

36th Annual Meeting of the Rocky Mountain Chapter of the Society of Environmental Toxicology and Chemistry

May 8-9, 2025 Alamosa, Colorado

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Dr. Paula Schaffer College of Veterinary Medicine and Biomedical Sciences Colorado State University

Thank you to our host venue for donating meeting space and to our sponsors!









http://rm.setac.org

Day 1: Thursday, May 8, 2025

Meeting:	Career Panel & Networking Social:	Dinner:
9am-4:30pm	5:30-7pm	7-8:30pm
Adams State University	The Roast	
McDaniel Hall Room 101	Private party room	
1500 First Street	420 San Juan Ave	
Alamosa, CO 81101	Alamosa, CO 81101	
Phone #	303-995-4155	
Parking pass required	Immediate parking is limited; carpool or plan extra walking time	
Light snacks provided but lunch on your own	Light snacks and beverages provided	At your expense

Parking Map at Adams State University:

Enter the McDaniel Lot marked by a star (\star) at the bottom center on the map below. Retrieve a parking pass from a RM SETAC representative.



Bring your reusable water bottle to reduce waste.

Day 1: Thursday, May 8, 2025 Agenda

9:00 am	Check in	
9:30 am	Welcome Remarks	
9:40 am	Advancing Toxicology Through The Adverse Outcome Pathway Framework: Applications From Lab to Field	
	Brett Blackwell, Los Alamos National Laboratory	
10:40 am	Poster Session & Break	
11:20 am	Wildfire and mining stressors: macroinvertebrate responses to sedimentation and toxic metals	
	Maricela Alaniz**, Colorado State University	
11:40 am	Toxicity of Direct Potable Reuse Water Compared to Local Drinking Water	
	Elliese Wright**, Colorado School of Mines	
12:00 pm	Topsoil remediation in Pueblo, CO: An analysis of health and socioeconomic	
	impacts	
	Leah Greteman**, Colorado State University Pueblo	
12:20 pm	Lunch On Your Own	
	(BOD Meeting)	
2:00 pm	Preliminary Results: Mixture and Concentration Effects on the Uptake of PFAS	
	from Legacy AFFF Sources	
	Joseph Tucker**, University of Colorado Denver	
2:20 pm	Hazard analysis of PFAS chemicals from Joint Base Cape Cod, MA	
	Kelsey Craig**, Adams State University	
2:40 pm	Poster Session & Break	
	Evaluation of per- and polyfluoroalkyl substances (PFAS) in Eggs of Higher Trophic	
3:20 pm	Level Birds	
·	Shaun A. Roark, Jacobs	
3:40 pm	Multi-omic analysis of gut-brain axis perturbation by neurotoxicant chlorpyrifos	
	Lourse Connette University of Colored Domain	
4:00 pm	Lauren Garrett, University of Colorado Denver RM SETAC 2026 and NA SETAC Updates	
4:20 pm	Awards and Closing Remarks	
4.20 pm	האימו עם מווע כוסטווא ולכווומו הס	

Day 2: Friday, May 9, 2025

Backcountry River Travel And Natural History Of The Rio Grande Narrow Ridge Outdoors - 5 Browns Drive, South Fork, CO

Itinerary:

- 9:00 am Depart Alamsoa for South Park. Travel is in personal vehicles. Let us know if you need transportation and we can help arrange carpooling.
- 10:00 am Arrive Narrow Ridge Outdoors: <u>5 Browns Drive, South Fork, CO</u>.
- 10:00 am 3 ish pm. Gear up, shuttle to river in Narrow Ridge vehicles, Raft.
- 3ish Depart South Fork.
 - Note: if you are returning to the Front Range, the most direct route home does not go back through Alamosa, so plan accordingly with vehicles and carpooling.

Please bring:

- Bring outdoor clothing to be comfortable in a variety of conditions. Weather in the mountains in May is unpredictable! I recommend synthetic or wool base layers, fleece mid-layers, and rain gear if you have it.
- Bring sunglasses, water bottle, sunscreen, and hat.
- Narrow Ridge Outdoors can supply splash gear, neoprene booties, and even wetsuits if you want one.
- Light lunch provided. Gluten free and vegan options will be available. Let us know if you have any additional dietary considerations.

KEYNOTE SPEAKER Brett Blackwell, PhD Los Alamos National Laboratory



Advancing Toxicology Through The Adverse Outcome Pathway Framework: Applications From Lab to Field

The adverse outcome pathway (AOP) framework provides a basis for understanding the potential biological effects chemical stressors pose to organisms, communities, or ecosystems. AOPs can bridge traditional toxicology with emerging data from in vitro and computational methods, supporting a shift away from animal testing toward predictive or tiered testing strategies. An example of an AOP-driven, tiered testing strategy for PFAS will be given as an example of how high throughput screening can be used to inform chemical hazard. AOPs can also play a role in environmental monitoring and provide a basis for identifying hazards from complex chemical mixtures. An example of a wastewater treatment upgrade along the Colorado River will be used to highlight the utility of such an approach. Together, these examples will highlight the value of AOPs and a strategic vision to advance chemical testing into the modern age.

ABSTRACTS

Alaniz, Maricela**

Wildfire and mining stressors: macroinvertebrate responses to sedimentation and toxic metals.

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Wildfire and mining are two major disturbances that degrade freshwater ecosystems, yet their combined effects on watersheds remain poorly understood. This study used a stream mesocosm experiment to examine how fire-related sediment and mining-related metals influence aquatic macroinvertebrate communities. Macroinvertebrates were collected from the upper Arkansas River near Leadville, CO (a historically mined but unburned watershed) and exposed to four treatments: control, metals, sediment, and combined sediment + metals. A double-pulse exposure introduced 2,200 g of sediment per pulse and metals (25 µg/L Cu, 500 µg/L Zn) over 24 hours. Dissolved zinc concentrations in water were lower in the sediment and sediment + metals treatments compared to the metals-only treatment, suggesting metal adsorption to sediment particles. Periphyton biomass was lower in treated mesocosms compared to controls, likely due to scouring from sediment and increased metal toxicity. Macroinvertebrates had higher mortality in all treated mesocosms, with sediment addition potentially having a greater impact than metals alone. Macroinvertebrates were likely affected by habitat loss, scouring from sediment, loss of food (e.g., periphyton), and increased metal toxicity. Compared to controls, macroinvertebrate drift was higher in the sediment and sediment + metals treatments but not in the metals-only treatment. Sediment may have had a greater impact on macroinvertebrates than metals since the Arkansas River has not historically experienced sedimentation, making this a novel stressor that had a more pronounced effect on the community.

Presentation format: Platform

Chavez, Molly and Ellie Heiser**

Analysis of microbial diversity in mountain creeks in the Sangre de Christo and San Juan mountains

Molly Chavez - Adams State University, mollychavez@adams.edu Ellie Heiser - Adams State University, heiserem@adams.edu Megan Garrett - Adams State University, garrettmp@adams.edu Nayeli Vazquez - Adams State University, vazquezn@adams.edu David Bertolatus - Adams State University, dbertolatus@adams.edu

Our presentation examines the microbiome diversity of two mountain streams to understand the health of the ecosystem in the San Luis Valley. For our research, we collected sediment DNA from streams in the San Juan and Sangre de Cristo Mountain Ranges using a commercial kit (PowerBead Pro). We processed the sediment DNA in the lab by doing a PCR test (polymerase chain reaction) to amplify the 16S rRNA genes, which allowed us to then identify the taxonomic units of the bacteria present in the water. We calculated a Shannon diversity index from 16S rRNA sequencing data using Excel to understand the diversity and abundance of bacteria taxonomic groups. For our presentation, we will discuss the relative abundance of alphaproteobacteria, actinobacteria, gammaproteobacteria, spirochaete, and clostridia in both mountain streams. As a group, we further analyzed each bacteria taxonomic unit. For the presentation, we will describe each unit and discuss the ecological significance of these bacteria within the stream sediment environment. We concluded that the San Juan stream had a higher diversity of bacteria, which in some cases can indicate healthier water quality. However, we will elaborate on why this is not always the case and what further research could be done about the microbial diversity to better understand the water guality of our mountain streams. We are motivated to do this research because we care about the ecological impacts on our water systems. We want to better understand the stressors on our environment and how we can monitor and protect the water quality. This research furthers our career interests in the health and environmental sciences by helping us understand the complex relationship between microbes, the ecosystem, and humans.

Presentation format: Poster

Craig, Kelsey**

Hazard analysis of PFAS chemicals from Joint Base Cape Cod, MA

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PFAS chemicals are a group of persistent per- and poly-fluoroalkyl compounds whose effects are difficult to predict and potentially harmful to humans and wildlife. Joint Base Cape Cod, MA contains a groundwater plume contaminated with PFAS from historic fire training activities. Significant research on this groundwater PFAS plume has provided environmental data of PFAS mixture concentrations, and ongoing research is examining the effects of exposure to these mixtures on fathead minnows. However, broader risk assessment is hindered by a lack of established toxicity reference values (TRVs) for mean PFASs. Ankley et al. (2021) published a review of PFAS ecotoxicology, which included an expansive compilation of existing TRVs for different organisms, PFAS congeners, and toxicity endpoints. Here, we have organized and filtered these data to conduct an initial risk assessment of the PFAS mixtures present at JBCC. TRVs from fish exposures for individual PFAS chemicals were sorted by endpoint and averaged, producing an average threshold concentration for each chemical/endpoint pair. Hazard quotients were calculated for individual chemicals and endpoints using environmental concentration from JBCC reported in Barber et al. (2023). To estimate the risk for mixture exposure, hazard indices were calculated by summing the hazard quotients for all measured chemicals within a specific endpoint. Ultimately, this work can define base toxicity values for each PFAS chemical and provide insight on whether a PFAS mixture will produce a toxic result for species exposed. Creating this risk assessment will allow new environmental data to be added and compared to a growing collection of baseline values. This will increase the scope of our understanding and could provide vital insight into PFAS reactions on a landscape or population level.

Presentation format: Platform

Garrett, Lauren G.

Multi-omic analysis of gut-brain axis perturbation by neurotoxicant chlorpyrifos

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The connection between gut activity and neural pathology is a growing field of inquiry. Chlorpyrifos is an organophosphate pesticide with known neurotoxic effects in various non-target aquatic species. Additionally, exposure often causes perturbation of the gut microbiome contributing to intestinal inflammation, increased membrane permeability of the gut mucosa and metabolic disruption. However, what impact dysbiosis has on the development of neurological disorders induced from chlorpyrifos remains elusive. By employing metagenomic and transcriptomic methodologies, we investigated the gut-brain axis by correlating 16S and RNAseg data from the respective gut communities and brain tissues in adult zebrafish exposed to chlorpyrifos across a dose curve (0.0001uM, 0.001uM, 0.01uM, 0.1uM, 1uM). Metagenomic and transcriptomic data were processed using nf-core piplines, amplised version 2.70 and rnased version 3.14, respectively. Differentially abundant features for both datasets (relative abundance and gene counts) were selected using Microbiome Multivariable Association with Linear Models (Maaslin2) by correlation across the dose curve. Original datasets were filtered by the differentially abundant features discovered and then correlated using Hierarchical-all-against-all correlation (HALLA) to determine which relationships between differentially abundant features were significant. We report significant dose-dependent correlations for taxa and genes, as well as significant positive correlations between *Rhodobacter* and genes associated with circadian rhythm (perb1 and crv2) as well as significant negative correlation between *Paraclostridium* and genes associated with lipid catabolism (lipib), the nervous system (fkbp5, slca22.2, slc20a1a), and hypoxia (hif1al). We describe the role of these two novel biomarkers, *Paraclostridium* and *Rhodobacter*, and their association with genes involved in processes previously shown to be altered by exposure to chlorpyrifos.

Presentation format: Platform

Greteman, Leah**

Topsoil remediation in Pueblo, CO: An analysis of health and socioeconomic impacts

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Vivian Carrell, Colorado State University Pueblo

Jim Carsella, Colorado State University Pueblo

Moussa Diawara, Colorado State University Pueblo

Since the 1860s, Pueblo, CO has been a hub for the steel-making industry. Between 1860 and 1912, six smelters operated in the center of town, creating toxic emissions and other byproducts that left a lasting impact. In 2014, the EPA listed an area in the center of town on the National Priorities List, indicating that this Superfund site was one of the 1,200 most contaminated sites in the country, citing high levels of lead and arsenic in topsoils and indoor dust. Remedial action has been ongoing since early 2015, and the EPA reported as of March 2025 that the topsoil cleanup is 94% complete. This study is meant to be a third and final look by this primary investigator into lead contamination and its effects in Pueblo. Researchers took 100 topsoil samples within the site, compiled over 600 child blood lead level (BLL) samples from the Pueblo Department of Public Health and the Environment across a 10-year span, and procured property value data from the Pueblo County Assessor's Office. The goal is to determine whether the success of the remediation can be measured using a multiple linear model predicting BLL based off of soil lead levels, property values, age, and location. Our findings aim to provide an independent look into the current state of the Superfund site using our methods. Data is in the midst of being analyzed and results are pending.

Presentation format: Platform

Hawkins, Fiona**

Chemical-Gene Interactions of PFAS chemicals

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PFAS (per- and polyfluoroalkyl substances) are a group of human-made chemicals used in a variety of products for their water- and oil-repellent properties. They have strong carbon-fluorine bonds which cause them to degrade slowly and they are persistent in the environment and living organisms . PFAS have been linked to several health issues, including cancer, liver damage, and immune system disruption. Despite being linked to these health issues, the exact mechanisms by which they act are unknown. This research project aims to explore the genetic impacts of PFAS exposure and to develop genetic markers for different PFAS compounds. Data on chemical-gene interactions for twenty three PFAS structures were gathered from the Comparative Toxicogenomics Database. Understanding the interaction between chemicals and genes, as well as the effects of chemical mixtures, can reveal the mechanisms through which these substances cause changes in organisms.

Presentation format: Poster

la, Ly and Sarah Santana**

Companion Animal Toys & Their Potential Toxicity

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Pet ownership is a cornerstone of American life, with approximately 70% of U.S. households owning at least one pet and national spending on pet care reaching an estimated \$147 billion in 2023. Among pet products, rubber-based toys are especially popular due to their durability and dental health benefits. However, unlike children's toys, pet toys are minimally regulated, raising concerns about the potential presence of hazardous chemicals. We have assessed the toxicological risks associated with common compounds found in rubber pet toys, with implications for both companion animal and human health. Several chemicals of concern were identified in common rubber pet toys, including methylene diphenyl diisocyanate (MDI) and 1,4-butanediol (BDO), both associated with adverse health effects in animal models and potential occupational risks for manufacturing workers. These findings point to significant gaps in the regulation of pet products and a lack of standardized safety testing. To address these concerns, we have highlighted a few recommendations including greater transparency in material disclosure, stricter oversight in manufacturing practices, and further research into the long-term exposure effects on companion animals. Through the strengthening of regulatory framework in pet products for both occupational safety and general usage of the product for industry personnel and consumers humans and animals will be better protected.

Presentation format: Poster

Jalali, Jacob

Analysis of PFAS in surface water and wastewater samples by automated solid Phase extraction and QSight LC-MS/MS based on US EPA method 1633

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Per- and polyfluoroalkyl substances (PFAS) are a group of human-made fluorinated organic compounds. Due to their unique chemical properties, long-term persistence in environment, and associated risks for human health, PFAS have been classified as persistent organic pollutants and have become the current hot topics around the world. Since surface waters are the primary sources of drinking water in many areas around the world, there have been many studies on PFAS exposure in these water resources and the PFAS concentrations were found in the range from low ng/L to µg/L levels. In this study, seven surface water samples collected in Toronto Lakeshore area (Lake Ontario, Humber Bay River, Mimico Creek, and Grenadier Pond) and eight wastewater samples from Ontario, Canada were analyzed using automated SPE and QSight LC-MS/MS based on US EPA1633 method.

Presentation format: Poster

Pieper, Nick and Devin Waters**

Grazing impact on water quality through macroinvertebrate analysis

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How does grazing impact water quality? We can assess this by examining macroinvertebrate communities due to their lack of mobility as well as the different tolerances that each taxon has to pollutants. Macroinvertebrate communities of two sites were sampled: North Crestone Creek (NCC), where grazing is prohibited, and San Francisco Creek (SFC), where grazing is allowed. Macroinvertebrate samples were taken using D-frame nets within a 100-meter section of each stream. Afterward, the macroinvertebrates were identified to the order level and enumerated. Four biotic indices were calculated to assess the macroinvertebrate community: percent Ephemeroptera, Plecoptera, and Tricoptera (%EPT), Macroinvertebrate Biotic Index (MBI), Shannon Diversity Index (H), and Shannon Evenness Index (Evenness). EPT taxa represent the most sensitive to pollution, so the %EPT gives an idea of what percentage of the community is composed of sensitive taxa. MBI represents the average sensitivity of macroinvertebrate communities. Shannon Diversity Index represents the general community diversity, and evenness represents the general equality within the community between taxa. %EPT was similar for both streams (68.9% at NCC and 63.7% at SFC). The results were also similar for MBI (NCC, 4.00 and SFC, 4.38). The last two results were the Shannon Diversity Index and the evenness. NCC resulted in an evenness of 0.264, whereas SFC's evenness was 0.283. NCC resulted in an H of 1.872 and SFC in an H of 1.902. Both streams sampled indicated a moderate water guality based on macroinvertebrate analysis, with no clear evidence that grazing affects water quality at these two sites during this time. However, further research could help to confirm these findings.

Presentation format: Poster

Roark, Shaun A.

Evaluation of per- and polyfluoroalkyl substances (PFAS) in Eggs of Higher Trophic Level Birds

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The goal of this ongoing study is to evaluate exposure of higher trophic level, water-associated birds, common loon (*Gavia immer*), double-crested cormorant (*Nannopterum auritum*), and osprey (Pandion haliaetus), to per- and polyfluoroalkyl substances (PFAS). PFAS have been measured in archived, abandoned eggs collected throughout Maine using high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS). To-date, analyses have been completed on 17, 14, and 32 loon, cormorant, and osprey eggs, respectively. PFOS was the most abundant compound in all eggs, comprising over 50% of total PFAS in cormorant samples, and exceeding a proposed egg toxicity reference value (TRV) of 92.4 ng/g wet weight in approximately 20% of samples. Several other persistent compounds were frequently detected, with some displaying species specificity. For example, 9Cl-PF3ONS and 11H-PF3OUdS were found only in osprey eggs, while ADONA was unique to loon eggs. Whole egg PFAS concentrations were compared to available chemical-specific bird egg TRVs. Embryonic development assessed according to a scaled system of embryological stage, will be compared with PFAS concentrations to assess the correlation of PFAS concentration and development stage at death. We will use findings to evaluate application of laboratory-based TRVs to wild bird populations and guide further investigation of PFAS exposure and effects in birds. Results will aid in understanding PFAS contamination and accumulation within aguatic ecosystems, inform PFAS risk assessments, and provide insights for contaminated site investigations.

Presentation format: Platform

Tucker, Joseph **

Preliminary Results: Mixture and Concentration Effects on the Uptake of PFAS from Legacy AFFF Sources

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Historical use of aqueous film-forming foams (AFFFs) during fire training activities at Joint Base Cape Cod has led to contamination of groundwater by complex mixtures of per- and polyfluoroalkyl substances (PFAS) near these fire training areas (FTA). In order to evaluate the uptake/depuration kinetics and effects of complex mixtures of PFAS, a series of fish exposure experiments were conducted using on-site, purpose-built, fish exposure mobile laboratories. Three groundwater wells, including one reference (REF) and two PFAS contaminated water sources (FTA-1, and FTA-2), were used as environmental source waters for a series of continuous-flow fish exposure experiments using a unique pair of on-site purpose-built mobile laboratories. Adult male fathead minnows were exposed to 100% REF, 90% REF:10% FTA-1, 100% FTA-1, 90% REF:10% FTA-2, or 100% FTA-2 for up to 6 weeks. After two weeks of exposure to contaminated water, half of the exposed fish were moved to additional aquaria receiving just REF water. Water and fish carcasses were sampled weekly for analysis of up to 40 PFAS for determination of mixture- and concentration dependent effects on PFAS uptake rates, depuration rates, and bioconcentration factors. Preliminary results on concentration- and mixture- dependent uptake of PFAS will be presented.

Presentation format: Platform

Vajda, Alan

<u>Reproductive disruption in fish exposed to PFAS-contaminated groundwater at a legacy</u> <u>fire-training area</u>

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants with recognized reproductive toxicity, yet the ecological impacts of complex PFAS mixtures remain insufficiently understood. This study evaluated the reproductive effects of PFAS-contaminated groundwater from a legacy fire-training area on male fathead minnows (*Pimephales promelas*) using a field-based flow-through exposure system conducted over three years (2018, 2019, 2021). Groundwater exposure ranged from Σ PFAS concentrations from 100 ng L⁻¹ to 72,800 ng L⁻¹, with PFOS, PFOA, and PFHxS as dominant contributors. Sperm motility declined by up to 50% at Σ PFAS concentrations of 20,000 ng L⁻¹ (FTA-1). At higher concentrations, such as 36,400 ng L⁻¹ (FTA-2 50%) and 72,800 ng L⁻¹ (FTA-2 100%), effects were more severe, including up to 35% mortality. Additional reproductive impairments included suppression of secondary sex characteristics, increased hepatosomatic index, reduced sperm density, and testicular apoptosis. Transcriptomic analysis of tests from FTA-2-exposed fish identified 1,373 differentially expressed genes, implicating disruptions in pathways related to oxidative stress, mitochondrial dysfunction, and immune regulation. Key upregulated genes included *nfkbil1* (+4.3-fold) and *gaa* (+3.8-fold), while *trip4* (-3.2-fold) and cox4nb (-2.7-fold) were downregulated. Mixtures enriched in PFOA and precursors such as FBSA were more toxic than those with similar concentration but dominated by PFOS. These findings provide critical insights into the reproductive toxicity of PFAS-contaminated sites and underscore the importance of considering mixture-specific effects in ecological risk assessments.

Presentation format: Poster

Wright, Elliese**

Toxicity of Direct Potable Reuse Water Compared to Local Drinking Water

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Tzahi Cath, Colorado School of Mines

James Rosenblum, Colorado School of Mines

Direct potable reuse (DPR) is emerging as an essential part of the solution to the increasing water scarcity in the Western United States and other parts of the world, but DPR presents unique treatment challenges. Wastewater, the impaired source water commonly used in potable reuse, can contain many types of contaminants of emerging concern (CECs) such as pharmaceuticals and per-fluoroalkyl substances (PFAS). The vast majority of these contaminants are unregulated in drinking water by the US EPA. In direct potable reuse systems, CECs are not diluted or naturally attenuated like they are in indirect reuse systems. Therefore, direct potable reuse water requires intensive monitoring to ensure that CECs are being removed to an acceptable level. The DPR Mobile Demonstration Lab, a mobile pilot system from Colorado School of Mines, is being used to study these types of problems. The DPR mobile demonstration lab treatment train consists of ozonation, biofiltration, ultrafiltration with coagulation, granular activated carbon (GAC), UV light and advanced oxidation, and chlorination.

Due to the high concentration of unregulated organic contaminants in DPR source water, it is important to rethink how DPR water is judged as safe and how it is compared to traditional drinking water. Previous research on the DPR Mobile Lab has used a combination of targeted chemical analysis and single-gene *in-vitro* bioassays to demonstrate that CECs and specific gene activities (estrogenic and aryl hydrocarbon) are completely removed through the treatment train. Complete removal has been demonstrated with relatively new GAC media, but after a certain number of bed volumes of water have passed through the GAC columns some CECs start to break through the columns and can be detected in the effluent. At this point, the GAC media will need to be replaced. As GAC-based DPR is more widely implemented, it will be critical to know exactly when to replace media to maximize the safety of consumers and minimize cost of media.

This next project used a toxicity-based water index and *in-vitro* bioassays to compare the DPR water as the GAC columns break through to local drinking water. The water index will be determined by dividing each chemical concentration by a standard toxicity measure and adding the values for each chemical together to get one value per sample. This will be used together with the data from the in-vitro bioassays.

This study was able to determine the level of TOC breakthrough at which the DPR treated water became worse quality, based on the water index and in-vitro bioassays, than the local drinking water. This study also determined what the most toxic compounds in the DPR treated water were and compared them to the most toxic compounds in local drinking waters. Next, this project demonstrated that activity measured in an in-vitro bioassay increases as the water index increases, which happens as the GAC is used up. Finally, this project demonstrated that TOC breakthrough in a GAC based DPR system is correlated with CEC breakthrough and *in-vitro* bioassay activity.

Presentation format: Platform