Office of Research and Development

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM

Water Systems

Project 1: Current Systems and Regulatory Support

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Task 1A – Current Resource Water (RW) Treatment Processes and Resource Recovery

Water Systems

1. Evaluating microbial and chemical contaminants in waste treatment streams

2. Antibiotic resistance in RW effluents that are discharged into the environment or recycled

3. Safe and sustainable management of waste residuals

Task Lead: Eric Villegas

1. Evaluating Microbial and Chemical Contaminants in RW Treatment Streams

Water Systems

FY16 Key Activities/Products/Impacts

- **Regional Applied Research Effort (RARE) with R6, NERL, NRMRL**
  - Characterization of microbial pathogens and emerging chemicals of concern (CECs) in TX RW effluents to refine risk assessment models for direct potable reuse.

- **Comprehensive study on microbial communities and CECs in RW effluent (FY16/17)**
  - High throughput sequencing to characterize bacteria, viruses, and protozoa.
  - Monitor pathogen community dynamics through the treatment stream.
  - Assess treatment efficacies of traditional WW treatment plants in the US that are considering water reuse.

2. Antibiotic Resistance in RW Effluents that are Discharged into the Environment or Recycled

Water Systems

FY16 Key Activities/Products/Impacts

- **Research on understanding the diversity and fate of ARBs in RW systems and elucidating mechanisms and transfer rates of antibiotic resistant genes**
  - Characterize the relative contribution of RW treatment systems on the persistence and transmission of antibiotic resistant organisms in the environment.
  - Manuscript (FY17) providing data on the prevalence and human health impact of antibiotic resistant bacteria (ARB) in RW systems
  - Final product from this research in FY18.

3. Safe and Sustainable Management of Waste Residuals

Water Systems

FY16 Key Activities/Products/Impacts

- **Semi-continuous activated sludge reactors established to characterize transformation and fate of perfluorinated alkyl substances (PFAS)**
  - Study completed. Data currently being analyzed to support future OW guidance.

- **Part 503-The Biosolids Rule**
  - Revise current guidance on treatment requirements for land application of biosolids. The most recent version of the rule was published in 2003.
  - Current research assesses newer and more accurate methods for monitoring the microbial characteristics of residuals for land application.
  - Research results (FY19) will provide support to program offices for updating lists of methods for compliance monitoring.

Task 1B – Monitoring/Analytical Tools

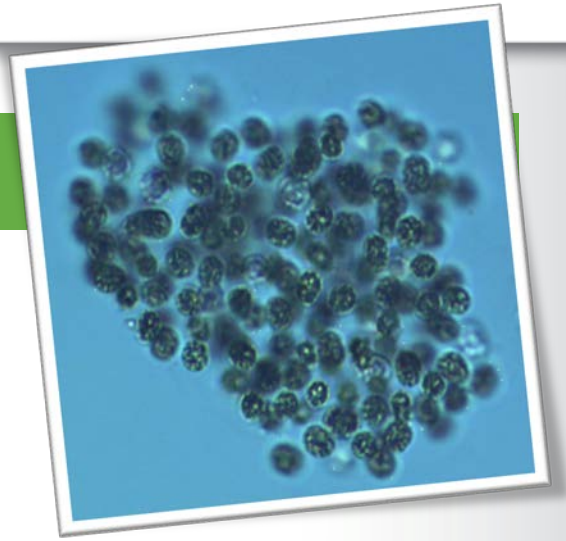
Water Systems

1. Methods development (chemicals and microbes)

2. Environmental/occurrence monitoring

Task Lead: Jody Shoemaker

1. Methods Development



FY16 Key Activities/Products/Impacts

- **Method 544-Microcystins (6 congeners) and nodularin in DW**
  - Public comment period complete. Completed and posted on-line.
  - Proposed for UCMR 4.
- **Chemical methods development for UCMR initiated for saxitoxin and related cyanobacterial neurotoxins**
  - Laboratory experiments began in July 2016; evaluating solid phase extraction sorbents.
- **UCMR method development for Legionella and Mycobacteria**
  - Methods are still undergoing verification, in second phases (extraction efficiency).
  - Recruiting for external participants in multi-laboratory study.

1. Methods Development

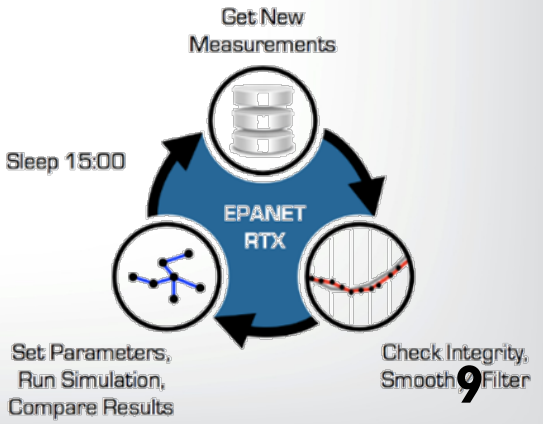
FY16 Key Activities/Products/Impacts

- **UCMR method for protozoa**
  - Method development to assess *Toxoplasma gondii* oocyst densities to support potential future ambient and drinking water regulations.
  - Conducted initial survey of marine and freshwater in coastal CA and the DE River watershed. Results revealed the presence of *T. gondii* oocysts, which can pose a significant human health risk in those regions.
- **Application of a salivary antibody multiplex immunoassay to assess exposure to microbial contaminants in premise plumbing**
  - Method development of a plasma and saliva test for 26 pathogens using antigens coupled to MicroPlex beads is complete. Premise plumbing saliva samples will be collected and analyzed by this method for the target pathogens in the near future.
- **Experiments on relative source contribution of Sr in contaminated DW (Late FY16)**

2. Environmental/Occurrence Monitoring

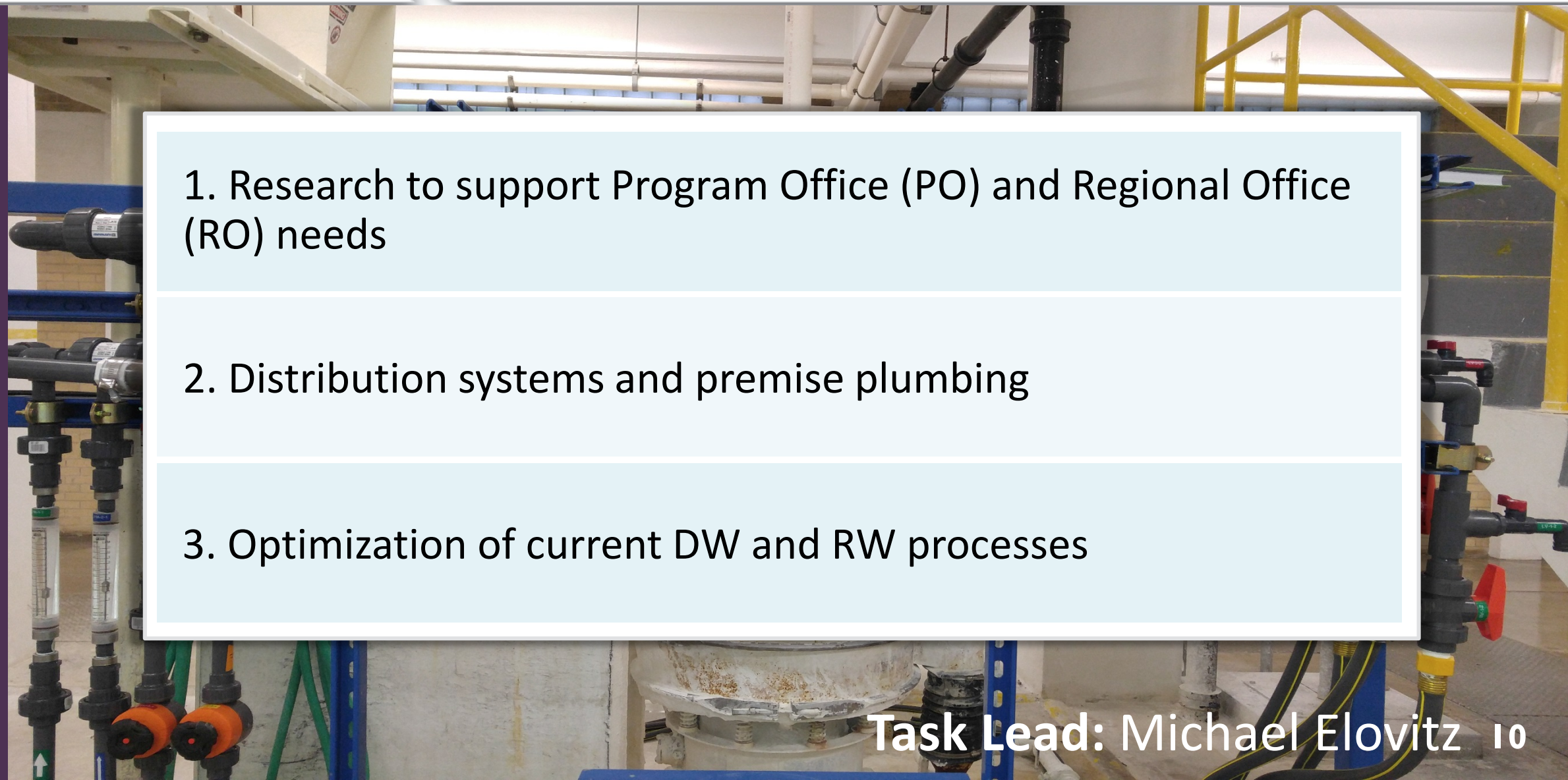
FY16 Key Activities/Products/Impacts

- **Water quality parameters and their influence on *Legionella* and non-tuberculous Mycobacteria (NTM) occurrence in water**
  - Research completed. Data being analyzed for journal publication.
- **Potable water occurrence study of NTM**
  - Research completed. Manuscript published in FY15: Donohue et al. (2015). Increased Frequency of Nontuberculous Mycobacteria Detection at Potable Water Taps within the United States. *Environ Sci Technol*. 19;49(10):6127-33.
- **Small DW distribution system modelling and assessment**
  - EPANET-RTX software development to link utility real-time monitoring data with a network infrastructure model to provide real-time water system status: *Analyze past operations and events, predict system behavior, simulate and evaluate "what-if" scenarios.*



Task 1C – Water Treatment

Water Systems



1. Research to support Program Office (PO) and Regional Office (RO) needs

2. Distribution systems and premise plumbing

3. Optimization of current DW and RW processes

Task Lead: Michael Elovitz

1. Research to Support PO/RO Needs

FY16 Key Activities/Products/Impacts

- **Flint, MI and the Lead and Copper Rule**
  - ***Technical support to Regions and OW***
    - Installation of pilot-scale pipe testing rig on-site in Flint to test optimal water quality for maintaining disinfectant levels and minimizing lead release.
    - Ongoing analysis of Pb pipe samples from Lake Mills (WI), Providence (RI), Flint (MI), Lansing (MI), Downer's Grove (IL), and Chicago (IL).
    - Pb profile sampling of houses with lead pipes in Cincinnati and Dayton (OH).

1. Research to Support PO/RO Needs

FY16 Key Activities/Products/Impacts

- **Flint, MI and the Lead and Copper Rule (cont.)**
  - ***Development of sampling protocols for identifying lead service lines***
    - Publication evaluating sampling methods and supporting techniques for tackling lead in DW in Alberta Province (*J. Water Supply*: Research and Technology-Aqua, 65(5):373-383)
    - Development of sampling protocols for identifying lead service lines. Report/ Standard Operating procedure targeted for end of 2017.
  - ***Development of improved exposure model to support OW decision on a Household Action Level for the revised LCR***
    - Couple the Stochastic Human Exposure and Dose Simulation (SHEDS) and the Integrated Exposure Uptake Biokinetic Model (IEUBK) models for prediction of DW lead concentrations leading to specific blood lead levels in children, infants, and adults

1. Research to Support PO/RO Needs

FY16 Key Activities/Products/Impacts

- **Perfluorinated alkyl substances (PFAS)**
  - Provided technical support to OW for DW Health Advisories for PFOA and PFOS toxicity studies and best available technologies for DW treatment.
- **Cyanotoxins**
  - Technical support to City of Toledo for optimizing DW treatment for microcystins.
- **Brominated Disinfection Byproducts (DBPs)**
  - Assessment of surface water bromide inputs from coal fired generating stations:
    - Coal generating stations use bromide additives to meet air pollution goals set by the Mercury Air Toxics Standards Rule.
    - Generating station WW samples will be collected in 2017 and 2018 to quantify bromide discharges.

2. Distribution Systems and Premise Plumbing

FY16 Key Activities/Products/Impacts

- **Tap and shower head sampling in building premise plumbing**
  - The overall occurrence and cell-equivalent quantities (CE L-1) of Mycobacterium spp. were highest (100%, 2.1x104), followed by *Vermamoeba vermiformis* (75%, 54), *Legionella* spp. (63%, 17), *P. aeruginosa* (12%, 3), and Acanthamoeba spp. (3%, 1).
  - Journal article in review: *Int. J. Environ.* Res. Public Health.
- **Distribution systems**
  - Publication: Resilience of microbial communities in a simulated drinking water distribution system subjected to disturbances: Role of conditionally rare taxa and potential implications for antibiotic-resistant bacteria. *Environ. Sci.: Water Res. Technol.* 2016(2):645-657.
  - Results show that the core distribution system microbiome is complimented by conditionally rare taxa that are low in number but increase under favorable conditions.
  - Rare taxa include microbes with unique functionality (e.g. nitrification, antibiotic resistance).

2. Distribution Systems and Premise Plumbing

FY16 Key Activities/Products/Impacts

- **NCER funding for examining the impacts of water conservation on water quality in premise plumbing and water distribution systems**
  - Water conservation trends over the past 20 years.
  - Research on impacts from low-flow fixtures, pipe materials, water chemistry, and water treatment on overall water quality delivered to consumers.
  - Development of a decision support tool to “right-size” plumbing and distribution systems with the “right” materials.

3. Optimization of Current DW & RW Processes

FY16 Key Activities/Products/Impacts

- **Maintenance and updates to EPA's Treatability Database**
  - Publically accessible data base for identifying effective technologies for removing chemical, microbial, and radiological contaminants (iaspub.epa.gov/tdb/pages/general/home.do).
  - Future plans to merge with OW cost models.
- **Evaluation of ultraviolet (UV) disinfection techniques for traditional WW treatment plants using bacteriophage to model treatment effectiveness**
  - Determination of dose-response curves for inactivation of multiple bacteriophages and dose required for 4-log inactivation: MS2 bacteriophage determined to be the most conservative indicator organism.
  - Metagenomic analyses to assess changes in phage diversity under different treatment conditions.
  - Compare predicted and observed photoinactivation of phages in field treatment systems.

3. Optimization of Current DW & RW Processes

FY16 Key Activities/Products/Impacts

- **UV disinfection in DW: Objectives**
  - Evaluate approaches for validating low pressure (LP) and medium pressure (MP) UV reactors for Adenovirus inactivation using MS2 and *Bacillus pumilis* test microbes.
  - Evaluate UV lamp monitoring sensor technology that accounts for contributions of low-and high-wavelength UV light for MP reactors, and assess algorithms for UV dose-monitoring (Crypto, Giardia, Adeno).
  - Develop ORD/OW recommendations document from lessons learned applicable to the Ground Water Rule/Surface Water Treatment Rule.
  - Work with EPA Water Technology Innovation Center, Regional State Regulatory Agencies & water utilities on harmonization of review/permitting protocols.

3. Optimization of Current DW & RW Processes

FY16 Key Activities/Products/Impacts

- **UV disinfection in DW: Results**
  - Use of Adeno microbes in conventional validations is impractical; if used the dataset should be large to assess high point-to-point variability/uncertainty.
  - >160 UV reactor runs conducted to-date varying flowrate, lamp output, UVT, and sleeve types.
  - In both LP & MP analyses, MS2 microbes alone provided good correlations and conservative predictions of Adeno inactivation, better than B. pumilus alone or combined with MS2.
  - A low-wavelength sensor paired with typical ÖNORM sensor monitoring appears to be effective, and combined with the analysis algorithm & Action Spectra Correction Factors, maps microbe datasets well, and shows promise for use in predicting Crypto & Adeno scenarios with test microbes.

Task 1D – Health Effects for Regulatory Decisions and Guidance

Water Systems

1. Regulated DBP's relation to bladder cancer
2. Regulated DBP exposure and colon cancer
3. Regulatory technical support
4. Internal dose/multi-route exposure from regulated DBPs through physiologically-based pharmacokinetic models
5. Impacts from varying source water quality and disinfection scenario on health impacts from DBPs

Task Lead: Jane Ellen Simmons

1. Regulated DBP's Relation to Bladder Cancer

Water Systems

FY16 Key Activities/Products/Impacts

- Human urothelial cells were engineered to stably overexpress Glutathione S-Transferase Theta 1 (GSTT1) a protein coding gene that plays a role in human carcinogenesis.
- Cytotoxicity of both bromodichloromethane (BDCM) and dibromochloromethane (DBCM) and induction of micronuclei were greater in cells that over-express GSTT1compared to parent cells (Pegram et al., 2016. Brominated trihalomethane toxicity in human urothelial cell lines. *The Toxicologist* 150: 96).
- This is the first report of BrTHM-induced genotoxicity in human urothelial cells, and the first demonstration of GSTT1-dependent BrTHM genotoxicity in eukaryotic cells.
- These findings provide evidence toward establishment of the biological plausibility of a hypothesis linking drinking water exposures to these prevalent DBPs to the etiology of human bladder cancer.
- Actual media concentrations were determined in the micronucleus assay system; this will greatly enhance our ability to extrapolate from this in vitro assay to humans.

2. Regulated DBP Exposure and Colon Cancer

FY16 Key Activities/Products/Impacts

- Development of updated and improved human colonocyte cytotoxicity assay.
  - Decreased variability, improved reproducibility, and more time-efficient for increasing assay throughput.
  - This task is currently in transition from method development to the data collection phase, where individual DBPs and environmentally realistic mixtures of DBPs will be assessed.

3. Regulatory Technical Support

Water Systems

FY16 Key Activities/Products/Impacts

- Technical support to the 6-Year DBP rule process, including being members of the Support Document writing team.

4. Internal Dose/Multi-Route Exposure from Regulated DBPs through PBPK Models

FY16 Key Activities/Products/Impacts

- A human PBPK model for bromodichloromethane (BDCM) was developed that is a significant advancement as it uses human-derived BDCM chemical-specific parameters.
- Analyses with the refined PBPK model emphasizes the need to consider the contribution of multiple routes of exposure to BDCM because dermal and inhalation exposures contribute significantly to internal dose, in particular for target organs other than liver (e.g. bladder, reproductive organs).
- The model provides an improved tool for assessing total exposure from all routes, which will facilitate and improve future evaluations of health risks from BDCM.
  - Kenyon, E.M., Leavens, T.L., Eklund, C., and Pegram. R.A. (2016). Development and Application of a Human PBPK Model for Bromodichloromethane to Investigate the Impact of Multi-Route Exposure. *Journal of Applied Toxicology,* 36(9):1095-111. doi: 10.1002/jat.3269.

5. Impacts from Varying Source WQ & Disinfection Scenarios on Health Impacts from DBPs.

Water Systems

FY16 Key Activities/Products/Impacts

- Integrated chemical and toxicological evaluation (in vitro, alternative rapid in vivo) assessment of the differing impacts of disinfection by chlorine or chloramine on the same source water; majority of chemistry and toxicology assays completed.
- Currently ongoing integrated assessment of the impact of varying levels of bromide and iodide on the DBPs formed and the toxicity of the mixtures formed during chlorination and chloramination.
- Developed methods for quantifying haloacetaldehydes and applied them to geographically diverse regions and linked source and drinking waters disinfected by either chlorine or chloramines.

Project Summary

Task 1A-RW	• Characterization of microbial communities • ARB • Biosolids (PFAS)
Task 1B-Monitoring and Analytical Methods	• UCMR/CCL • Advancing methods (e.g. salivary immunoassays) • Opportunistic pathogens (Legionella, Mycobacteria) • Distribution system modeling network for monitoring
Task 1C-Treatment	• LCR • PFAS • Cyanotoxins • Distribution Systems-opportunistic pathogens • Treatability database • UV
Task 1D-Health Effects	• DBPs • Application of cell-based assays • PBPK modeling • Source water impacts: iodinated and brominated DBPs