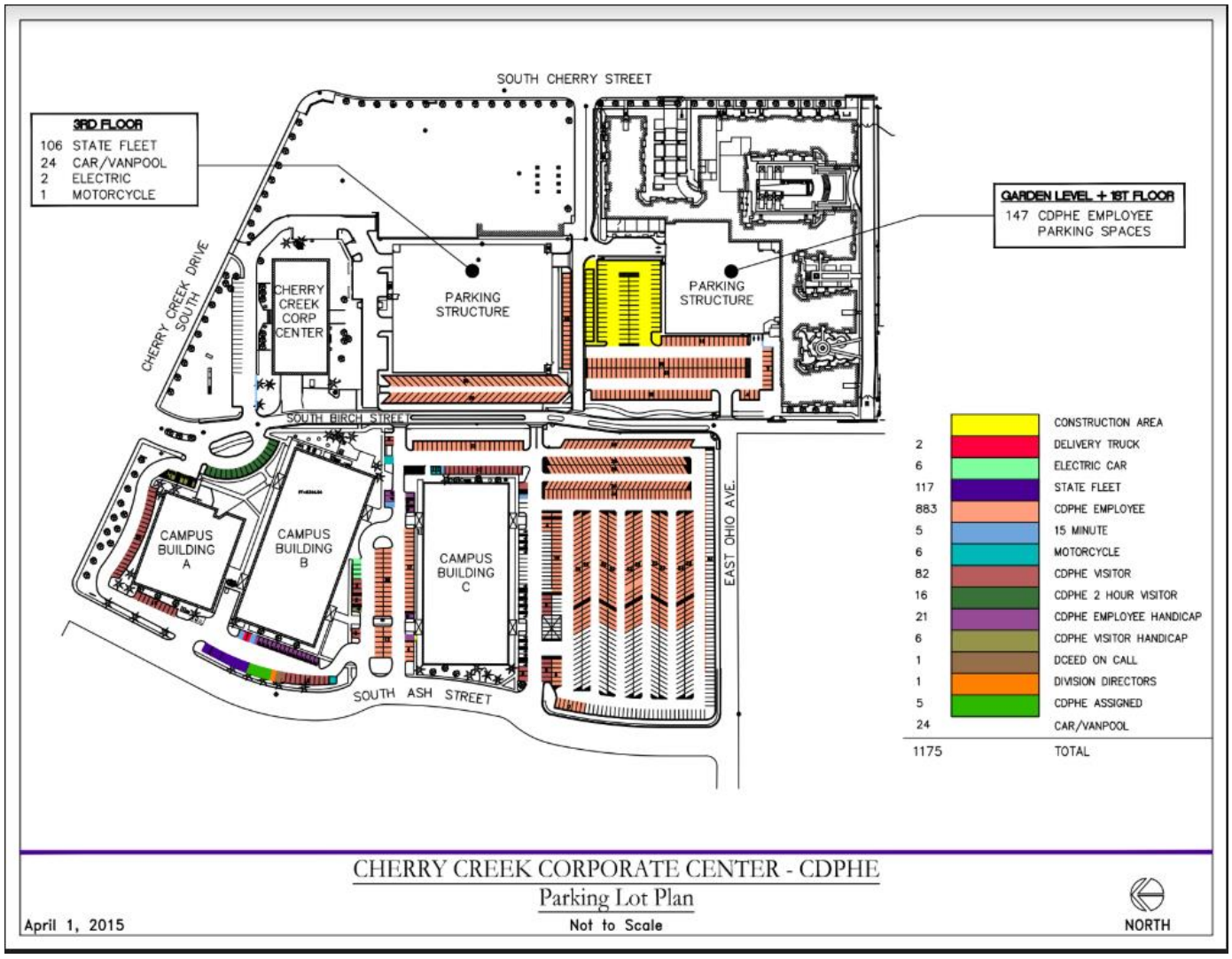
You **do not need** to check in at the **security desk**.

Day 1: PFAS Session – Department Operations Center (directly left as you enter the building)

Day 2: General Session – Sabin-Cleere Meeting Rooms (down the main hall on the left)



Meeting Agenda

Colorado Department of Public Health and Environment

	Time	Activity
Day 1: PFAS Session Thursday, April 25 Department Operations Center (directly left after you enter the building)	8:30-9:15	Registration
	9:15-9:30	Opening Remarks
	9:30-10:15	Plenary Presentation
	10:15-10:55	Oral Presentations
	10:55-11:25	Posters/Break
	11:25-12:05	Oral Presentations
	12:05-1:30	Lunch
	1:30-2:30	Oral Presentations
	2:30	Adjourn Day 1
Day 2: General Session Friday, April 26 Sabin-Cleere Meeting Rooms (down the main hall on the left)	8:30-9:15	Registration
	9:15-9:30	Opening Remarks
	9:30-10:15	Plenary Presentation
	10:15-10:55	Oral Presentations
	10:55-11:25	Posters/Break
	11:25-12:05	Oral Presentations
	12:05-1:45	Lunch & BoD Meeting
	1:45-2:00	SETAC Update
	2:00-2:40	Oral Presentations
	2:40-2:55	Posters/Break
	2:55-3:35	Oral Presentations
	3:35-4:00	Student Awards and Raffle
	4:00	Adjourn Day 2

RMSETAC Schedule - Thursday, April 25, 2019
CDPHE Department Operations Center

9:30	-	10:15	**Robert Bilott**. *Taft Stettinius & Hollister LLP*. **Plenary:** Evolving Legal, Regulatory, and Scientific Challenges of PFAS Contamination
10:15	-	10:35	**John Adgate**. *Colorado School of Public Health*. Exposure and health effects from per- and polyfluoroalkyl substances in Colorado water: preliminary results
10:35	-	10:55	**Kristy Richardson**. *Colorado Department of Public Health and Environment*. Colorado's approach to addressing PFAS groundwater contamination
10:55	-	11:25	***Poster Review and Break***
11:25	-	11:45	**Carrie McDonough**. *Colorado School of Mines*. Novel and legacy per- and polyfluoroalkyl substances (PFASs) in human serum and drinking water from El Paso County, CO
11:45	-	12:05	**Alan Vajda**. *University of Colorado Denver*. Uptake and effects of PFASs in fish exposed to Cape Cod PFAS plume
12:05	-	1:30	***Lunch***
1:30	-	1:50	****Juliane Brown**. *Colorado School of Mines*. Development of Risk Based Screening Criteria for Consumption of Food Impacted by Poly and Perfluoroalkyl Substances
1:50	-	2:10	**Bharat Chandramouli**. *SGS AXYS Analytical Services, Ltd*. Ether PFAS replacements for Perfluoroalkyl acids (PFAAs): Methods, properties, toxicity and fate
2:10	-	2:30	****Anastasia Nickerson**. *Colorado School of Mines*. A novel extraction method for comprehensive characterization of PFASs in AFFF-impacted soils
	2:30		***Meeting Adjourn Day 1***

** Indicates presentations being considered for best student platform presentation

RMSETAC Schedule - Friday, April 26, 2019
CDPHE Sabin-Cleere Meeting Rooms

9:30	- 10:15	**Aaron Roberts**. *University of North Texas*. **Plenary:** Deepwater Horizon and the Rocky Mountains: What a marine oil spill can teach us about PAH toxicity in high elevation systems
10:15	- 10:35	****Christopher Kotalik**. *Colorado State University*. The influence of life stage on the sensitivity of aquatic insects to metals in streams
10:35	- 10:55	**William Adams**. *Red Cap Consulting*. Towards an Updated Water Quality Criterion for Iron: Multiple Linear Regression (MLR) Models for Predicting Chronic Toxicity
10:55	- 11:25	***Poster Review and Break***
11:25	- 11:45	****David Bertolatus**. *University of Colorado Denver*. A landscape-based approach to assess impacts from exposure to complex mixtures in the Shenandoah River watershed
11:45	- 12:05	**David Norris**. *University of Colorado Boulder*. Historical incidence of "intersex" bass in North America
12:05	- 1:45	***Lunch and BoD Meeting***
1:45	- 2:00	**William Clements**. *Colorado State University*. **Update:** SETAC North America Board update to RMSETAC
2:00	- 2:20	****Andrea Kingcade**. *Colorado State University*. Assessment of two antilipidemic drug subclasses (fibrates and statins) on embryogenesis in two model fish species (Danio rerio and Pimephales promelas)
2:20	- 2:40	****Logan Rand**. *Colorado School of Mines*. Incidental iron oxide nanoparticle characteristics and stability during remediation of a mining-impacted stream
2:40	- 2:55	***Poster Review and Break***
2:55	- 3:15	**Yong Liu**. *University of Colorado Denver*. Kinetics study of heterogeneous reactions of n-butylamine with succinic acid using an ATR-IR flow reactor
3:15	- 3:35	**Allie Bamber**. *Colorado Department of Public Health and Environment*. Evaluation of health symptoms and air exposures in communities near oil and gas operations in Colorado
3:35	- 4:00	***Student Awards and Raffle***
4:00		***Meeting Adjourn Day 2***

** Indicates presentations being considered for best student platform presentation

Day 1: PFAS Session

Thursday, April 25, 2019

Plenary Presentation:

Evolving Legal, Regulatory, and Scientific Challenges of PFAS Contamination

Robert Bilott, Taft Stettinius & Hollister LLP

https://www.taftlaw.com/people/robert-a-bilott?tab=experience

Rob Bilott is a partner at Taft Stettinius & Hollister LLP, where he has practiced for over 28 years, representing a diverse array of clients from individuals to multinational corporations. Rob brought the very first PFAS environmental exposure case in 1999 that resulted in the discovery and public disclosure of PFOA in drinking water supplies of approximately 70,000 people in West Virginia and Ohio. That discovery spurred national regulator interest and investigation into PFAS and the filing in 2001 of the nation's first class action litigation on behalf of individuals exposed to PFOA in their drinking water.

Rob was appointed by the court as one of the Class Counsel for the plaintiffs and helped negotiate and obtain a class settlement in 2004 that secured benefits for the class valued in excess of $300 million including water filtration systems for impacted private and public water supplies, funding of independent scientific health studies for PFOA, blood testing of 69,000 people, and eventual medical monitoring and confirmation of probable links to human disease that resolved issues of general causation for the class of members' personal injury claims.

As a result of that ground-breaking class settlement and probable link findings on PFOA, over 3,500 class members were able to pursue individual personal injury claims against DuPont for diseases linked to their PFOA drinking water exposures, all of which were eventually consolidated into Multi-District Litigation (MDL) proceedings in federal court in Ohio. Rob's ligation also spurred the first regulatory investigations, assessments, and enforcement actions involving PFAS, leading to the largest civil administrative penalty ever obtained by EPA at the time for withholding of PFAS toxicity and risk information.

To date, Rob has helped secure over $1 Billion in benefits for his clients exposed to PFOA in their drinking water. In 2017, Rob was a recipient of the international Right Livelihood Award, also known as the "Alternative Nobel Prize", for his work on PFAS contamination issues.

Oral Presentation Abstracts
Thursday, April 25, 2019
(in the order they appear in the schedule)

Exposure and health effects from per- and polyfluoroalkyl substances in Colorado water: preliminary results

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Per- and polyfluoroalkyl substances (PFAS) are a class of chemicals widely used in industrial and commercial applications because of their unique physical and chemical properties. Between 2013 and 2016 PFAS were detected in public water systems and private wells in El Paso County, Colorado. The contamination was likely due to aqueous film forming foams (AFFF) used at a nearby Air Force base. In June 2018, serum PFAS concentrations were quantified and questionnaires administered in 213 non-smoking adult participants residing in three affected water districts. Eighteen PFAS were quantified and those detected in a majority of participants included: perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorononanoate (PFNA) and perfluoroheptane sulfonate (PFHpS). Study participants' median PFHxS serum concentration (14.8 ng/mL) was approximately 12 times as high as the U.S. national average. Both the PFOS median (9.7 ng/mL) and the PFOA median (3.0 ng/mL) concentrations were approximately twice as high as the U.S. national average, while PFNA median concentration (0.4 ng/mL) were lower than the national average. While PFOA and PFOS concentrations are comparable to those found in other highly exposed communities in the U.S., serum PFHxS concentrations establish these Colorado communities as the most highly PFHxS-exposed of several sites around the U.S. with recently discovered PFAS drinking water contamination.

Colorado's approach to addressing PFAS groundwater contamination

Kristy L. Richardson, State Toxicologist, Disease Control and Environmental Epidemiology Division, Colorado Department of Public Health and Environment, Denver, Colorado 80246, Kristy.Richardson@state.co.us.

Groundwater contamination by per- and polyfluoroalkyl substances (PFAS) is a growing concern among public health and environmental agencies. In Colorado, PFAS contamination has been identified in groundwater affecting three Colorado communities. In each community, we have responded with a multi-step process to establish a collaborative response that reduces risk and works towards identifying solutions in the absence of national regulations. Additionally, we are building a better understanding of the scope of PFAS contamination statewide that may inform future regulation of PFAS in the state.

Novel and legacy per- and polyfluoroalkyl substances (PFASs) in human serum and drinking water from El Paso County, CO

Carrie A. McDonough, Department of Civil & Environmental Engineering, Colorado School of Mines, Golden, CO 80401, cmcdonough@mines.edu. **Kelsey Barton**, Department of Environmental & Occupational Health, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, kelsey.barton@ucdenver.edu. **Anne Starling**, Department of Epidemiology, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, anne.starling@ucdenver.edu. **John Adgate**, Department of Environmental & Occupational Health, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, john.adgate@ucdenver.edu. **Christopher Higgins**, Department of Civil & Environmental Engineering, Colorado School of Mines, Golden, CO 80401, chiggins@mines.edu.

More information on bioaccumulation, biotransformation, and biological effects of per- and polyfluoroalkyl substances (PFASs) is rapidly needed to understand health risks posed by widespread, long-term aqueous firefighting foam (AFFF) contamination of drinking water. Three public water systems in El Paso County, Colorado were found to be contaminated with PFASs from AFFF above the EPA health advisory level of 70 ng/L between 2013 and 2016, with contamination likely beginning before 2013. In this study, untreated drinking water and human blood samples from throughout the region were analyzed via high resolution mass spectrometry (HRMS) to better understand human exposure to PFASs resulting from this contamination event. Drinking water samples were collected from 10 private and 28 public wells throughout the region. Blood samples were collected from a cohort of 220 individuals living in the area. Samples were analyzed using liquid chromatography with quadrupole-time-of-flight mass spectrometry (LC-QToF-MS) in data-independent acquisition mode. Quantitative data were obtained for PFASs for which analytical standards were available. Data were also screened for >1000 previously characterized and theoretical PFASs using an HRMS fragmentation library and an extensive extracted ion chromatogram (XIC) list. C3-C8 perfluoroalkane sulfonates (PFSAs) and C4-C8 perfluoroalkanoic acids (PFCAs) were frequently detected in raw drinking water samples at concentrations exceeding 70 ng/L. C5-C11 PFCAs and C3-C8 PFSAs were frequently detected in serum from residents. HRMS suspect screening suggests the presence of additional PFASs in human serum, such as unsaturated and oxy-PFSAs.

Uptake and effects of PFASs in fish exposed to Cape Cod PFAS plume

Alan Vajda, **David Bertolatus**, and **Paige Friedentag**, University of Colorado Denver, CB 171, Denver, CO 80207, 720-939-9871, Alan.vajda@ucdenver.edu; **Larry Barber** and **Jeramy Jasmann**, U.S. Geological Survey, 3215 Marine Street, Boulder, CO 80303; **Denis Le Blanc** and **Andrea Tokranov**, U.S. Geological Survey, New England Water Science Center, Northborough, MA 01532; **Elsie Sunderland** and **Heidi Pickard**, John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138 and Department of Environmental Health, Harvard T. H. Chan School of Public Health, Harvard University, Boston, MA 02115.

The primary objective of this investigation is the evaluation of perfluorinated alkyl substances (PFAS) uptake, accumulation, and biological effects on a model fish, the fathead minnow. Translational biomarkers of immune, metabolic, oxidative stress, and reproductive function will be assessed to inform human health risk of chronic exposure to environmental PFAS mixture. Biomarker response will be evaluated to link adverse higher order

environmental health outcomes to aqueous and tissue contaminant concentrations. These experiments leverage from a mobile laboratory/water-quality characterization approach to assess the biological effects of contaminant exposure on aquatic organisms developed jointly by the USGS and University of Colorado Denver For this ongoing research, mobile laboratories will be deployed on-site to assess (1) the uptake and accumulation of PFAS in the fathead minnow (*Pimephales promelas*), a model aquatic organism, and (2) potential adverse biological outcomes associated with exposure to PFASs. The experiments will involve 28-day on-site, continuous-flow *in vivo* fish exposure exposures along a Fire Training Area contamination gradient where high concentrations of PFAS also have been documented. The interdisciplinary research team brings a broad range of scientific expertise together to focus on the fundamental relationships between the geosphere, hydrosphere, biosphere, and anthropogenosphere. The research team includes key partners with federal, state, and local science and resource management agencies, community-based organizations, and local property owners.

Development of Risk Based Screening Criteria for Consumption of Food Impacted by Poly and Perfluoroalkyl Substances

Juliane B. Brown, PhD Candidate, Colorado School of Mines, Civil and Environmental Engineering, Golden, Colorado 80401, jbbrown@mines.edu. **Jason Conder**, PhD, Senior Scientist, Geosyntec Consultants, Huntington Beach, California 92648, JConder@geosyntec.com. **Jennifer Arblaster**, Scientist, Geosyntec Consultants, South Burlington, VT 05403, jarblaster@geosyntec.com. **Christopher Higgins**, PhD, Professor, Civil and Environmental Engineering, Colorado School of Mines, Golden, CO 80401, chiggins@mines.edu.

Poly and perfluoroalkyl substances (PFASs) have been identified as chemicals of concern in agricultural soils, biosolids-amended soil, and irrigation water due to their toxicity, persistence, and bioaccumulation potential. The agricultural use of PFAS-contaminated water, municipal and industrial biosolids, and municipal compost can all contribute to the exposure of PFASs to food crops. Soil irrigated with contaminated water may become contaminated over time resulting in additional exposure pathways to food crops. The unintended uptake and accumulation of PFASs in food crops is an important and continuing concern for protecting human health. There is an urgent need for regulatory agencies to accurately understand accumulation in the edible portion of food crops as these foods are often consumed fresh or with minimal processing. Recognition of PFASs in food as an 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 PFASs into crops. Using available crop-specific transfer factors (TFs) from plant bioaccumulation studies, exposure data, and consumption rates for lettuce – a food crop often locally grown in gardens and farms – exposure intakes are estimated for a range of concentrations using Monte Carlo Simulation in a tiered stochastic modeling approach. For PFAS 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 other risk-based pathways and available health advisories or criteria.

Ether PFAS replacements for Perfluoroalkyl acids (PFAAs): Methods, properties, toxicity and fate

Bharat Chandramouli (bharat.chandramouli@sgs.com), **Coreen Hamilton** and **Million Woudneh**, SGS AXYS Analytical Services, Ltd. Sidney, BC Canada.

The phaseout of persistent and toxic PFOS and PFOA, and their replacement by newer chemistry means that "novel" PFAS are being detected in the environment. PFOA in fluoropolymer manufacture is being replaced with a diverse set of per-/polyfluoroether carboxylic acids including GenX (2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid or HFPO-DA) , ADONA (3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid]), PMPA (tetrafluoro-2-(hexafluoro-2-(trifluoromethoxy)propoxy]-propanoate) and others. Chrome plating alternatives for PFOS include F-53B (6:2 chlorinated polyfluorinated ether sulfonate) and others. Of these, GenX is under increased scrutiny, especially in North America after the discovery of HFPO-DA in watersheds adjacent to a fluoropolymer manufacturing facility in North Carolina. In this study, we present the results of method development and validation for three such replacements, HFPO-DA, ADONA and F-53B in water, solids and tissue. A review of occurrence, properties, fate and toxicology of these compounds will also be presented. Methods for HFPO-DA, ADONA and F53-B addition to routine PFAS monitoring of PFAAs and precursors were developed and validated in water, soil and tissue using isotope dilution LC-MS/MS. Aqueous samples were extracted using weak anion exchange (WAX). Soil/tissue samples were extracted using methanol, cleaned up using WAX prior to analysis. Extracts were analyzed using a Waters Acquity UPLC interfaced with a Waters TQ-S tandem mass spectrometer. Results from validation show good accuracy and precision for ether PFAS in conjunction with 29 other PFAS. Preliminary tests on oxidative fate show that HFPO-DA is resistant to hydroxyl radical oxidation.

A novel extraction method for comprehensive characterization of PFASs in AFFF-impacted soils

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A novel method was developed to enhance extraction of a wider range of poly- and perfluoroalkyl substances (PFASs) in soils. Previous methodology has shown to be insufficient for complete extraction of strongly sorbing species, such as the many cationic and zwitterionic PFASs in aqueous film forming foam (AFFF)-impacted soils. Two extraction methods were compared via liquid chromatography-quadrupole time of flight high resolution mass spectrometry (LC-QTOF-HRMS) analysis of field-collected soils. A spike recovery experiment using a mixture of AFFFs was also performed. A wide variety of PFASs from an extensive extracted ion chromatography (XIC) list and custom HRMS spectral library were analyzed in both negative and positive ionization modes. Identified compounds lacking analytical standards were semi-quantified and assigned levels of confidence based on their exact mass, isotope ratios, and fragmentation.
The new extraction method was applied to soil core samples from a former fire training area to understand how AFFF-derived PFAS plumes move and transform within the subsurface. The results show that cations and zwitterions make up a large percentage of soil concentrations at the source zone, up to 95% at certain depths. Without exhaustive extraction techniques and LC-QTOF-HRMS analysis, the majority of the PFAS mass may be missed near fire training area source zones. Enhanced extraction of all PFASs more accurately captures the total environmental concentrations.

(alphabetical order by first author)
** Indicates presentations being considered for best student poster presentation

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Assessing the histological and molecular impacts of exposure to Polyfluorinated Alkyl Substances in aquatic systems using fathead minnows, *Pimephales promelas*

****Paige Friedentag**, **Alan Vajda**, and **David Bertolatus**, University of Colorado Denver, CB 171, Denver, CO 80207, 720-939-9871, Paige.Friedntag@ucdenver.edu; **Larry Barber** and **Jeramy Jasmann**, U.S. Geological Survey, 3215 Marine Street, Boulder, CO 80303; **Denis Le Blanc** and **Andrea Tokranov**, U.S. Geological Survey, New England Water Science Center, Northborough, MA 01532; **Elsie Sunderland** and **Heidi Pickard**, John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138 and Department of Environmental Health, Harvard T. H. Chan School of Public Health, Harvard University, Boston, MA 02115.

Polyfluorinated alkyl substances (PFAS), a group of anthropogenic chemicals with endocrine disrupting properties, are becoming a primary focus in the aquatic toxicology field. A series of on-site, flow-through exposure experiments are underway to evaluate the influence of a gradient of PFAS-contaminated groundwater on histological and molecular impacts related to reproductive and metabolic function on fathead minnows *(Pimephales promelas)*. Fish exposed to the contaminated water will be collected and dissected on days 0, 4, 7, 14 and 21; water and tissue samples will be collected to evaluate contaminant occurrence and concentration. Histological review of gonads and livers will be performed to identify if cellular changes occurred. Transcriptomic response of liver will be analyzed by microarray to identify impacts on gene expression impacting reproduction, metabolism, and immune function. This research uniquely addresses PFAS impacts in the context of complex environmental mixtures, and will inform assessments of PFAS hazard to human and ecosystem health.

Closing the PFAS mass balance: Developing a spectral library for PFAS identification using High Resolution Mass Spectrometry

****Sarah Mass**, MS Student, Colorado School of Mines, sarahmass@mymail.mines.edu, (720) 403 4589

PFAS analysis by High Resolution Mass Spectrometry (HRMS) is an excellent tool for identifying and quantifying individual PFASs in environmental samples quickly and accurately. HRMS often uses targeted analysis, where masses of compounds in samples are compared to masses of analytical standards. However, analytical standards only exist for tens of PFASs, but thousands of PFASs have been released into the environment. Targeted PFAS analysis can therefore provide only a partial picture of total PFAS load at a contaminated site, and research about toxicity, recalcitrance, and transformation of PFASs without analytical standards is limited. To address this issue, fragmentation spectra for more than 350 PFASs were acquired and compiled in an internal library from AFFFs, neat standards, commercial products, and environmental samples. The library can be used to screen for PFASs without commercially available analytical standards, increasing analytical capacity from ~50 compounds to ~350 compounds. The end goal is to acquire fragmentation spectra for all known PFASs, a moving target as more compounds are discovered each year. The library has been shared with researchers all over the world in hopes that it will help the scientific community as a whole gain a better understanding of total PFAS load in the environment.

Development of Per and Polyfluoroalkyl Substances (PFAS) Ecological Risk Based Screening Levels

Jean Zodrow, Arcadis, Highlands Ranch, CO, Jean.Zodrow@arcadis.com. **Meredith Frenchmeyer**, Arcadis, Portland, OR, Meredith.Frenchmeyer@arcadis.com. **Paul Anderson**, Arcadis, Chelmsford, MA, Paul.Anderson@arcadis.com. **Victoria Lazenby**, Arcadis, Melbourne, Australia, Victoria.Lazenby@arcadis.com. **Erica Houtz**, Arcadis, San Francisco, CA, Erica.Houtz@arcadis.com. **Craig Divine**, Arcadis, Irvine, CA, Craig.Divine@arcadis.com.

Contamination of soils and waters by per- and polyfluoroalkyl substances (PFASs) has become a widespread problem, especially 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, there is little information on the uptake and toxicity of PFASs to these receptors. The goal of this project is to develop risk-based screening levels (RBSLs) for assessing PFAS risk to T&E species, considering the known fate and transport of these chemicals and the receptors most likely to be impacted at DoD sites. Standard risk assessment approaches, as are applied in the United States and internationally, will be used to develop risk-based screening criteria for PFASs. Ecological receptors, including representative T&E species and surrogate receptors, will be selected based on consideration of PFAS-specific risk factors (e.g., biomagnification, trophic level exposures, and species-specific toxicity). Species-specific exposure parameters, uptake, and toxicity data will be compiled from existing databases as well as primary literature. For uptake, empirical data will be used to recommend bioaccumulation, bioconcentration, and biomagnification factors for relevant environmental media and trophic levels. Media-specific risk-based screening levels will then be developed for each receptor and PFAS. For PFASs lacking adequate data to develop a compound specific RBSL, RBSLs based on surrogate compounds or classes of compounds will be considered.

Day 2: General Session

Friday, April 26, 2019

Plenary Presentation:

Deepwater Horizon and the Rocky Mountains: What a marine oil spill can teach us about PAH toxicity in high elevation systems

Dr. Aaron Roberts, University of North Texas

https://robertslab.weebly.com/about.html

Dr. Roberts' is a Professor in the Department of Biological Sciences & Director of the Advanced Environmental Research Institute at the University of North Texas. Dr. Roberts earned a BS in Biology from the University of Missouri and his MS and PhD in Zoology from Miami University. He has been a faculty member at UNT since 2006. Dr. Roberts' laboratory studies the effects of a wide range of chemical contaminants including mercury, nanoparticles, and PAH on aquatic biota including plankton and fish.

Dr. Roberts is also a Co-Principle Investigator with the RECOVER consortium (http://recoverconsortium.org/) where his research is focused on determining how ultraviolet radiation in sunlight enhances the toxicity of oil to pelagic fish. This interaction, called photo-induced or photo-enhanced toxicity, can increase the toxicity of PAH by several times. In open ocean systems where UV exposure may be quite high, this phenomenon likely plays an important role in the impact and subsequent recovery of ecosystems following oil spills. RECOVER represents a unique and exciting opportunity to study the mechanisms by which photo-induced toxicity occurs as well as adaptations that may allow species to recover from toxic impacts.

Oral Presentation Abstracts
Friday, April 26, 2019
(in the order they appear in the schedule)

The influence of life stage on the sensitivity of aquatic insects to metals in streams

Christopher Kotalik, Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, Colorado 80523, cjkotalik@gmail.com. **William H. Clements**, Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, Colorado 80523, william.clements@colostate.edu. **Pete Cadmus**, Colorado Parks and Wildlife, 317 W. Prospect Rd., Fort Collins CO 80526, pete.cadmus@state.co.us.

Evaluations of aquatic insect responses to contaminants typically use larval life stages to characterize taxa sensitivity, but the effects of contaminants on the morphological transition of larvae to emerged terrestrial adults has received less attention. We compare the lethal and sublethal effects of metals to larval and emerging adult aquatic insects using stream mesocosms and field biomonitoring at an acid mine drainage (AMD) remediation site. Mesocosm results showed differences in metal tolerance among dominant taxa and life stages. In addition, the timing of adult emergence was highly altered in all dominant taxa, and sex ratios were changed in mayflies. Field results showed differences in the recovery propensity of larvae compared to adults at sites downstream of the AMD inputs. Our results demonstrate that metal tolerance in aquatic insects is life-stage dependent and that taxa sensitivity is influenced by a combination of physiology and phylogeny. Failure to quantify emergence can mischaracterize contaminant effects on aquatic insect population dynamics and aquatic subsidies to terrestrial ecosystems. Regulatory frameworks would benefit by including test results that account for the effects of contaminants on metamorphosis and adult insect emergence.

Towards an Updated Water Quality Criterion for Iron: Multiple Linear Regression (MLR) Models for Predicting Chronic Toxicity

[1]William J. Adams, [2]David K. DeForest, [3]Kevin V. Brix, [2]Lucinda M. Tear, [4]William Stubblefield, [4]Allison Cardwell, [5]Patricio Rodriguez
[1]Red Cap Consulting - Lake point, UT, [2]Windward Environmental LLC- Seattle, WA, [3]EcoTox - Miami, FL, [4]Oregon State University – Corvallis, OR, [5]PHR Consulting-Santiago, Chile

We evaluated the ability of multiple linear regression (MLR) models to predict Fe toxicity to green algae, *Ceriodaphnia*, and fathead minnows as a function of combinations of water pH, DOC, and hardness. Individual water chemistry terms (pH, ln[DOC], ln[hardness]) were included as independent variables in the analysis, either EC10s or EC20s were included as the dependent variable. Akaike Information Criterion (AIC) was used to identify which combination of terms resulted in the best models for predicting toxicity for the three-species tested. MLR models were able to predict Fe EC10/20s within a factor of two of the observed values for >90% of the toxicity tests. The MLR models were used to normalize species sensitivity distributions (SSDs) such that Fe HC5 (concentration hazardous to 5% of the species) values could be derived for site-specific conditions of interest. Evaluation of the regression models indicate that the predicted R2 for *C. dubia* and fathead minnows were lower than the adjusted R2 indicating that these models are either unduly influenced by individual data points or over-parameterized. Additional testing is planned to expand the datasets and improve model robustness.

A landscape-based approach to assess impacts from exposure to complex mixtures in the Shenandoah River watershed

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Aquatic habitats are often contaminated with complex mixtures of chemicals originating from diverse sources. Here, we employed integrated chemical and biological analyses to determine how mixture composition and exposure effects varied between sites with different land use. Adult fathead minnows were exposed to water from locations within the Shenandoah River watershed in mobile laboratories. Exposure locations included agricultural, urban, waste-water impacted, mixed-use, and forested sites. Multiple biological endpoints were measured, including gonadosomatic index, number of nuptial tubercles, plasma vitellogenin, and hepatic gene expression. Water samples were taken during the fish exposure and analyzed for more than 460 chemical constituents. Each location had a unique chemical profile that was generally consistent with landuse. Whole-organism and molecular responses also differed between the sites. At a waste-water impacted site, biomarkers of endocrine disruption were significantly affected, including decreased GSI, altered secondary sex characteristics, and decreased sperm abundance. However, molecular biomarkers of estrogen exposure showed no difference between sites, suggesting that estrogen receptor activation was likely not a mechanism for these adverse outcomes. Fish exposed at an agriculture-impacted site had increased mortality and decreased GSI relative to initial controls, which was accompanied by differential regulation of immune and metabolism related pathways at the transcript level. Lastly, hierarchical clustering of hepatic transcriptome profiles showed a site-specific pattern, demonstrating that sites with differing landuse exert unique exposure effects at the transcript level. The continued study of the relationships between land use, chemical occurrence, and exposure effects is expected to yield predictive tools for risk assessment and management.

Historical incidence of "intersex" bass in North America

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Testicular oocytes (TOs) in testes of teleost fishes have been reported in surveys conducted since 2000 across the USA with 0 to 100% of largemouth (LMB) and smallmouth bass (SMB) exhibiting TOs. Because sexual differentiation is a labile process in teleosts, with numerous species known to change sexes or be hermaphroditic, we hypothesized that examination of individuals collected 50 to 100 years ago would exhibit TOs. We arranged to excise one gonad from specimens of LMB and SMB stored at six natural history museums: Chicago Field Museum, Harvard University, Tulane University, University of Colorado, University of Kansas, University of Michigan. Gonads were removed, placed in 70% ethanol and returned to our laboratories for histological examination. We analyzed 138 female and 67 male LMB as well as 17 female and 21 male SMB. All ovaries were normal. Examination of over 4000 sections of testes revealed TOs in only 13 LMB (19%) and 4 SMB (19%). One SMB and one LMB exhibited TOs to the extent seen today. These fish were from different museums but they both were collected in Tennessee in the 1920s. This suggests that fishes inhabiting certain regions may historically have exhibited a high intensity of TOs similar to recent observations. We find that TOs have been present in LMB and SMB prior to the detection of estrogenic endocrine-disrupting chemicals in the environment and that the incidence of TOs in some populations was of comparable intensity to those reported in the 21st century.

Assessment of two antilipidemic drug subclasses (fibrates and statins) on embryogenesis in two model fish species (*Danio rerio* and *Pimephales promelas*)

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Embryogenesis in fish is a critical process in development that begins within hours of fertilization and progresses through important stages including gastrulation and organogenesis. The antilipidemic drug category is one of many pharmaceutical classes detected in effluent and surface water downstream of wastewater treatment plants. To elucidate the effects antilipidemic drugs may have on these sensitive life stages, two mixture exposure studies with nine antilipidemic drugs were performed separately with zebrafish (*Danio rerio*, ZF) embryos and fathead minnow (*Pimephales promelas*, FHM) embryos. Nine additional studies were performed with ZF embryos to better understand the role each drug may have on development. ZF embryos were exposed for 72 hours at concentrations up to 0.5µM or 1µM and FHM embryos were exposed for 144 hours at concentrations up to 0.05µM. Up to 15 observations were divided into four categories and evaluated: developmental toxicity, and muscle, yolk, and cardiovascular abnormalities and will be summarized. The observations illustrate that (a) embryos are sensitive to antilipidemic drug exposures during embryonic stages of development, (b) differences in species sensitivities occurred, and (c) differences between mixture and individual exposures of drugs were observed. These significant sublethal phenotypes would likely impact individual fish development and potentially the population as well if environmental concentrations increased.

This model represents a potential tool for assessing sensitive, sublethal effects of pharmaceuticals in the environment.

Incidental iron oxide nanoparticle characteristics and stability during remediation of a mining-impacted stream

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Acid mine drainage (AMD), which poses a significant water quality threat to streams, causes the precipitation of Fe (hydr)oxides to which toxic metals sorb. Given that this process is a result of mining activities, we can classify the resulting precipitates as incidental nanoparticles (INPs) and their colloidal aggregates. We examined the characteristics, abundance, surface properties, and aggregation of INPs during remediation of the AMD inputs to the North Fork of Clear Creek, Colorado. Particle sizes and concentrations were measured concurrent with water chemistry monitoring during the first six months of lime water treatment plant operation. Single particle ICP-MS (spICP-MS) analyses showed Fe and Cu INP number concentrations in impacted stream water decrease from 10^7 to 10^5 particles mL^{-1}, before and after treatment respectively. The number-weighted mode Fe INP size measured with spICP-MS was 182 ± 7 nm during treatment. STEM imaging revealed aggregates of spherical 5-10 nm Fe INPs. We also studied surface chemistry changes during the treatment period using hematite, a model Fe INP, suspended in filtered field waters. Changes in zeta potential and INP size, measured by dynamic light scattering, support that the pretreatment stream chemistry promoted rapid aggregation while improved water quality inhibited aggregation. We also observed impacts to chemistry and particle behavior resulting from the seasonal stream hydrology, namely the occurrence of spring snowmelt shortly after treatment began. This work provides new insight into AMD-generated INP behavior before and during remediation in a dynamic alpine stream.

Kinetics study of heterogeneous reactions of n-butylamine with succinic acid using an ATR-IR flow reactor

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Kinetics of heterogeneous reaction of n-butylamine (BA) with succinic acid (SA) were studied via a flow system combined with attenuated total reflection infrared Fourier-transformed spectroscopy (ATR-FTIR) over wide ranges of BA concentrations (0.145-32.5 ppm), thin film thicknesses (0.05-0.15 μm), temperatures (263-295 K), and relative humidities (0-75% RH) under atmospheric pressure condition for the first time. Pseudo-first-order rate constants k_{app} and overall reactive uptake coefficients γ values were derived according to the changes in absorbance from peak located near 1634 cm^{-1}, which can be assigned to $-COO^-$ antisymmetric stretch ($\nu_{as}(-COO^-)$). The results show that the heterogeneous reaction of SA with BA follows Langmuir-Hinshelwood mechanism and k_{app} is largely dominated by surface reaction over bulk phase reaction. The studies of the influence of temperature and RH on the reaction of SA with BA show that both the k_{app} and γ values display very strong temperature and RH dependence. Low temperature promotes the reaction mainly due to the physisorption of BA predominated in the whole reaction. With an increasing RH, both the k_{app} and γ values increase indicating that the water vapor enhances the reaction. In addition, the water uptake results indicate that the hygroscopic behavior of the thin film is enhanced after BA exposure.

Evaluation of health symptoms and air exposures in communities near oil and gas operations in Colorado

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Oil and natural gas (OG) development activity in Colorado is increasing in areas with large population growth. Community scale exposures to substances emitted from OG operations is a public health concern. The Colorado Department of Public Health and Environment has received over 400 citizen reports of acute health symptoms and/or odors during the upstream development phases of drilling, hydraulic fracturing, and flowback by people living near OG sites. We have completed 11 community exposure investigations, including 6 with the Colorado Air Monitoring Mobile Lab to measure exposures to volatile organic compounds (VOCs) during different phases of OG operations. For each investigation, we conducted screening level health risk evaluations to assess the potential for acute and chronic non-cancer and cancer health effects to occur in those communities. To date, we have not found any concentrations of VOCs above short or long-term health guideline values. Using currently available measurement technology and risk assessment methods, we are unable to document conditions that suggest an ongoing health hazard at this time, however, these conclusions are only relevant to the time period and location in which these samples were collected. More extensive site specific community level air monitoring is needed to further characterize exposure concentrations during different phases at different locations to fully understand the relationship between reported acute health symptoms and exposures to OG related substances in Colorado.

Poster Presentation Abstracts
Friday, April 26, 2019
(alphabetical order by first author)
** Indicates presentations being considered for best student poster presentation

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Assessing the histological and molecular impacts of exposure to Polyfluorinated Alkyl Substances in aquatic systems using fathead minnows, *Pimephales promelas*

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Polyfluorinated alkyl substances (PFAS), a group of anthropogenic chemicals with endocrine disrupting properties, are becoming a primary focus in the aquatic toxicology field. A series of on-site, flow-through exposure experiments are underway to evaluate the influence of a gradient of PFAS-contaminated groundwater on histological and molecular impacts related to reproductive and metabolic function on fathead minnows *(Pimephales promelas)*. Fish exposed to the contaminated water will be collected and dissected on days 0, 4, 7, 14 and 21; water and tissue samples will be collected to evaluate contaminant occurrence and concentration. Histological review of gonads and livers will be performed to identify if cellular changes occurred. Transcriptomic response of liver will be analyzed by microarray to identify impacts on gene expression

impacting reproduction, metabolism, and immune function. This research uniquely addresses PFAS impacts in the context of complex environmental mixtures, and will inform assessments of PFAS hazard to human and ecosystem health.

Impacts of environmental change on freshwater nitrite oxidation

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Microbial communities play an important role in determining the fragility or resilience of freshwater ecosystems facing environmental challenges. Nitrogen cycling microbes can remove nitrogen pollution in contaminated systems, but removal rates are dictated in part by response to physicochemical parameters in the system. Here, we characterized the ecophysiology of a novel nitrite-oxidizing bacteria (NOB, which metabolize nitrite to nitrate) from the *Candidatus* Nitrotoga genus in order to determine growth limits and response to environmental change. *Ca.* Nitrotoga sp. CP45 was enriched from the South Platte River system (Colorado), which is heavily impacted by wastewater treatment effluent and runoff from animal feed operations and agriculture. CP45 oxidized nitrite at temperatures ranging from 4-28˚C, with maximum rates at 25˚C. Nitrite oxidation occurred at pH 5-8, but the upper and lower pH limits were not reached within the conditions tested. Rates were slowed significantly in the presence of light, likely due to photo-inhibition. The addition of organic carbon in the form of acetate and dextrose significantly elevated rates, while formate halted nitrite oxidation activity. Exposure to antibiotics commonly found within the river (5-500 nM erythromycin, sulfamethoxazole, and trimethoprim, significantly higher than *in situ* river concentrations) resulted in elevated nitrite oxidation rates. Mesocosm experiments showed that endogenous riverine sediment communities were also able to maintain nitrite oxidation throughout exposure to elevated antibiotic concentrations. These results suggest that freshwater NOB are resilient and are able to maintain nitrogen cycling—a critical ecosystem service—in the face of antibiotic pollution from wastewater and agricultural runoff.

Toxicological and chemical assessment of water quality downstream of NPDES oil and gas produced water discharges

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Produced water (PW) is the largest waste stream associated with oil and gas operations. This complex fluid contains petroleum hydrocarbons, heavy metals, salts, naturally occurring radioactive (NORMs) and any remaining drilling, stimulation or well maintenance chemicals, as well as their potential transformation products. In the United States, west of the 98th meridian, the federal National Pollutant Discharge Elimination System (NPDES) exemption allows release of PW for agricultural beneficial reuse. Contents and concentrations of chemicals in PW vary by location and time. As a result, treatment strategies vary, and PW NPDES releases are poorly characterized. The goal of this study is to characterize potential environmental impacts and toxicity of PW discharges on downstream water quality. Water samples were collected from three NPDES PW discharges and surrounding watersheds in a Wyoming oil field. Engineered wetlands were located downstream of each discharge. Thus, the efficiency of using wetlands for onsite treatment of PW was also assessed. PW discharge streams were characterized using chemical analyses and toxicological bioassays. Contaminants including benzene, NORMS and surfactants were identified at elevated concentrations at the NPDES discharges. Concentrations of these chemicals generally decreased with increasing distance from the discharge. Hydrophilic compounds, including surfactants, were significantly attenuated in the wetlands. Mutation bioassays revealed higher chronic toxicity at the discharge than implied by chemical analysis, showing that bioassays are a useful tool for assessing complex water quality. The results of this study can be used to help industry and regulators effectively and safely manage PW discharges for agricultural beneficial reuse.