Biogeochemical Transformations at Critical Interfaces

ORNL Mercury Science Focus Area (SFA) 2017 Annual Report

Mercury Program Overview

Anthropogenic releases and changing environmental conditions profoundly affect the biogeochemical cycling of trace metals, such as mercury (Hg). Mercury can be methylated to form methylmercury (MeHg), a neurotoxin that bioaccumulates in the food web, endangering humans and other biota. While Hg contamination in most natural environments is mainly the result of atmospheric processes (Mason et al. 2006; Mason et al. 2002; Fitzgerald and Lamborg 1998; Lindberg and Stratton 1998), mining and industrial processes can lead to severe local pollution. The United Nations Environment Programme (UNEP) recently highlighted the risk of this contamination to human and ecosystem health (UNEP 2013). On the Oak Ridge Reservation (ORR), for example, Hg pollution in the East Fork Poplar Creek (EFPC) watershed is caused by historical Hg use at the Y-12 National Security Complex where large quantities of Hg were lost to the environment during the 1950s and 1960s. Understanding the watershed-scale processes that control Hg fate and transformation in these systems is critical to generating new tools, knowledge, and remediation approaches that will enable efficient cleanup of one of the most pressing environmental challenges facing the Department of Energy (DOE), the United States, the state of Tennessee, and the city of Oak Ridge: remediating Hg contamination on the ORR.

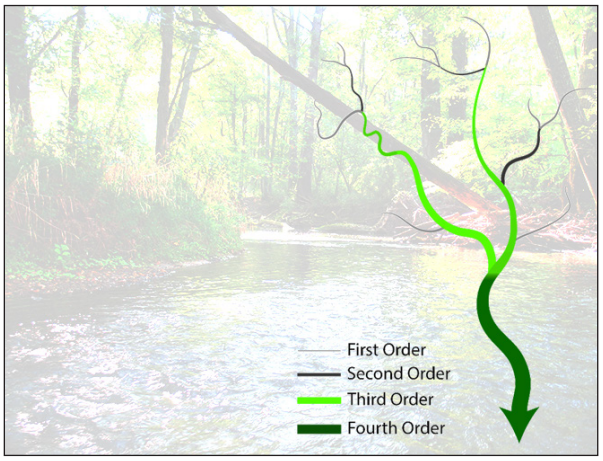

Fig. 1: Low-Order Streams. Streams are ranked based on a hierarchical network of channels within a watershed. Low-order streams (i.e., first- through fourth-order streams) are located in the headwater areas and typically convey small volumes of water. Such streams play a dominant role in the flow, biogeochemistry, and water quality of downstream high-order reaches. East Fork Poplar Creek (EFPC) is a third-order stream being studied as a representative use case.

Developing a process-rich, predictive capability that integrates field, laboratory, and modeling studies of Hg fate and transformation dynamics across broad spatiotemporal scales in low-order streams is the overarching aim of the Critical Interfaces Science Focus Area (CI-SFA) at Oak Ridge National Laboratory (ORNL). The project's overarching aim will be accomplished over three successive 3-year phases. The **Phase I** focus is to **determine the fundamental mechanisms and environmental factors that control Hg biogeochemical transformations at key interfaces in terrestrial and aquatic ecosystems.**

Low-order freshwater streams, such as EFPC (the project's representative use case), constitute nearly 90% of the total stream length in the United States and are the most frequently occurring stream type (>85%; see Fig. 1, this page). Because of their low hydraulic radius (cross-sectional area and wetted perimeter) and low

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average water velocity, these stream systems have high water–sediment contact times, which promote in-stream biogeochemical interactions and exchanges (see Fig. 2, this page). Questions being addressed in **Phase I** of the CI-SFA plan include:

- What is the role of EFPC periphyton biofilms in Hg transformations? Which Hg-methylating microbial groups dominate in different EFPC ecosystem compartments?
- What are the key geochemical and biochemical variables, and how do their interactions affect mercury-dissolved organic matter (Hg-DOM) complexation, Hg-cell surface interactions, cellular uptake, and methylation?
- How do cell-cell interactions and microbial community metabolism influence net MeHg production? What is the native function of Hg-methylation gene pair *hgcAB*?
- Which metabolic pathway feeds into reactions involving the HgcAB protein? What are the molecular-scale drivers that control the behavior of Hg-natural organic matter (Hg-NOM) complexation?

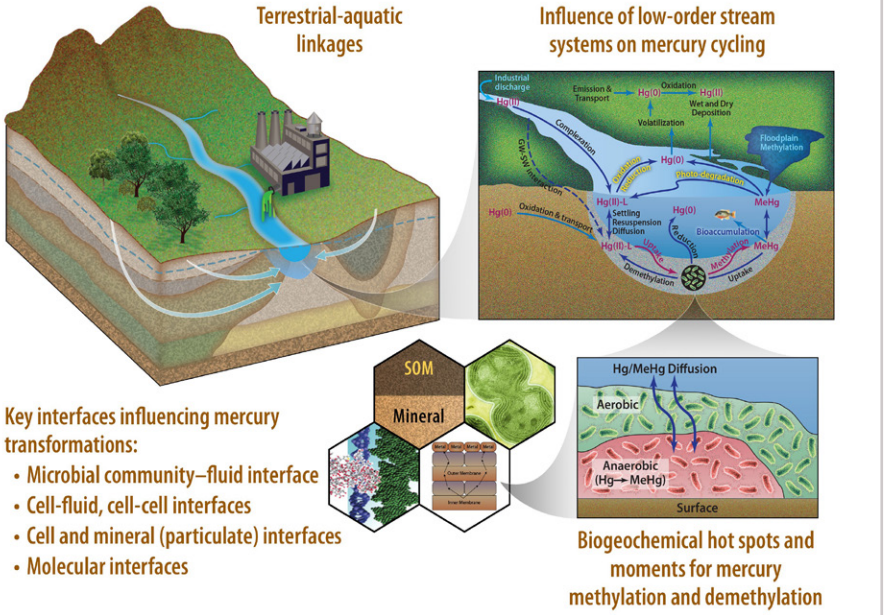


Fig. 2. Key Biogeochemical Interfaces. Interfaces are common boundaries between two or more system compartments or phases where steep gradients develop and govern the fate and transformation of material crossing those gradients.

Accomplishments from July 2016 to June 2017 include a series of recent publications that highlight the role of in-stream processes, specifically algal biofilms (periphyton), on net MeHg production in EFPC; the discovery of an iron-reducing bacterium *Geobacter bemidjiensis* Bem capable of both methylating Hg and degrading MeHg; the design of *hgcAB* biomarkers, and first-of-a-kind measurements of Hg-DOM complexes present in EFPC that influence Hg bioavailability. In addition to these publications, new results collected include characterizing mercury sulfide (HgS) aggregates isolated from EFPC soils, delineating the global proteomic profiles of *Geobacter sulfurreducens* PCA after *hgcAB* gene deletion, elucidating the roles of thiol ligands in Hg cellular sorption and bioavailability, applying the *hgcAB* biomarkers to a range of environmental systems [e.g., EFPC and Spruce and Peatland Responses Under Changing Environments (SPRUCE)], probing the transformation mechanisms from dimethylmercury to MeHg with density functional theory (DFT), and developing a prototype simulation tool to predict Hg fate and transformation in low-order streams. Although the CI-SFA uses Hg and EFPC as representative use cases, the information generated and the integrated multiscale approach can be extended to understanding biogeochemical processes that affect fate, toxicity, and fluxes of nutrients and other trace metals and radionuclides in complex, heterogeneous, and multiscale environmental systems.

Snapshot of FY2017 Accomplishments

See Appendices A–D, pp. 21–24, for details on progress from July 2016 to June 2017, including:

- 11 papers published or in press
- 5 submitted manuscripts
- 2 book chapters published
- 19 presentations, abstracts, or posters delivered or accepted
- 5 invited talks

The activities outlined in the CI-SFA Phase I Science Plan represent collaborative and complementary research that supports four thrusts:

- Ecosystem Features Influencing Hg Transformation
- Biogeochemical Mechanisms Controlling Hg Uptake and Methylation
- Microbial Community Functions and Geochemical Influences on Hg Transformations
- Molecular Structure, Dynamics, and Mechanisms of Hg Transport and Transformations

This annual report summarizes accomplishments from July 2016 to June 2017, a period representing the second year following the program's triennial review in April 2015 and proposal acceptance in August 2015 by the DOE's Office of Biological and Environmental Research. The ORNL CI-SFA program produced a total of 26 new peer-reviewed publications during the initial 2 years of this 3-year renewal cycle for a total of 85 publications since the program began in 2009.

Scientific Progress

Task 1: Ecosystem Features Influencing Mercury Transformation

The general objectives of Task 1 in the CI-SFA project are to (1) identify ecosystem compartments (e.g., channel margin, floodplain, periphyton) and hydro-biogeochemical conditions that govern net MeHg concentration in EFPC, and (2) understand the extent to which groundwater–surface water exchange drives Hg transformations in EFPC. These objectives are addressed through a set of hypotheses-driven field and laboratory investigations. Additionally, we are developing a process-rich numerical model to integrate past results and challenge our current understanding of watershed processes occurring through space and time. The model will be used in an iterative fashion with experiments to help inform the design of experiments and subsequently to refine the model based on experimental results. Task 1 research examines the biogeochemical controls on Hg methylation and demethylation within the context of the flowing creek system and its connection with the surrounding watershed. Emphasis is on field-based investigations with supporting laboratory work to elucidate mechanisms.

FY16–FY17 Accomplishments

Working from the watershed to the laboratory microcosm scale, significant progress was made toward Task 1 milestones, with two papers and three technical reports published and five poster presentations. Detailed experiments carried out across all four seasons provided new insights into the role of periphyton as sources of MeHg to EFPC (Olsen et al. 2016). Mercury transformations are one of many element and nutrient transformation cycles in low-order, freshwater streams. Mercury cycling in these systems is intimately linked with other element cycles (e.g., those of carbon and sulfur). Numerous techniques are used to measure nutrient uptake metrics and kinetics at the reach scale (on the order of hundreds of meters in stream length). However, the uncertainty in these estimates is rarely evaluated; moreover, these estimates are frequently based on simplifying limiting assumptions or direct violations of the underlying statistical theory. We developed a robust Monte Carlo–based method to quantify uncertainty in nutrient-uptake metrics (e.g., ambient uptake lengths and maximum areal uptake rates) that is free of these limitations. The approach is generally applicable to other metrics and provides a foundation for evaluating our hypothesis regarding the relationship between MeHg concentration and reach-scale estimates of whole-stream metabolism (Brooks et al. 2017).

Role of Periphyton in EFPC Mercury Cycling

Previous work in EFPC led us to hypothesize that key controls on net methylation occur within the stream or on the streambed, and specifically, that periphyton may play an important role in MeHg production. This hypothesis has been tested by measuring the rate of Hg methylation and MeHg demethylation using periphyton samples collected from the field. Mercury methylation and MeHg demethylation activity of periphyton biofilms from the industrially contaminated EFPC were measured during 2014–2016 using stable Hg isotopic rate assays. $^{201}Hg^{II}$ and $MM^{202}Hg$ were added to intact periphyton samples in ambient streamwater and the formation of $MM^{201}Hg$ and loss of $MM^{202}Hg$ were monitored over time and used to calculate first-order rate potentials for methylation and demethylation (see Fig. 3, p. 4). The influences of location, temperature and season, light exposure, and biofilm structure on methylation and demethylation potentials were examined. Between-site differences in net methylation for samples collected from an upstream versus downstream location were driven by differences in the demethylation rate potential (k_d). In contrast, the within-site temperature-dependent difference in net methylation was driven by changes in the methylation rate potential (k_m). Samples incubated in the dark had lower net methylation due to lower k_m values than those incubated in the light. Disrupting the biofilm structure decreased k_m and resulted in lower net methylation. Overall, the measured rates resulted in a net excess of MeHg generated, which could account for 3.71–7.88 mg d^{-1} MeHg flux in EFPC and suggests intact, actively

photosynthesizing periphyton biofilms harbor zones of MeHg production (Olsen et al. 2016).

The data were divided into a model training dataset and validation dataset. Methylation (k_m) and demethylation (k_d) rate constants were determined by fitting a series of increasingly complex kinetic models to the training data. Model formulations progressed as Model 1: methylation and demethylation were assumed to be irreversible over the incubation time course, and each methylation and demethylation dataset was modeled in isolation. Model 2: Hg was assumed to cycle between Hg and MeHg during experiments, and paired methylation-demethylation rate data were modeled simultaneously. Model 3: same as Model 2 with the added assumption of a time-dependent accumulation of Hg in a pool that is unavailable for methylation. Model 3 provides a superior fit to the observations relative to Models 1 and 2. We are currently updating the model to include time-dependent accumulation of MeHg in a pool that is unavailable for demethylation. The modeling approach adopted here feeds directly into the modeling framework for Hg transport and transformation (see next section).

In conjunction with our methylation-demethylation assays, we are collaborating with Task 3 of the CI-SFA. In this collaboration, companion periphyton samples from our assays are provided to Task 3 for (1) assessment of microbial community composition using 16s sequencing, and (2) qualitative and quantitative analysis of *hgcAB*, the two-gene cluster that encodes for Hg methylation, using a novel primer set. Additionally, 18s sequencing is being conducted to determine eukaryotic members of the microbial community. Dominant algae present in the biofilms are being determined via microscopic examination.

Modeling Framework for Mercury Transport and Transformation

In FY17, the Task 1 team continued to develop a data-informed modeling framework for Hg transport and transformation at the field scale. The long-term goal of that subtask is an integrative framework that combines evolving process understanding across multiple scales with field-scale measurements of transport and mass-exchange characteristics. We developed a new modeling approach that extends the highly successful travel-time based methods for characterizing and representing the transport of nonreacting tracers through the stream corridor. Existing approaches use tracer tests to infer retention time in sediments and surface storage zones. We extended that approach to incorporate multicomponent biogeochemical reactions. The key idea is to solve an auxiliary one-dimensional reactive transport subgrid system on each stream channel grid cell. The

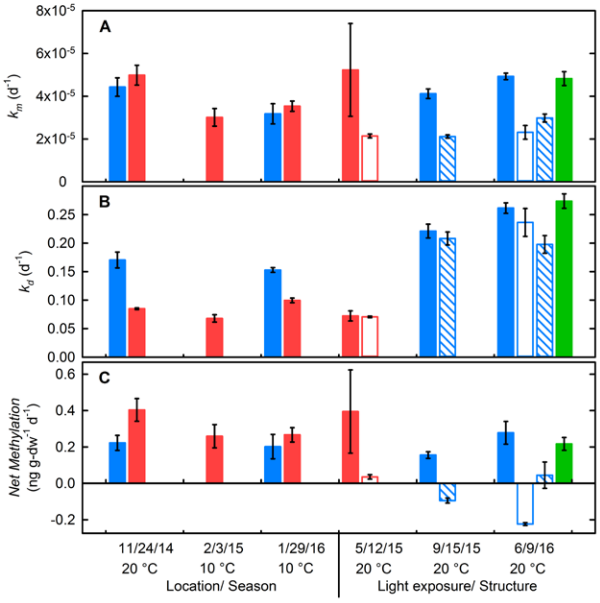


Fig. 3. Role of Periphyton in EFPC Mercury Cycling. Effect of treatments on (A) methylation rate potentials, (B) demethylation rate potentials, and (C) net methylation (mean ± standard error) in periphyton samples from EFPC determined using single time point assay (t = 48 h). Dates represent the day samples were taken and assays began. Blue bars = upstream site; red bars = downstream site; green bars = rocks from creek; filled bars = intact, incubated in light; open bars = intact, incubated in dark; and diagonal fill = disturbed, incubated in light.

auxiliary subgrid system is written in a Lagrangian (travel-time) framework and represents an ensemble of hyporheic pathways that leave and return to the stream channel. In contrast to existing travel-time approaches, hyporheic and surface storage zones are explicitly represented, which makes it possible to use existing reactive transport codes to represent mass transfer, biogeochemical speciation, kinetic reactions, and biomass dynamics. We used a simplified biogeochemical model to demonstrate that the new modeling framework can represent the dynamics and field-scale effects of locally reducing zones that act as biogeochemical hotspots without resolving the difficult-to-characterize three-dimensional geometry of the hyporheic zones. We are now implementing this approach as a subgrid model in the Advanced Terrestrial Simulator (ATS) software and plan to couple it to PFLOTRAN's biogeochemical capability. The resulting approach has the advantages of being extensible to watershed scales and tractable given practical constraints on stream corridor characterization. Although development is focused on Hg, the approach is broadly applicable to metal and nutrient transport in streams.

Status of FY17 Milestones

In the near-term we will:

Milestone 1a. Continue methylation and demethylation assays in creek periphyton to complete a year of seasonal data coupled with microbial community data. We envision several manuscripts resulting from these efforts. Additionally, in cooperation with Task 3, we are trying to isolate several novel methylating bacteria and Archaea, which currently show up as uncultured representatives in our samples.

We are planning a next phase of experimental work in which methylation and demethylation rates will be quantified for sediment samples.

We are working on methods to quantify changes over time in reactive Hg and MeHg that will inform our rate estimates. New equipment to conduct these studies has been ordered, and we hope to restart this work within the next several weeks.

Milestone 1i. Ongoing activities include development of our data-informed modeling framework for mercury transport and transformation at the field scale.

Manuscripts

Published or In Press

Brooks, S.C., C.C. Brandt, and N.A. Griffiths. 2017. "Estimating Uncertainty in Ambient and Saturation Nutrient Uptake Metrics from Nutrient Pulse Releases in Stream Ecosystems." *Limnology and Oceanography: Methods*. **15**(1):22–37. DOI:10.1002/lom3.10139.

Olsen, T.A., C.C. Brandt, and S.C. Brooks. 2016. "Periphyton Biofilms Influence Net Methylmercury Production in an Industrially Contaminated System." *Environmental Science & Technology* **50**(20):10843–10850. DOI:10.1021/acs.est.6b01538.

In Preparation or Submitted

Demers, J.D., J.D. Blum, S.C. Brooks, P.M. Donovan, C.L. Miller, A.L. Riscassi, W. Zheng, and B. Gu. "Hg Isotopes Reveal In-Stream Processing and Legacy Inputs in East Fork Poplar Creek, Oak Ridge, TN, USA." *In review*.

Dickson, J.O., S.C. Brooks, D.B. Watson, T.L. Mehlhorn, E.M. Pierce, and M.A. Mayes. "Diffuse Streambank Source of Mercury Loading to a Freshwater Stream: Implications for Targeted Remedial Actions." *In preparation*.

Olsen, T.A. and S.C. Brooks. "Seasonal Variations in Net Methylmercury Production by Algal Biofilms in a Low Gradient Stream." *In preparation*.

Olsen, T.A., K. Muller, S.L. Painter, and S.C. Brooks. "Measurement and Modeling of Methylmercury Production in Periphyton Biofilms." *In preparation*.

Painter, S.L., et al. "Modeling Multicomponent Reactive Transport in Streams." *In preparation*.

Riscassi, A.L., C.L. Miller, and S.C. Brooks. "Diel Mercury Concentration Variations in a Mercury-Impacted Stream." *In preparation*.

Task 2: Biogeochemical Mechanisms Controlling Mercury Uptake and Methylation

The overarching goal of Task 2 is to gain a fundamental understanding of the key biogeochemical mechanisms controlling Hg sorption, uptake, and transformation at the microbe-fluid and particulate (mineral)-DOM interfaces. We attempt to answer the following specific scientific questions:

- What are the key biogeochemical variables and their interactions affecting Hg-DOM complexation, Hg–cell surface interactions, cellular uptake, and methylation?
- How does photoredox transformation of Hg-DOM influence Hg reactivity and bioavailability?
- What are the specific molecules in DOM and proteins in and out of the cell membrane that complex Hg?
- Under what conditions do suspended particles become a net sink or source of bioavailable Hg in EFPC?

FY16 – FY17 Accomplishments

- Published five technical manuscripts, including one in *Science Advances* (Lu et al. 2017), two in *Environmental Science & Technology* (Liu et al. 2016; Chen et al. 2017), one in *Journal of Proteome Research* (Qian et al. 2016), and one in *Environmental Pollution* (Luo et al. 2017).
- Submitted two additional manuscripts for review. They include one on studies of the effects of DOM on Hg methylation by *G. sulfurreducens* PCA and *Desulfovibrio desulfuricans* ND132, and a book chapter on "Mercury in Water" in *Encyclopedia of Water: Science, Technology, and Society*.
- Gave two invited presentations at the 252nd American Chemical Society national meeting in Philadelphia.
- Published two additional technical manuscripts in collaboration with university investigators.

Most notably, we discovered that some methanotrophs (see Fig. 4, this page) can take up and degrade MeHg in the environment (Lu et al. 2017). While numerous studies have characterized the basis of Hg methylation, to date, no studies have examined MeHg degradation by methanotrophs, despite their ubiquitous presence in the environment. Most studies have focused on demethylation by the organomercurial lyase (MerB) pathway that is only effective at very high Hg concentrations and at above neutral pH conditions. We found that some methanotrophs such as *Methylosinus trichosporium* OB3b can take up and degrade MeHg rapidly, whereas others such as *Methylococcus capsulatus* Bath can take up but not degrade MeHg. Demethylation by *M. trichosporium* OB3b increased with increasing MeHg concentrations, but was abolished in mutants deficient in the synthesis of methanobactin, a metal-binding compound used by some methanotrophs such as *M. trichosporium* OB3b. Further, addition of methanol (> 5 mM) as a competing one-carbon (C1) substrate inhibited demethylation. Our results indicate a new demethylation pathway, which is remarkably different from the canonical MerB pathway and is effective at much more environmentally relevant conditions (i.e., low or nanomolar Hg concentrations and circumneutral pH). We proposed that MeHg degradation by methanotrophs may involve an initial bonding of MeHg by methanobactin followed by cleavage of the C–Hg bond in CH_3Hg^+ by the methanol dehydrogenase. This new demethylation pathway by methanotrophs indicates possible broader involvement of C1-metabolizing aerobes in the degradation and cycling of toxic MeHg in the environment. Our work thus provides new insights into hitherto unknown, yet potentially widespread biological mechanisms of MeHg uptake and demethylation due to methanotrophs' prevalence in the environment, and it may open new opportunities to explore how nature detoxifies MeHg.

For the first time, we demonstrated the utilization of ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) coupled with electrospray ionization to positively identify individual Hg-DOM complexes (Chen et al. 2017). This work was performed in collaboration with DOE's Environmental Molecular Sciences Laboratory (EMSL). The measurements were performed by direct infusion of DOM in 1:1 methanol:water solution at a Hg-to-dissolved-organic-carbon (DOC) molar ratio of 3×10^{-4}. Heteroatomic molecules, especially those containing multiple sulfur and nitrogen atoms, were found to be among the most important in forming strong complexes with Hg. Major Hg-DOM complexes of $C_{10}H_{21}N_2S_4Hg^+$ and $C_8H_{17}N_2S_4Hg^+$ were identified based on both the exact molecular mass and patterns of Hg stable isotope distributions detected by FTICR-MS. In collaboration with Task 4, DFT was used to predict the solution-phase structures of candidate molecules. Our study represents the first step to unambiguously identify specific DOM molecules in Hg binding, which are critically important in affecting Hg biological uptake and conversion to neurotoxic MeHg in the environment.

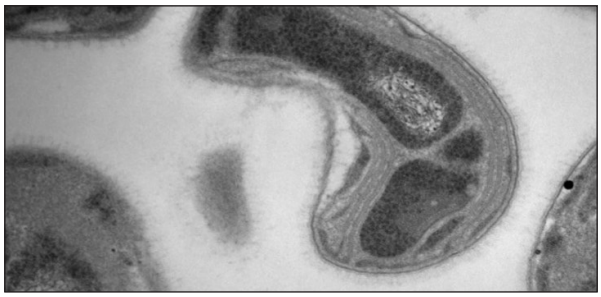


Fig. 4. Methanotroph. Image by Jeremy Semrau, University of Michigan.

We studied the cellular sorption and bioavailability of Hg on the biosynthesis of MeHg by *D. desulfuricans* ND132. We found that, although mercuric Hg(II) can be taken up rapidly by ND132 cells, a large fraction of the Hg(II) is unavailable for methylation because of strong cellular sorption (Liu et al. 2016). However, thiols, such as cysteine, glutathione, and penicillamine, added either together with Hg(II) or after cells have been exposed to Hg(II), can effectively desorb or mobilize the bound Hg(II), leading to substantially increased MeHg production. Contrary to previous observations, ND132 cells do not preferentially take up Hg(II)-thiol complexes, but Hg(II)-ligand exchange between these complexes and the cell-associated membrane proteins likely constrains Hg(II) uptake and methylation. We thus suggest that cellular binding and exchange of Hg(II) with complexing ligands in solution is an important controlling mechanism of Hg(II) bioavailability.

In collaboration with Task 4 and Robert Hettich at ORNL, we utilized the shotgun proteomics approach to compare global proteome profiles between wild-type *G. sulfurreducens* PCA and its two mutant strains: a Δ*hgcAB* mutant, which is deficient in two genes known to be essential for the biosynthesis of MeHg toxin, and a Δ*omcBESTZ* mutant, which is deficient in five outer membrane c-type cytochromes and thus impaired in its ability for dissimilatory metal ion reduction. We delineated the global response of *G. sulfurreducens PCA* in both mutants and identified cellular networks and metabolic pathways that were affected by the loss of these genes (Qian et al. 2016). We found that deletion of *hgcAB*

increased the relative abundances of proteins implicated in extracellular electron transfer, including most of the c-type cytochromes, PilA-C, and OmpB, whereas deletion of *omcBESTZ* significantly increased relative abundances of various methyltransferases, suggesting that a loss of dissimilatory reduction capacity results in elevated activity among C1 metabolic pathways. Our results support the hypothesis that the function of HgcA and HgcB is linked to C1 metabolism through the folate branch of the acetyl-CoA pathway by providing methyl groups required for Hg methylation. This is the first study comparing differences in the proteomes of Δ*hgcAB,* Δ*omcBESTZ,* and wild-type strains of *G. sulfurreducens* PCA, and the result provided new insights into the impact of these gene deletions on key metabolic processes.

Additionally, we recently identified a novel pathway of abiotic photochemical formation of HgS from photolysis of the complexes between Hg and naturally DOM, resulting in rapidly decreased Hg reactivity and bioavailability (Luo et al. 2017). We demonstrated that Hg reactivity and bioavailability decreased by photochemical reactions between Hg and DOM. Photo-irradiation of Hg-DOM complexes resulted in HgS precipitation, loss of Hg reactivity, and an up to 80% decrease in MeHg production by the methylating bacterium *G. sulfurreducens* PCA. Loss of Hg reactivity proceeded at a faster rate with decreasing Hg/DOM ratio. These results not only suggest a novel pathway of abiotic photochemical formation of HgS, but also explain a mechanism whereby freshly deposited Hg is readily methylated but, over time, progressively becomes less available for microbial uptake and methylation.

Status of FY16-FY18 Milestones

Milestone 2a. Molecular profiling of Hg-DOM interactions. *90% complete*; one manuscript published in *Environmental Science & Technology Letters*.

Milestone 2b. Proteomics analyses and profiling of cell membrane proteins and functional groups. *90% complete*; one manuscript published in *Journal of Proteome Research*.

Milestone 2c. Hg-cell ligand exchange and competitive interactions. *90% complete*; one manuscript published in *Environmental Science & Technology*.

Milestone 2d. Hg-cell surface interactions on Hg uptake and methylation. *70% complete*; one manuscript in review.

Milestone 2e. Cell imaging and surface characterization. *Work initiated (5%)*.

Milestone 2f. Environmental factors (e.g., thiols and DOM) affecting Hg methylation and demethylation. *70% complete*; one manuscript published in *Science Advances*.

Milestone 2g. Photochemical transformation of Hg and bioavailability. *100% complete*; one manuscript published in *Environmental Pollution*.

Milestone 2h. Hg reactions and reactivity at particulate–water interfaces. *Work initiated (5%)*.

Future Goals, Vision, and Plans

- In collaboration with Tasks 3 and 4, complete and submit a technical manuscript on quantitative proteome analysis and functional properties of *D. desulfuricans* ND132 and its *hgcAB* mutant.
- Revise or publish one book chapter and one technical manuscript related to DOM effects of Hg bioavailability and MeHg production.
- Continue studies and identification of specific Hg-DOM molecules or complexes using the FTICR-MS technique.
- Continue studies on Hg-cell surface interactions and examine cell membrane proteins responsible for Hg binding and uptake.
- Continue investigations of MeHg degradation mechanisms by selected methanotroph strains in collaboration with university investigators.
- Investigate Hg stable isotope fractionation during abiotic dark oxidation of elemental Hg(0) in collaboration with university investigators.
- In collaboration with Task 1, examine roles of suspended particles on Hg sorption, desorption and bioavailability in EFPC, and assemblages and compositions of periphyton films using spectroscopic techniques.

Manuscripts

Published or In Press

Chen, H., R.C. Johnston, B.F. Mann, R.K. Chu, N. Tolic, J.M. Parks, and B. Gu. 2017. "Identification of Mercury and Dissolved Organic Matter Complexes Using Ultrahigh Resolution Mass Spectrometry." *Environmental Science & Technology Letters* **4**(2): 59–65. DOI:10.1021/acs.estlett.6b00460.

Liu, Y., X. Lu, L. Zhao, J. An, J.Z. He, E.M. Pierce, A. Johs, and B. Gu. 2016. "Effects of Cellular Sorption on Mercury Bioavailability and Methylmercury Production by *Desulfovibrio desulfuricans* ND132." *Environmental Science & Technology* **50**:13335–13341. DOI:10.1021/acs.est.6b04041.

Lu, X., W. Gu, L. Zhao, F.M. Ul Haque, A.A. DiSpirito, J.D. Semrau, and B. Gu. 2017. "Methylmercury Uptake and Degradation by Methanotrophs." *Science Advances* **3**(5):e1700041. DOI:10.1126/sciadv.1700041.

Luo, H.-W., X. Yin, A.M. Jubb, H. Chen, X. Lu, W. Zhang, H. Lin, H.-Q. Yu, L. Liang, G.-P. Sheng, and B. Gu. 2017. "Photochemical Reactions Between Mercury (Hg) and Dissolved Organic Matter Decrease Hg Bioavailability and Methylation." *Environmental Pollution*, **220**:1359–1365. DOI:10.1016/j.envpol.2016.10.099.

Qian, C., A. Johs, H. Chen, B.F. Mann, X. Lu, P.E. Abraham, R.L. Hettich, and B. Gu. 2016. "Global Proteome Response to Deletion of Genes Related to Mercury Methylation and Dissimilatory Metal Reduction Reveals Changes in Respiratory Metabolism in *Geobacter sulfurreducens* PCA." *Journal of Proteome Research* **15**(10):3540–3549. DOI:10.1021/acs.jproteome.6b00263.

In Preparation or Submitted

Zhao, L., H. Chen, X. Lu, H. Lin, G.A. Christensen, E.M. Pierce, and B. Gu. "Contrasting Effects of Dissolved Organic Matter on Mercury Methylation by *G. sulfurreducens* PCA and *D. desulfuricans* ND132." *Environmental Science & Technology. In review*.

Gu, B., X. Lu, A. Johs, and E.M. Pierce. "Mercury in Water." In *Encyclopedia of Water: Science, Technology, and Society*. P. Maurice (Ed), Wiley. *In review*.

Task 3: Microbial Community Functions and Geochemical Influences on Mercury Transformations

The overarching goals of Task 3 are twofold: (1) Determine the breadth and depth of Hg-methylating species in a range of environmental systems, and (2) determine the native biochemical function(s) of HgcA and HgcB and their participation in other cellular biochemical pathways. Our research is designed to resolve three specific questions:

- How widespread is the ability to methylate Hg, and what are the relative contributions to the overall net pool of MeHg generated?
- What is the native biochemical function of HgcA and HgcB, and in which biochemical pathways do they participate?
- Under what conditions are the expression of HgcA and HgcB increased or decreased?

To this end, we are:

- Developing and deploying new tools and techniques that will enable quantification of Hg-methylating microbes that contain the two gene cluster in diverse environment(s) and form collaborations for wide-scale assessment of these procedures.
- Constructing new mutants and strains to characterize the genes, related biochemical pathways and, in coordination with task 4, elucidate the mechanism of methylation in *hgcAB*.
- Determining the influence of both the presence of other organisms and different geochemical factors on *hgcAB* gene abundance of the 2-gene cluster and the methylation activity in collaboration with Task 1.

FY16 – FY17 Accomplishments

In the past year, we have made considerable progress in (1) validating and optimizing our molecular primers for the *hgcAB* genes with environmental samples and (2) creating deletion or mutant strains to determine the native function of *hgcAB* genes and to elucidate the mechanism of Hg methylation (in collaboration with Task 4). A progress summary is presented in the following two sections.

Recently, we completed the development of qualitative and quantitative polymerase chain reaction (PCR)-based methods for identifying Hg-methylators via *hgcAB* and are beginning to validate them using a range of environmental samples (Christensen et al. 2017). We are validating the PCR-based protocols on (1) samples collected from the SPRUCE site located at the Marcell Experimental Forest in Minnesota in collaboration with researchers at the University of Minnesota, (2) soil samples from a Hg-contaminated rice paddy in Guizhou, China, (3) samples collected from the Great Salt Lake in Utah in collaboration with researchers from Montana State University, (4) filtered ocean samples collected from two ocean cruises in collaboration with Carl Lamborg, University of California, Santa Cruz, and David Krabbenhoff, U.S. Geological Survey, and (5) periphyton samples collected from EFPC in collaboration with Task 1. The periphyton samples collected from EFPC are being used to optimize our PCR-based protocols, which included a series of initial steps to isolate bacterial genomic DNA (a minor component in the sample) from the larger fraction of algal DNA present in the complex periphyton. In addition to validating our PCR-based protocols, we are also using the periphyton samples to isolate and enrich EFPC-relevant Hg methylating strains from each clade (i.e., a *Deltaproteobacteria,* a *Firmicutes,* and a methanogenic *Archaea*). It is important to note that if successful, the isolates from EFPC will be used in our proposed co-culture studies; if the isolation process is not successful, we plan to proceed with using bacterial strains that are closely related to EFPC-identified strains. Based on the results collected to-date on samples from a range of environmental systems, the protocol described in Christensen et al. (2017) will be updated to include the optimization steps that are

required when applying these PCR-based protocols. Details associated with the optimization steps also will be posted to the CI-SFA website.

Furthermore, we are characterizing *hgcAB* and HgcAB to determine the native function of these genes and proteins; that is, what biochemical function do they perform in the absence of Hg. To this end, we have employed several tactics including deletion mutagenesis of ~14 genes separately that we suspect are involved in the methyl group transfer to Hg, as well as the two electrons required for the reduction of the cobalamin cofactor in the active site of HgcA. All these mutants have been cultivated under several different regimes of carbon and electron sources and electron acceptors to determine the effects on cell metabolism as well as Hg methylation. The results are still being processed. We have also constructed a new *hgcAB* deletion strain that will be used for making strains with multiple deletions (i.e., *hgcAB* deletion and an additional deletion) to address the effect of additional gene knockouts in the *hgcAB* deletion strain background. With the completion of this strain, we have begun to examine gene fitness via TnLE-seq in response to Hg. The results are still being processed.

Lastly, we have prepared two additional manuscripts for publication. The first is in review at *Applied and Environmental Microbiology* and focuses on examining microbial community response to carbon substrate amendment in Hg-impacted sediments. The second, currently in author review, utilizes our recently developed PCR-based protocols on eight unique sites along with metagenomes and 16s ribosomal RNA (rRNA) gene sequencing and site geochemistry to determine the efficacy of our primers in the environment, and sensitivity and robustness of the primers and their quantitative accuracy as compared to other methods, as well as attempts to link Hg or MeHg to Hg-methylator abundance. We are also putting together a Hg-methylation review (*Trends in Microbiology*) to describe the future of Hg and Hg-methylation research.

Status of FY16-FY18 Milestones

Milestone 3a. Purification and proteomic identification of HgcAB functional complex using genetically attached tandem affinity tags. *Ongoing collaboration with Task 4.*

Milestone 3b. Differential growth and geochemical perturbation regimes and the effect on *hgcAB* expression. *To begin in FY2017. D. desulfuricans* ND132 was grown in standard medium containing pyruvate and fumarate and hgcA expression was determined to be very low (~10-50 copies per 1 x 10^6 cells). Next steps include growing *D. desulfuricans* ND132 on different electron donors and acceptors for comparison and across growth (i.e., early and late exponential, stationary). On a larger scale, *D. desulfuricans* ND132 wild-type and *hgcAB* deletion have been grown in batch in various metabolic conditions. Analyses to follow include RNA sequencing, organic acid identification, and metabolomics.

Milestone 3c. Transposon liquid enrichment sequencing (TnLE-seq) fitness studies to determine essential gene complement in the presence and absence of *hgcAB*. *Ongoing, University of Missouri. Expected completion in summer 2017.*

Milestone 3d. ^{13}C studies to determine HgcAB biochemical pathways, and identify CH_3 carbon source in MeHg. *Modified, not using ^{13}C. Manuscript being prepared.*

Milestone 3e. Single species bioreactor studies as a foundation for synthetic microbial communities. *To begin in FY2017.* Microorganisms have been selected, and the experiment is in setup.

Milestone 3f. Multispecies cultivations (dual, tri, quad, and penta) to attain a microbial community with full redox and functional representation. *To begin in FY2017.* To follow success of Milestone 3e.

Milestone 3g. Lab-based biofilm reactor studies of EFPC periphyton samples. *Ongoing, collaboration with Task 1.* Protocols for bacterial isolation from periphyton and PCR-based protocols for *hgcAB* have been optimized.

Milestone 3h. Lab-based biofilm reactor studies of samples from other relevant sites. *To begin in FY2017.*

Milestone 3i. Isolation of new and novel Hg-methylating bacteria. *Ongoing, ORNL and Smithsonian Environmental Research Center.*

Future Goals, Vision, and Plans

- Complete biochemical studies to move toward determining carbon flow using ^{13}C experiments in model organisms.
- Utilize field representatives in model communities. Hypotheses generated will be tested in the field to close the lab-to-field gap and validate the relevance of lab-based work.
- Complete initial tests with periphyton biofilms from the field (Task 1) for in-lab measurements of overall metabolism; Hg(II) methylation; MeHg demethylation; temporal community populations; and temporal genomic, transcriptomic, and proteomic complements.
- Isolate and characterize Hg(II)-methylating organisms from the field biofilms.

Manuscripts

Published or In Press

Christensen, G.A., A.C. Somenhally, J.G. Moberly, C. Miller, A.J. King, A.V. Palumbo, S.C. Brooks, and D.A. Elias. Carbon Amendments Alter Microbial Community Structure and Net Mercury Methylation Potential in Sediments. *Applied and Environmental Microbiology. Accepted*.

In Preparation or Submitted

Vishnivetskaya, T.A., H. Hu, J.D. Van Nostrand, A.M. Wymore, X. Xu, G. Qiu, X. Feng, S.D. Brown, C.C. Brandt, M. Podar, J. Zhou, B. Gu, and D.A. Elias. "Microbial Community Structure with Trends in Methylation Gene Diversity and Abundance in Mercury-Contaminated Rice Paddy Soils in Guizhou, China." *PLOS One. In review*.

Christensen, G.A., A.J. King, J.G. Moberly, C. Miller, A.C. Somenhally, M. Podar, S.D. Brown, A.V. Palumbo, C.C. Brandt, A.M. Wymore, S.C. Brooks, C.C. Gilmour, J.D. Wall, M.D. Fields, S.J. Callister, and D.A. Elias. "An Effort to Link *hgcA* Abundance in the Environment to Methyl- and Total Mercury Concentrations." *In preparation*.

Task 4: Molecular Structure, Dynamics, and Mechanisms of Mercury Transport and Transformations

The goal of Task 4 is to investigate structures, reactions, energetics, and dynamics to understand at the molecular scale how Hg is transformed and transported by biological macromolecules and abiotic species encountered in natural and contaminated environments. Currently, there are two main focus areas in Task 4: (1) bacterial Hg methylation, and (2) permeability of Hg-containing complexes. These areas are being addressed with both experimental and computational approaches. We are also using computational approaches to study Hg-ligand interactions and the mechanisms of abiotic formation of dimethylmercury. We briefly discuss the progress made from July 2016 to July 2017.

Background

Microbial Hg methylation is an important contaminant transformation process that occurs in hypoxic environments. The proteins HgcA and HgcB are essential for MeHg production by anaerobic bacteria.

Cellular uptake and export are important steps in the biotransformation of Hg by microorganisms. However, the mechanisms of transport across biological membranes remain unclear. Membrane-bound transporters are known to be relevant, but passive diffusion may also be involved. Inorganic Hg^{II} and MeHg are commonly complexed with thiolate ligands, so these species are of particular interest.

FY16–FY17 Accomplishments

Hg Methylation Assays

Experimental work in Task 4 has focused on delineating the biochemistry of Hg methylation and interdependent metabolic pathways. The goal of our recent experiments is to determine rates and mechanisms of intracellular Hg methylation in relation to methylation activities previously described for whole cells only. We have developed a protocol to conduct methylation activity assays in cell lysates of *D. desulfuricans* ND132, which enables us to directly determine intracellular methylation rates at physiologically relevant Hg(II) concentrations. Preliminary results indicate that methylation activity is primarily associated with the membrane fraction. Additional experiments are underway to quantify the cellular levels of HgcA and HgcB and determine factors that impact methylation rates in cell lysates.

The overall rate of Hg methylation is also controlled by external factors, such as Hg bioavailability (i.e., Hg-cell interactions, Milestone 4f). The level of MeHg in pure culture studies typically reaches a maximum a few hours to a day after addition of Hg(II), even if provided in large excess. We have collaborated with Task 2 to investigate the interplay between Hg speciation and cellular sorption by *D. desulfuricans* ND132. In this study, we show that mobilization of Hg(II) by adding small molecule thiols results in a substantial increase in MeHg production. It is proposed that the Hg(II)-ligand exchange reactions between complexing ligands and the cellular interface control Hg bioavailability and Hg methylation rates (Liu et al. 2016).

Proteomics

Furthermore, we published a joint paper with Task 2 describing the impact of *hgcAB* gene deletions on the proteome of *G. sulfurreducens* PCA (Qian et al. 2016). We used shotgun proteomics to compare global proteome profiles between wild-type *G. sulfurreducens* PCA and Δ*hgcAB* and Δ*omcBESTZ* mutants. We identified cellular networks and metabolic pathways that were affected by the loss of these genes. Deletion of *hgcAB* increased the relative abundances of proteins implicated in extracellular electron transfer and various methyltransferases. The results are consistent with an increase in Hg reduction in the Δ*hgcAB* mutant associated with elevated activity among C1 metabolic pathways. We showed that *G. sulfurreducens* PCA encodes only the folate branch of the acetyl-CoA pathway, and proteins associated with the folate branch were found at lower abundance in the Δ*hgcAB* mutant. This observation supports the

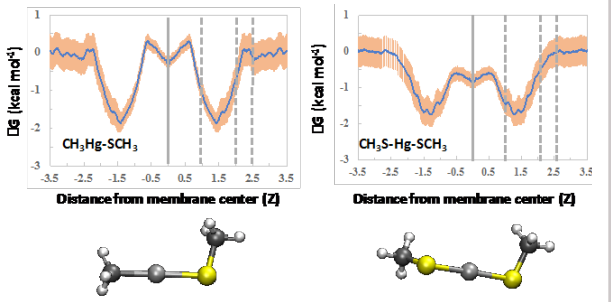


Fig. 5. Calculated free energy profiles for the passive transport of Hg-containing solutes across a model cytoplasmic membrane.

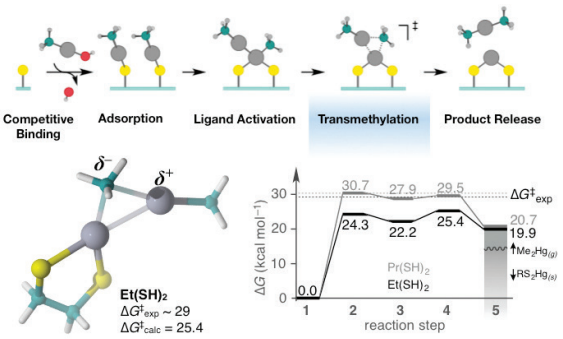


Fig. 6. Proposed mechanism of dimethylmercury formation on reduced sulfur surfaces.

hypothesis that the function of HgcA and HgcB is linked to cellular C1 metabolism through the folate branch of the acetyl-CoA pathway, which constitutes a source of methyl groups for biochemical methylation reactions.

Membrane Permeability of Hg-Containing Molecules

We have performed extensive molecular dynamics (MD) simulations of the passive diffusion of Hg^{II} and MeHg complexes with thiolate ligands through a model bacterial cytoplasmic membrane. We found that the differences in free energy between the individual complexes in bulk water and at their most favorable position within the membrane are ~2 kcal mol^{-1} (see Fig. 5, this page), and that favorable interactions with the carbonyl and tail groups of the phospholipids stabilize Hg-containing solutes at the tail-head interface of the membrane. From the MD results, we computed permeability coefficients for the neutral compounds $CH_3S–Hg^{II}–SCH_3$ and $CH_3Hg–SCH_3$, which are on the order of 10^{-5} cm s^{-1}. These values provide evidence that small, non-ionized Hg-containing species can diffuse readily through cytoplasmic membranes.

Abiotic Formation of Dimethylmercury

Recently, it was shown that mineral surfaces with exposed reduced sulfur sites could facilitate the formation of dimethylmercury from MeHg (Jonsson et al. 2016). To provide mechanistic insight, we performed DFT calculations on representative models. We found that the transfer of a methyl group from one Hg to the other proceeds through an unusual, transient Hg–Hg bond (see Fig. 6, this page). The computed energetic barriers are consistent with the experimental data, suggesting that the proposed mechanism is reasonable.

Status of FY16-FY18 Milestones

Milestone 4a and 4b. The characterization of heterologously expressed HgcB is performed in the lab of our collaborator Steve Ragsdale at the University of Michigan through a subcontract from Task 4. HgcB has been expressed in a *Escherichia coli* expression host with the pRKISC plasmid containing the iron sulfur cluster maturation genes *iscRSUA-hscBA-fdx-iscX*. The purified protein is 75% replete in [4Fe-4S] and direct electron transfer from a pyruvate-formate oxidoreductase has been demonstrated. Furthermore, the stoichiometry of Hg(II) binding to HgcB was found to be at a 1:1 molar ration as determined by titration experiments. Ongoing work aims to increase the yields of purified protein and characterization of the midpoint potentials of the Fe-S clusters.

The experimental identification and characterization efforts are supported by a hypothesis-driven co-occurrence analysis. The analysis aims to identify proteins associated with metabolic pathways potentially relevant to the function of HgcA and HgcB. The approach takes advantage of microbial genome sequence data from strains containing homologs of HgcA and HgcB available at Joint Genome Institute Integrated Microbial Genomes system, National Center for Biotechnology Information, and European Molecular Biology Laboratory. Preliminary results are shown as a heat map (See Fig. 7, pg. 12) representing E-values relative to clade-specific template sequences (Date et al. *In preparation*).

Milestone 4d. Ongoing experiments aim to isolate native HgcA and HgcB from *D. desulfuricans* ND132 using tandem affinity purification, immunostaining, and mass spectroscopy. In collaboration with Task 3, we have obtained *D. desulfuricans* ND132 strains engineered with 3xFLAG/TEV/StrepII tags in for tandem affinity purification of HgcA and HgcB. Tandem affinity purification, immunoblotting, crosslinking, and pull-down assays in combination with mass spectrometry are being used to identify cellular proteins that directly interact with HgcA and HgcB. The results are expected to reveal protein-protein interactions, which will complement results obtained from methylation assays with ND132 cell lysates

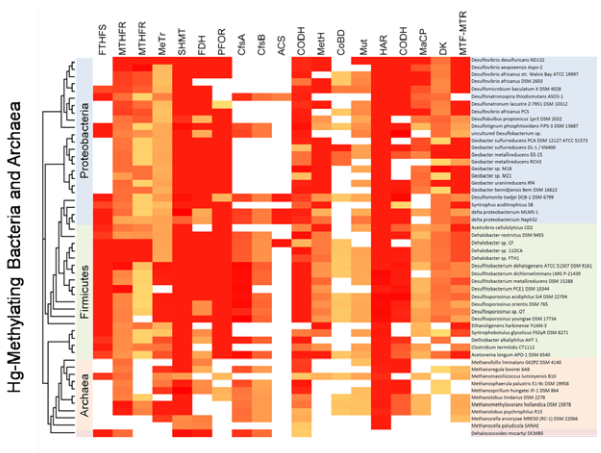


Fig. 7. Co-occurrence analysis of genes in mercury methylating bacteria and archaea

and help delineate the roles of HgcA and HgcB in the context of microbial metabolism. We have submitted a user proposal to EMSL requesting access to mass spectrometry capabilities for sample analysis.

Milestone 4e. Thermodynamic formation constants (log β) describe the stabilities of Hg-ligand complexes in solution and are therefore critical for understanding Hg speciation. The predominant forms of Hg^{2+} in solution are exceptionally strong complexes of low-molecular-weight biological thiols such as cysteine (Cys) and glutathione. Determining accurate log β values is a challenge experimentally due to their high thermodynamic stability. The lack of reliable thermodynamic data in the literature limits the use of geochemical models, which are parameterized with these data, in predicting Hg speciation and transport. For example, literature values for log β of $Hg(Cys)_2$ span >6 orders of magnitude. Therefore, our objective is to use quantum chemical calculations to determine Hg:Cys log β values from first principles to predict Hg speciation in solution and assign confidence to reported experimental values.

We have developed a computational approach based on DFT and continuum solvation for computing accurate reduction potentials, pK_as, and log β values to ~1 log unit (Johnston et al. 2016). In collaboration with Task 2, we applied that approach to assign putative three-dimensional structures to Hg-DOM chemical formulas identified by ultrahigh-resolution electrospray mass spectrometry (Chen et al. 2017). We have submitted an EMSL user proposal to work with the developers of the program NWChem to use highly accurate relativistic quantum chemical approaches to investigate mechanisms of MeHg formation, Hg-ligand interactions, and other Hg transformations.

Future Goals

- Use crosslinking to determine intermolecular interactions of HgcA and HgcB with other cellular components (Milestone 4d).
- Hg-DOM complexation (Milestone 4e). Computation of log β values of increasingly realistic Hg-DOM models.

Manuscripts

In Preparation or Submitted

Asaduzzaman, A.M, D. Riccardi, A.T. Afaneh, J.C. Smith, F. Wang, J.M. Parks, and G. Schreckenbach. "Environmental Mercury Chemistry – *in silico*." *Accounts of Chemical Research. In review.*

Zhou, J., M.D. Smith, S.J. Cooper, X. Cheng, J.C. Smith, and J.M. Parks. "Modeling of the Passive Diffusion of Mercury and Methylmercury Complexes Through a Bacterial Cytoplasmic Membrane." *Environmental Science & Technology. In review.*

Date, S., J.M. Parks, and A. Johs. "Co-occurrence Analysis of Genes Implicated in Mercury Methylation and One-Carbon Metabolism in Anaerobic Bacteria." *In preparation.*

Pavlova, A., J.M. Parks, and J.C. Gumbart. "Development of CHARMM-Compatible Force-Field Parameters for Cobalamin and Related Cofactors from Quantum Mechanical Calculations." *In preparation.*

Johnston, R.C., T.A. Olsen, S. Jonsson, S.C. Brooks, and J.M. Parks. "Quantum Chemical Insights into Dimethylmercury Formation on Reduced Sulfur." *In preparation.*

Select Research Highlights

Research Highlight

May 31, 2017

A New Demethylation Pathway by Methanotrophs in the Environment

Certain methanotrophs can take up and degrade toxic methylmercury.

The Science

We discovered that some methanotrophs can take up and degrade methylmercury (CH_3Hg^+) rapidly, whereas others can take up but not degrade CH_3Hg^+. Demethylation increases with increasing CH_3Hg^+ concentrations but was abolished in mutants deficient in the synthesis of methanobactin, or inhibited by methanol as a competing one-carbon substrate. A new demethylation pathway is proposed, which is remarkably different from the canonical organomercurial lyase pathway.

The Impact

Methanotrophs likely play an important role in controlling net methylmercury production *in situ*. The work provides new insights into hitherto unknown, yet potentially widespread biological mechanisms of CH_3Hg^+ uptake and demethylation due to methanotrophs' prevalence in the environment.

The Summary

Methylmercury (CH_3Hg^+) is a potent neurotoxin produced by certain anaerobic microorganisms in natural environments. While numerous studies have characterized the basis of mercury methylation, no studies have examined CH_3Hg^+ degradation by methanotrophs, despite their ubiquitous presence in the environment. We report that some methanotrophs such as *Methylosinus trichosporium* OB3b can take up and degrade CH_3Hg^+ rapidly, whereas others such as *Methylococcus capsulatus* Bath can take up but not degrade CH_3Hg^+. Demethylation by *M. trichosporium* OB3b increases with increasing CH_3Hg^+ concentrations but was abolished in mutants deficient in the synthesis of methanobactin, a metal-binding compound used by some methanotrophs such as *M. trichosporium* OB3b. Further, addition of methanol (> 5 mM) as a competing one-carbon (C1) substrate inhibits demethylation, suggesting that CH_3Hg^+ degradation by methanotrophs may involve an initial bonding of CH_3Hg^+ by methanobactin, followed by cleavage of the C–Hg bond in CH_3Hg^+ by the methanol dehydrogenase. This new demethylation pathway by methanotrophs indicates possible broader involvement of C1-metabolizing aerobes in the degradation and cycling of toxic CH_3Hg^+ in the environment.

Publication

Lu, X., W. Gu, L. Zhao, M. Farhan Ul Haque, A.A. DiSpirito, J.D. Semrau, and B. Gu. 2017. "Methylmercury Uptake and Degradation by Methanotrophs." *Science Advances* **3**(5):e1700041. DOI:10.1126/sciadv.1700041.

Research Highlight

January 9, 2017

Identifying Mercury (Hg) and Dissolved Organic Matter (DOM) Complexes Using Ultra-High Resolution Mass Spectrometry

Heteroatomic molecular complexes between Hg and DOM are identified in water.

The Science

For the first time, we positively identify major Hg-DOM complexes such as $C_{10}H_{21}N_2S_4Hg^+$ and $C_8H_{17}N_2S_4Hg^+$ based on both the exact molecular mass and patterns of Hg stable isotope distributions. Heteroatomic molecules, especially those containing multiple sulfur and nitrogen atoms in DOM, are among the most important in forming strong complexes with Hg.

The Impact

The study represents the first step to unambiguously identify specific DOM molecules in Hg binding, which are critically important in affecting Hg biological uptake and conversion to neurotoxic MeHg in the environment.

Summary

The chemical speciation and bioavailability of Hg is markedly influenced by its complexation with naturally DOM in aquatic environments. However, to date, analytical methodologies capable of identifying such complexes are scarce. For the first time, we utilize ultra-high

resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) coupled with electrospray ionization to identify individual Hg-DOM complexes. The measurements were performed by direct infusion of DOM in 1:1 methanol:water solution at a Hg-to-dissolved-organic-carbon (DOC) molar ratio of 3×10^{-4}. Heteroatomic molecules, especially those containing multiple S and N atoms, were found to be among the most important in forming strong complexes with Hg. Major Hg-DOM complexes of $C_{10}H_{21}N_2S_4Hg^+$ and $C_8H_{17}N_2S_4Hg^+$ were identified based on both the exact molecular mass and patterns of Hg stable isotope distributions detected by FTICR-MS. Density functional theory was used to predict the solution-phase structures of candidate molecules. These findings represent the first step to unambiguously identify specific DOM molecules in Hg binding, so as to fully understand environmental factors affecting Hg biological uptake and conversion to neurotoxic MeHg in the natural environment.

Publication

Chen, H., R.C. Johnston, B.F. Mann, R.K. Chu, N. Tolic, J.M. Parks, and B. Gu. 2017. "Identification of Mercury and Dissolved Organic Matter Complexes Using Ultrahigh Resolution Mass Spectrometry." *Environmental Science & Technology Letters* **4**(2):59–65. DOI:10.1021/acs.estlett.6b00460.

Research Highlight

November 18, 2016

Effects of Cellular Sorption on Mercury (Hg) Bioavailability and Methylmercury (MeHg) Production by *Desulfovibrio desulfuricans* ND132

The work explains why thiol compounds such as cysteine (Cys) enhance Hg methylation.

The Science

Mercury cellular sorption by *Desulfovibrio desulfuricans* ND132 is found to be the main cause of limited Hg biological uptake and methylation, but addition of thiols such as Cys can liberate the sorbed Hg for enhanced MeHg production. Cells do not preferentially take up Hg-thiol complexes, but Hg binding and exchange between cells and complexing thiol ligands in solution is an important controlling mechanism for Hg uptake and methylation.

The Impact

We explain a long-standing question: "Why do thiols such as Cys enhance Hg methylation?" We suggest a new mechanism of Hg biological uptake and methylation resulting from Hg ligand exchange between cell surface binding proteins or transporters and thiols and naturally dissolved organic matter in the environment.

Summary

Microbial conversion of inorganic mercury (IHg) to MeHg is a significant environmental concern because of the bioaccumulation and biomagnification of toxic MeHg in the food web. Laboratory incubation studies have shown that, despite the presence of large quantities of IHg in cell cultures, MeHg biosynthesis often reaches a plateau or a maximum within hours or a day by an as yet unexplained mechanism. Here we report that mercuric Hg(II) can be taken up rapidly by cells of *D. desulfuricans* ND132, but a large fraction of the Hg(II) is unavailable for methylation because of strong cellular sorption. Thiols, such as Cys, glutathione, and penicillamine, added either simultaneously with Hg(II) or after cells have been exposed to Hg(II), effectively desorb or mobilize the bound Hg(II), leading to a substantial increase in MeHg production. The amount of thiol-desorbed Hg(II) is strongly correlated to the amount of MeHg produced ($r = 0.98$). Cells do not preferentially take up Hg(II)-thiol complexes, but Hg(II)-ligand exchange between these complexes and the cell-associated proteins likely constrains Hg(II) uptake and methylation. We suggest that, aside from aqueous chemical speciation of Hg(II), binding and exchange of Hg(II) between cells and complexing ligands such as thiols and naturally dissolved organics in solution is an important controlling mechanism of Hg(II) bioavailability, which should be considered when predicting MeHg production in the environment.

Publication

Liu, Y.-R., X. Lu, L. Zhao, J. An, J.-Z. He, E.M. Pierce, A. Johs, and B. Gu. 2016. "Effects of Cellular Sorption on Mercury Bioavailability and Methylmercury Production by *Desulfovibrio desulfuricans* ND132." *Environmental Science & Technology* **50** (24):13335–13341. DOI:10.1021/acs.est.6b04041.

Research Highlight

January 6, 2017

Photochemical-Driven Mercury Sulfide (HgS) Formation Decreases Mercury (Hg) Bioavailability and Methylation

The work identifies a novel pathway of abiotic photochemical formation of HgS and explains why freshly deposited Hg is more readily methylated than aged Hg in water.

The Science

For the first time, we found that photolysis of the complexes between Hg and naturally dissolved organic matter (DOM) results in rapidly decreased Hg reactivity and bioavailability, resulting in up to 80% decreased methylmercury (MeHg) production by a methylating bacterium *Geobacter sulfurreducens* PCA. Additionally, we identify a new pathway of photolytic formation of HgS as the main cause of the loss of Hg reactivity and bioavailability.

The Impact

This research not only identifies a novel pathway of abiotic photochemical formation of HgS, but also provides a mechanism explaining why freshly deposited Hg is more readily methylated than aged Hg in natural water and sediments.

Summary

Atmospheric deposition of Hg to surface water is the dominant source of Hg in aquatic environments and ultimately drives MeHg toxin accumulation in fish. It is known that freshly deposited Hg is more readily methylated by microorganisms than preexisting Hg; however, the underlying mechanism is unclear. We report that Hg reactivity and bioavailability are both decreased by photochemical reactions between Hg and DOM. Photo-irradiation of Hg-DOM complexes results in HgS precipitation, loss of Hg reactivity, and an up to 80% decrease in MeHg production by the methylating bacterium *G. sulfurreducens* PCA. Loss of Hg reactivity proceeds at a faster rate with decreasing Hg/DOM ratio. These results not only suggest a novel pathway of abiotic photochemical formation of HgS, but also provide a mechanism whereby freshly deposited Hg is readily methylated. Over time, however, it progressively becomes less available for microbial uptake and methylation.

Publication

Luo, H.-W., X. Yin, A.M. Jubb, H. Chen, X. Lu, W. Zhang, H. Lin, H.-Q. Yu, L. Liang, G.-P. Sheng, and B. Gu. 2017. "Photochemical Reactions Between Mercury (Hg) and Dissolved Organic Matter Decrease Hg Bioavailability and Methylation." *Environmental Pollution*, **220**:1359–1365. DOI:10.1016/j.envpol.2016.10.099.

Research Highlight

July 28, 2016

Unravelling Proteome Response to Deletion of Genes Related to Mercury (Hg) Methylation and Dissimilatory Metal Reduction in *Geobacter sulfurreducens* PCA

The work provides insight into the impact of deleting the Hg methylation genes on key metabolic processes in *G. sulfurreducens* PCA.

The Science

For the first time, comparative proteomics was conducted to delineate the global response of *G. sulfurreducens* PCA after deletion of genes related to Hg methylation and dissimilatory metal reduction. We identified cellular networks and metabolic pathways that are affected by the loss of these genes.

The Impact

This is the first study comparing differences in the proteomes of DhgcAB, DomcBESTZ ,and wild-type strains of *G. sulfurreducens* PCA and provides insight into the impact of these gene deletions on key metabolic processes. Our results support the hypothesis that the function of HgcA and HgcB is linked to C1 metabolism through the folate branch of the acetyl-CoA pathway by providing methyl groups required for Hg methylation.

Summary

In this work, shotgun proteomics was used to compare global proteome profiles between wild-type *G. sulfurreducens*

PCA and two mutant strains: a DhgcAB mutant, which is deficient in two genes known to be essential for the biosynthesis of methylmercury toxin, and a DomcBESTZ mutant, which is deficient in five outer membrane c-type cytochromes and thus impaired in its ability for dissimilatory metal ion reduction. We were able to delineate the global response of *G. sulfurreducens* PCA in both mutants and identify cellular networks and metabolic pathways that were affected by the loss of these genes. Deletion of *hgcAB* increased the relative abundances of proteins implicated in extracellular electron transfer, including most of the c-type cytochromes, PilA-C, and OmpB, whereas deletion of omcBESTZ significantly increased relative abundances of various methyltransferases, suggesting that a loss of dissimilatory reduction capacity results in elevated activity among C1 metabolic pathways. Our results support the hypothesis that the function of HgcA and HgcB is linked to C1 metabolism through the folate branch of the acetyl-CoA pathway by providing methyl groups required for Hg methylation.

Publication

Qian, C., A. Johs, H. Chen, B.F. Mann, X. Lu, P.E. Abraham, R.L. Hettich, and B. Gu. 2016. "Global Proteome Response to Deletion of Genes Related to Mercury Methylation and Dissimilatory Metal Reduction Reveals Changes in Respiratory Metabolism in *Geobacter sulfurreducens* PCA." *Journal of Proteome Research* **15**(10):3540–3549. DOI:10.1021/acs.jproteome.6b00263.

Table 1. Top Cited Publications

Title	Year	Citations
The Genetic Basis for Bacterial Mercury Methylation, *Science*	2013	163
Mercury Methylation by Novel Microorganisms from New Environments, *Environmental Science & Technology*	2013	108
Mercury Reduction and Complexation by Natural Organic Matter in Anoxic Environments, *Proceedings of the National Academy of Sciences*	2011	89
Sulfate-Reducing Bacterium *Desulfovibrio desulfuricans* ND132 as a Model for Understanding Bacterial Mercury Methylation, *Applied and Environmental Microbiology*	2011	75
Kinetic Controls on the Complexation Between Mercury and Dissolved Organic Matter in a Contaminated Environment, *Environmental Science & Technology*	2009	51
Mercury and Other Heavy Metals Influence Bacterial Community Structure in Contaminated Tennessee Streams, *Applied and Environmental Microbiology*	2011	49

National and International Impact

ORNL CI-SFA team members attended strategic U.S. conferences to gain insights into the state of the science, share project findings and strategies with the broader Hg research community, and identify collaborative opportunities. From July 2016 to June 2017, CI-SFA scientists delivered or published 19 presentations, abstracts, or posters and gave 5 invited talks (see Appendix C, page 22, for details). Described in the following section are team members' contributions to the Environmental System Science Principal Investigators Meeting, American Geophysical Union Fall Meeting, and the American Chemical Society Spring Meeting.

Environmental System Science Principal Investigators (PI) Meeting

ORNL CI-SFA staff supported by DOE's Subsurface Biogeochemical Research (SBR) program attended the annual PI meeting April 25–26, 2017, at the Bolger Center in Potomac, Maryland, and gave eight poster presentations. Additionally, all CI-SFA members participated in the DOE SBR program's town hall held at the PI meeting. During the town hall, Eric Pierce gave an invited presentation titled "DOE Cleanup and Stewardship Challenge: The Importance of Understanding Watershed Function" as part of two breakout sessions.

International Conference on Mercury as a Global Pollutant

Every 2 years, since the first meeting in Gavle, Sweden, in 1990, the International Conference on Mercury as a Global Pollutant (ICMGP) convenes researchers, policymakers, and industrial organizations in diverse locations around the world to discuss important advances in Hg research and facilitate international collaborations. The 13th ICMGP 2017 will be held in Providence, Rhode Island, July 16–21, and represents the second meeting since the 2013 signing of the Minamata Convention on Mercury, a global treaty to protect human health and the environment from the adverse effects of Hg.

ORNL CI-SFA members will attend and present 17 abstracts. Baohua Gu will co-host a special session (along with Sofi Jonsson, University of Connecticut) titled, "Mercury Methylation: Microbial and Geochemical Constraints (Session 2f.)," which focuses on recent advances in understanding the mechanisms and controls on Hg chemical speciation, microbial uptake and methylation, and biotic and abiotic demethylation in both natural and contaminated marine and freshwater ecosystems. Topics may include, but are not limited to, coupled biological and geochemical interactions affecting Hg reduction and oxidation, biochemical pathways and mechanisms of Hg methylation and demethylation, biomolecular and genetic research, marine and freshwater bioaccumulation of Hg, and novel analytical tools including modeling approaches from molecular to watershed scales.

Ongoing Collaborative Research Activities

The ORNL mercury CI-SFA continues to engage with a number of key collaborators in the project. In FY2017, Task 1 worked informally with Carl Mitchell (University of Toronto) to test novel air samplers throughout the EFPC watershed to quantify evasion of Hg(0) from the creek. Task 1 continues to collaborate with the U.S. Geological Survey (USGS) by deploying air samplers to quantify Hg stable isotope fractionation patterns in Hg(0) coming from the creek. This is part of a continental-scale network of air sampling being conducted by the USGS. In addition, Task 1 staff attended an annual all-hands meeting and participated in quarterly web meetings of the DuPont-sponsored South River Science Team to discuss and compare approaches to studying Hg cycling in the similar South River watershed. Task 1 hosted several summer faculty and students from New Mexico State University, a minority-serving institution; they included K.C. Carroll and Dale Rucker from HGI hydroGEOPHYSICS, Inc.; postdoctoral fellow Mitra Khadka; graduate student Peter (Chia-Hsing) Tsai; and undergraduate intern Justin Milavec. Their work on hyporheic zone-surface water interactions complements our work in understanding Hg transport and transformation in EFPC. Guests from the University of Michigan included Jason Demers and his graduate student Elizabeth Crowther. Demers has an SBR-funded project to apply molecular-scale information gained from measuring Hg stable isotope fractionation to watershed-scale process information. ORNL's Scott Brooks is a co-investigator on the University of Michigan project. Finally, we hosted Ming Ye and one of his graduate students from Florida State University for several days last August. We are working with them to perform uncertainty quantification calculations of Hg aqueous speciation that considers thermodynamic data uncertainty, model uncertainty, and forcing function uncertainty.

With the release of the ORNL PCR-based protocols, Task 3 has been establishing external collaborations to assess more samples from other field sites, including week-long training sessions at ORNL or at our collaborators' laboratories. These collaborations include: (1) Eric Boyd, Montana State University, and his graduate student Melody Lindsay to confirm Hg-methylators in the Great

Fig. 8. Key ORNL SFA partners.

Salt Lake, Utah; (2) Carl Lamborg, University of California, Santa Cruz, and postdoctoral student Katlin Bowman to confirm Hg-methylators in the Arctic Ocean; (3) Brandy Toner, University of Minnesota, to confirm Hg-methylators in soil core samples from the Marcell Experimental Forest in Minnesota associated with the SPRUCE experiment; (4) Dave Krabbenhoft, USGS, (Task 3) worked to confirm Hg-methylators in the oxygen-minimum zone (filtered seawater) collected during a cruise from Iceland to Brazil; and (5) Helen Hsu-Kim, Duke University, and her postdoctoral student, Udonna Ndu to assay Hg-methylators from marsh samples (20 samples). In each case, the Task 3 team members are also serving as consultants for establishing these protocols in their laboratory.

Task 4 recently initiated a subcontract to Steve Ragsdale at the University of Michigan to assist with heterologous expression and functional characterization of HgcA and HgcB (Milestones 4a and 4b). Ragsdale was an unfunded collaborator with Task 4 for the past three years.

Although the CI-SFA's primary objective is fundamental science, it is important that project personnel have the opportunity to translate scientific discovery into information relevant to DOE's Office of Environmental Management (EM) and the broader DOE complex. Currently, Eric Pierce serves as ORNL's point of contact for the DOE EM headquarters and Oak Ridge EM (OREM) applied research and technology development programs. In this role, Pierce and others will have the opportunity to interact with local OREM staff (Elizabeth Phillips, Brian Henry, and Laura Wilkerson), EM headquarter staff (Rod Rimando and Kurt Gerdes), and the site-specific advisory panels. These interactions provide a way to inform DOE EM on how Hg is transformed in environmental systems, which is a need recently outlined in the DOE EM report titled *Technology Plan to Address EM Mercury Challenge*.

Program Structure and Advisory Committee

Organization and Leadership

CI-SFA scientific objectives are aligned to the four integrated research tasks of Ecosystem Features (Task 1); Biogeochemical Mechanisms (Task 2); Microbial Community Functions and Geochemical Influences (Task 3); and Molecular Structure, Dynamics, and Mechanisms (Task 4). These tasks are managed across the CI-SFA as an integrated team effort. Eric Pierce is the Laboratory Research Manager and the point of contact with DOE SBR program managers. He speaks to Paul Bayer biweekly on CI-SFA progress and potential issues. Task leaders are Scott Brooks, Baohua Gu, Dwayne Elias, and Jerry Parks, who lead Tasks 1–4, respectively. These leaders meet tri-weekly to discuss research directions, staffing, budget, and cost issues. CI-SFA staff also meet triweekly to discuss task progress. See website for a complete organization chart (www.esd.ornl.gov/programs/rsfa/contacts.shtml).

Fig. 9. ORNL CI-SFA Staff and SAC Members.

Media Mentions

Newly Identified Microbial Process Could Reduce Toxic Methylmercury Levels—*ORNL News*, May 31, 2017

- *ChemEurope*, June 1, 2017
- *Bioengineer.org*, May 31, 2017
- *News Medical Life Sciences*, June 1, 2017
- *Phys.org*, May 31, 2017
- *Medindia*, June 4, 2017
- *Bionity.com*, June 1, 2017
- *Lab Manager*, June 1, 2017
- *Military Technologies*, May 31, 2017
- *Postwaves*, June 2, 2017

A Lifetime of Learning: Eric Pierce—*ORNL Research Highlight*, March 15, 2017

Seven ORNL Researchers Elected AAAS Fellows—*ORNL News*, November 22, 2016

Unraveling the Mystery of Methylmercury—*Earthzine*, October 1, 2016

New ORNL Tool Probes for Genes Linked to Toxic Methylmercury—*ORNL News*, July 18, 2016

- *Science Daily*, July 18, 2016
- *EurekAlert! AAAS*, July 18, 2016

Methylmercury Sleuths Armed with New Spotlight—*DOE Office of Science*, January 23, 2017

Scientific Advisory Committee

The FY2017 Scientific Advisory Committee (SAC) meeting was held March 30–31, 2017, at ORNL. Members include committee chair Dave Krabbenhoft, USGS; Alex MacKerell, University of Maryland; Richard Sparling, University of Manitoba; Carl Lamborg, University of California, Santa Cruz; and Elizabeth Phillips, DOE OREM. All currently funded CI-SFA participants attended and highlighted recent progress toward objectives and goals.

Fig. 10: Newly Identified Microbial Process Could Reduce Toxic Methylmercury Levels—*ORNL News*, May 31, 2017.

National Laboratory Investments

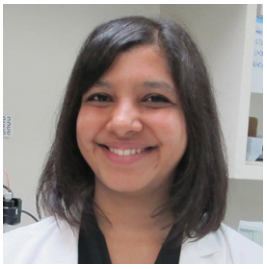
ORNL is committed institutionally to the success of the mercury CI-SFA program. In FY2017, ORNL funded a 2-year, $400,000 investment to develop an integrated surface-subsurface watershed modeling capability based on the highly parallel community flow and transport code PFLOTRAN. Specific goals are to add a new integrated surface-subsurface flow modeling capability and develop an improved representation of root water uptake, both applicable at the watershed scale.

Staff Award

Baohua Gu was elected a 2016 Fellow of the American Association for the Advancement of Science (AAAS) for his *distinguished contributions to molecular-scale mechanisms that control cycling of natural organic matter, contaminants, and toxic metals and for technology innovations to remediate contaminants in the environment*. Dr. Gu received this honor at the February AAAS 2017 Annual Meeting in Boston.

Postgraduate Spotlight

Swapneeta Date



We welcomed Swapneeta Date as a new member of the CI-SFA team. She received her Ph.D. in Biomedical Sciences, Cell Physiology and Molecular Biophysics from Texas Tech University in March 2016 and has extensive experience with the functional and structural characterization of membrane protein complexes. Her aim is to characterize the molecular mechanisms of Hg methylation and understand the complex interplay between various cellular components and pathways surrounding Hg methylation. She joined Task 4 as a postdoctoral researcher on August 29, 2016.

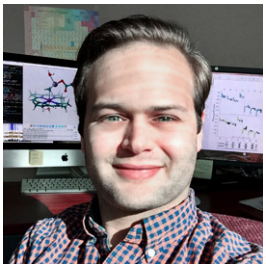
Todd Olsen

Todd Olsen received his bachelor degrees in Chemistry and Applied Mathematics from the University of Montana, Missoula, and his master's degree in Environmental Engineering from the University of Illinois at Urbana-Champaign. His master's thesis was titled "Investigations of and Performance Improvements in the Mercury Thiourea Complex Ion Chromatography Method for Mercury Speciation." In his thesis, a new system chemistry for the Hg thiourea-ion chromatography method is described that enables reliable quantification of MeHg and unexpectedly demonstrates the presence of previously unknown Hg species with low net charge that are chemically different from both MeHg and Hg^{II}. He was appointed in 2014 as an ORNL postmaster's research associate under the mentorship of Scott Brooks to study the impact periphyton biofilms have on Hg cycling in EFPC. His work at ORNL includes periphyton characterization, methylation and demethylation rate assays on periphyton, biogeochemical redox profiling of periphyton, and monitoring the dynamics of low molecular weight thiol concentrations in periphyton. Todd recently accepted a position with Geosyntec Consultants, in Sacramento, California.

Geoff Christensen

Geoff Christensen received his undergraduate degree from University of Illinois at Urbana-Champaign (2008) and his doctorate from the University of Missouri-Columbia (2014), both in Biochemistry. He worked with Judy Wall at Missouri, where he characterized the redox repressor Rex and its role in regulating the metabolism of the sulfate reducer *Desulfovibrio vulgaris* Hildenborough. In pursuit of his degree, he developed skills in molecular modeling; culturing aerobes and anaerobes; mutagenesis; and purification and analysis of DNA, RNA, and proteins. As a graduate student at Missouri, Geoff has been recognized for his efforts, including the National Institute of General Medical Sciences (NIGMS) Training Grant, Charles W. Gehrke Jr. Memorial Scholarship Fund, and the Federation of European Microbiological Societies (FEMS) Young Scientist Grant. Geoff joined ORNL in January 2015 under the tutelage of Dwayne Elias. Currently, Geoff is focused on developing and validating molecular probes for accessing Hg methylation in the environment, further refining his molecular and microbiology techniques. Geoff presented his findings at the 2015 American Geophysical Union Conference.

Ryne C. Johnston



Ryne C. Johnston is a computational chemist who received his Ph.D. in Chemistry from Oregon State University in 2015. He applies quantum chemical methods to glean energetic, mechanistic, or structural insights into the reactivity and speciation of organic molecules and (post-)transition metal complexes relevant to Hg biogeochemistry. One of his major research thrusts is to elucidate the enzymatic and abiotic pathways of Hg methylation by cobalamin (i.e., cofactor B12). He also is developing accurate predictive models *ab initio* to study the aqueous speciation behavior of Hg in natural aquatic environments. These projects fit within his broader interests in geology and bioinformatics. Ryne recently accepted a position at Schrödinger, Inc., in Portland, Oregon.

Appendix A. Cited References

Fitzgerald, W.F., and C.H. Lamborg. 1998. "Geochemistry of Mercury in the Environment." In *Environmental Geochemistry*. Eds: H.D. Holland and K.K. Turekian, 107–48. San Diego. Academic Press.

Jonsson, S., N.M. Mazrui, and R.P. Mason. 2016. "Dimethylmercury Formation Mediated by Inorganic and Organic Reduced Sulfur Surfaces." *Scientific Reports* **6**:27958. DOI:10.1038/srep27958.

Lindberg, S.E., and W.J. Stratton. 1998. "Atmospheric Mercury Speciation: Concentrations and Behavior of Reactive Gaseous Mercury in Ambient Air." *Environmental Science & Technology* **32**:49–57.

Mason, R.P., C.L. Miller, C.C. Gilmour, and A. Heyes. 2002. "Factors Controlling the Production, Fate, and Transport of Mercury and Methylmercury in Sediments." In *American Chemical Society 223rd National Meeting*. Orlando, Fla. American Chemical Society Press.

Mason, R.P., E.H. Kim, J. Cornwell, and D. Heyes. 2006. "An Examination of the Factors Influencing the Flux of Mercury, Methylmercury, and Other Constituents from Estuarine Sediments." *Marine Chemistry* **102**:96–110.

UNEP. 2013. *Mercury: Time to Act*. Rep. No. DTI/1623/GE. United Nations Environment Programme. www.unep.org/PDF/PressReleases/Mercury_TimeToAct.pdf.

Christensen, G.A., A.M. Wymore, A.J. King, M. Podar, R.A. Hurt Jr., E.U. Santillan, A. Soren, C.C. Brandt, S.D. Brown, A.V. Palumbo, J.D. Wall, C.C. Gilmour, and D.A. Elias. 2017. "Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment." *Applied and Environmental Microbiology* **83**:6068–6078. DOI:10.1128/AEM.01271-16.

Appendix B. CI-SFA Publications

See website for complete list (www.esd.ornl.gov/programs/rsfa/).

This annual report summarizes accomplishments from July 2016 to June 2017, a period representing the second year following the program's triennial review in April 2015 and proposal acceptance in August 2015 by DOE BER. The ORNL SFA program has produced a total of 26 new peer-reviewed publications during the initial 2 years of this 3-year renewal cycle for a total of 85 publications since the program started in 2009.

Manuscripts

Brooks, S.C., C.C. Brandt, and N.A. Griffiths. 2017. "Estimating Uncertainty in Ambient and Saturation Nutrient Uptake Metrics from Nutrient Pulse Releases in Stream Ecosystems." *Limnology and Oceanography: Methods* **15**(1):22–37. DOI:10.1002/lom3.10139.

Chen, H., R.C. Johnston, B.F. Mann, R.K. Chu, N. Tolic, J.M. Parks, and B. Gu. 2017. "Identification of Mercury and Dissolved Organic Matter Complexes Using Ultrahigh Resolution Mass Spectrometry." *Environmental Science & Technology Letters* **4**(2):59–65. DOI:10.1021/acs.estlett.6b00460.

Christensen, G.A., A.C. Somenahally, J.G. Moberly, C.L. Miller, A.J. King, A.V. Palumbo, S.C. Brooks, and D.A. Elias. "Carbon Amendments Alter Microbial Community Structure and Net Mercury Methylation Potential in Sediments." *Applied and Environmental Microbiology. Accepted.*

Frontalini, F., D. Curzi, E. Cesarini, B. Canonico, F.M. Giordano, R. De Matteis, J.M. Bernhard, N. Pieretti, B. Gu, J.R. Eskelsen, A.M. Jubb, L. Zhao, E.M. Pierce, P. Gobbi, S. Papa, and R. Coccioni. 2016. "Mercury-Pollution Induction of Intracellular Lipid Accumulation and Lysosomal Compartment Amplification in the Benthic Foraminifer *Ammonia parkinsoniana*." *PLOS ONE* **11**(9):e0162401. DOI:10.1371/journal.pone.0162401.

Jiang, P., Y.B. Li, G.L. Liu, G.D. Yang, L. Lagos, Y.G. Yin, B.H. Gu, G.B. Jiang, and Y. Cai. 2016. "Evaluating the Role of Re-adsorption of Dissolved Hg^{2+} During Cinnabar Dissolution Using Isotope Tracer Technique." *Journal of Hazardous Materials* **317**:466–75. DOI:10.1016/j.jhazmat.2016.05.084.

Johnston, R.C., J. Zhou, J.C. Smith, and J.M. Parks. 2016. "Toward Quantitatively Accurate Calculation of the Redox-Associated Acid–Base and Ligand Binding Equilibria of Aquacobalamin." *Journal of Physical Chemistry B* **120**(30):7307–18. DOI:10.1021/acs.jpcb.6b02701.

Liu, Y.-R., X. Lu, L. Zhao, J. An, J.-Z. He, E.M. Pierce, A. Johs, and B. Gu. 2016. "Effects of Cellular Sorption on Mercury Bioavailability and Methylmercury Production by *Desulfovibrio desulfuricans* ND132." *Environmental Science & Technology* **50**(24):13335–41. DOI:10.1021/acs.est.6b04041.

Lu, X., W. Gu, L. Zhao, M. Farhan Ul Haque, A.A. DiSpirito, J.D. Semrau, and B. Gu. 2017. "Methylmercury Uptake and Degradation by Methanotrophs." *Science Advances* **3**(5). DOI:10.1126/sciadv.1700041.

Luo, H.-W., X. Yin, A.M. Jubb, H. Chen, X. Lu, W. Zhang, H. Lin, H.-Q. Yu, L. Liang, G.-P. Sheng, and B. Gu. 2017. "Photochemical Reactions Between Mercury (Hg) and Dissolved Organic Matter Decrease Hg Bioavailability and Methylation." *Environmental Pollution*, **220**:1359–65. DOI:10.1016/j.envpol.2016.10.099.

Olsen, T.A., C.C. Brandt, and S.C. Brooks. 2016. "Periphyton Biofilms Influence Net Methylmercury Production in an Industrially Contaminated System." *Environmental Science & Technology*. DOI:10.1021/acs.est.6b01538.

Qian, C., A. Johs, H. Chen, B.F. Mann, X. Lu, P.E. Abraham, R.L. Hettich, and B. Gu. 2016. "Global Proteome Response to Deletion of Genes Related to Mercury Methylation and Dissimilatory Metal Reduction Reveals Changes in Respiratory Metabolism in *Geobacter sulfurreducens* PCA." *Journal of Proteome Research*. DOI:10.1021/acs.jproteome.6b00263.

Submitted Manuscripts

Asaduzzaman, A.M., D.M. Riccardi, A.T. Afaneh, J.C. Smith, F. Wang, J.M. Parks, and G. Schreckenbach. "Environmental Mercury Chemistry – *in silico*." *Accounts of Chemical Research. In review*.

Demers, J.D., J.D. Blum, S.C. Brooks, P.M. Donovan, C.L. Miller, A.L. Riscassi, W. Zheng, and B. Gu. "Hg Isotopes Reveal In-Stream Processing and Legacy Inputs in East Fork Poplar Creek, Oak Ridge, TN, USA." *Submitted*.

Zhao, L., H. Chen, X. Lu, H. Lin, G.A. Christensen, E.M. Pierce, and B. Gu. "Contrasting Effects of Dissolved Organic Matter on Mercury Methylation by *G. sulfurreducens* PCA and *D. desulfuricans* ND132." *Environmental Science & Technology. In review.*

Zhou, J., M.D. Smith, S.J. Cooper, X. Cheng, J.C. Smith, and J.M. Parks. "Modeling of the Passive Diffusion of Mercury and Methylmercury Complexes Through a Bacterial Cytoplasmic Membrane." *Environmental Science & Technology. In review*.

Vishnivetskaya, T.A., H. Hu, J.D. Van Nostrand, A.M. Wymore, X. Xu, G. Qiu, X. Feng, S.D. Brown, C.C. Brandt, M. Podar, J. Zhou, B. Gu, and D.A. Elias. "Microbial Community Structure with Trends in Methylation Gene Diversity and Abundance in Mercury-Contaminated Rice Paddy Soils in Guizhou, China." *PLOS One. In review*.

Book Chapters

Gu, B., X. Lu, A. Johs, and E.M. Pierce. 2017. "Mercury in Water." In *Encyclopedia of Water: Science, Technology, and Society*. P. Maurice, Ed., Wiley.

Smith, J.C. and J.M. Parks. 2016. "Modeling Mercury in Proteins." In *Computational Approaches for Studying Enzyme Mechanism Part B, Methods in Enzymology*, **578**:103–22. G.A. Voth, Ed., Elsevier, B.V.

Technical Reports

Brooks, S.C., V. Eller, J. Dickson, J. Earles, K. Lowe, T. Mehlhorn, T. Olsen, C. DeRolph, D. Watson, D. Phillips, and M.J. Peterson. 2017. "Mercury Content of Sediments in East Fork Poplar Creek: Current Assessment and Past Trends." Oak Ridge National Laboratory, ORNL/TM-2016/578. DOI:10.2172/1338545.

Peterson, M.J., S.C. Brooks, T.J. Mathews, M.A. Mayes, A. Johs, D.B. Watson, M.D. Poteat, J.G. Smith, T. Mehlhorn, B. Lester, J. Morris, K. Lowe, J.O. Dickson, V. Eller, and C.R. DeRolph. 2016. "Mercury Remediation Technology Development for Lower East Fork Poplar Creek — FY 2015 Progress Report." Oak Ridge National Laboratory, ORNL/TM-2016/48.

Watson, D., M. Bevelhimer, C.C. Brandt, C.R. DeRolph, S.C. Brooks, M.A. Mayes, T. Olsen, J.O. Dickson, M.J. Peterson, and R. Ketelle. 2016. "Evaluation of Lower East Fork Poplar Creek Mercury Sources – Model Update." Oak Ridge National Laboratory, ORNL/SR-2016/503.

Appendix C. Presentations and Conferences

Published or Accepted Conference Abstracts or Presentations

Chen, H., R.C. Johnston, B. Mann, R. Chu, N. Tolic, J.M. Parks, and B. Gu. "Identification of Mercury and Dissolved Organic Matter Complexes Using Ultra-High Resolution Mass Spectrometry." International Conference on Mercury as a Global Pollutant. July 16–21, 2017. Providence, R.I.

Christensen, G.A., A.M. Wymore, A.J. King, S.D. Brown, M. Podar, C.C. Brandt, S.C. Brooks, A.V. Palumbo, J.D. Wall, A. Soren, C.C. Gilmour, U. Ndu, H. Hsu-Kim, and D.A. Elias. "Application of *hgcAB* Biomarkers in the Environment." ORNL Mercury Science Focus Area Science Advisory Committee Meeting. March 30–31, 2017. Oak Ridge, Tenn.

Christensen, G.A., A.M. Wymore, A.J. King, S.D. Brown, M. Podar, C.C. Brandt, S.C. Brooks, A. . Palumbo, J.D. Wall, A. Soren, C.C. Gilmour, U. Ndu, H. Hsu-Kim, and D.A. Elias. "Application of *hgcAB* Biomarkers in the Environment." Environmental System Science Principal Investigator Meeting. April 26–27, 2017. Potomac, Md.

Date S., S.D. Smith, K.W. Rush, J.M. Parks, J.D. Wall, S.W. Ragsdale, and A. Johs. "The Biochemistry of Mercury Methylation in Anaerobic Bacteria." Environmental System Science Principal Investigators Meeting. April 25–26, 2017. Potomac, Md.

Date S., S.D. Smith, K.W. Rush, J.M. Parks, S.W. Ragsdale, J.D. Wall, and A. Johs. "Insights into the Biomolecular Mechanism of Microbial Mercury Methylation." International Conference on Mercury as a Global Pollutant. July 16–21, 2017. Providence, R.I.

Dickson, J.O., P.D. Ayers, T.L. Mehlhorn, L.G. Rodriguez, E.M. Pierce, S.C. Brooks, M.J. Peterson, and M. Mayes. "The Role of Bank Erosion in Mercury Flux into a Contaminated Stream." The American Society of Agronomy, Crop Science

Society of America, and Soil Science Society of America Annual Meeting. November 6–9, 2016. Phoenix, Ariz.

Gu, B., Y. Liu, X. Lu, L. Zhao, J. An, A. Johs, and E.M. Pierce. "Effects of Thiol Ligands on Mercury Cellular Sorption, Bioavailability, and Methylation by Anaerobic Bacteria." International Conference on Mercury as a Global Pollutant. July 16–21, 2017. Providence, R.I.

Gu, B., Z. Yang, D.E. Graham, and S.D. Wullschleger. "Rising Temperatures, Thawing Permafrost, and Mercury/Methylmercury Transformations in the Arctic." International Conference on Arctic Science: Bringing Knowledge to Action. April 24–27, 2017. Reston, Va.

Johnston, R.C., T. Olsen, S.C. Brooks, and J.M. Parks. "Quantum Chemical Insights into Dimethylmercury Formation on Reduced Sulfur Reveal Common Themes in Mercury Methylation and Demethylation." International Conference on Mercury as a Global Pollutant. July 16–21, 2017. Providence, R.I.

Johs, A., V. Eller, K. Muller, C. Lee, T. Mehlhorn, C. Miller, B.K. Robertson, D. Harper, S.C. Brooks, M.A. Mayes, E.M. Pierce, and M.J. Peterson. "Evaluation of Sorbent Materials for Removal of Mercury from a Contaminated Freshwater Ecosystem." 19th Symposium on Separation Science and Technology for Energy Applications. October 10–12, 2016. Gatlinburg, Tenn.

Liang, L., B. Gu, R.C. Johnston, A. Johs, K. Neupane, J.M. Parks, K.W. Rush, and S.J. Tomanicek. "Mercury Methylation by Methylcobalamin: Kinetics and Mechanisms Revisited." Goldschmidt Conference 2016. June 26–July 1, 2016. Yokohama, Japan.

Lu, X., L. Zhao, W. Gu, Y. Liu, A. Johs, J.D. Semrau, and B. Gu. "Microbial Demethylation in the Environment: Roles of Iron-Reducing Bacteria and Methanotrophs." International Conference on Mercury as a Global Pollutant. July 16–21, 2017. Providence, R.I.

Lynes, M.M., A.J. King, G.A. Christensen, A.M. Wymore, A.V. Palumbo, T.A. Olsen, S.C. Brooks, and D.A. Elias. "Using Molecular Probes to Detect *hgcAB* from Enriched Periphyton Biofilm Samples from the East Fork Poplar Creek Mercury Contaminated System." ASM Microbe 2017. June 1–5, 2017. New Orleans, La.

Olsen, T.A., K.A. Muller, and S.C. Brooks. "Measurement and Modeling of Methylmercury Production in Periphyton Biofilms." Environmental System Science Principal Investigator Meeting. April 25–26, 2017. Potomac, Md.

Painter, S.L., and S.C. Brooks. "Modeling Solute Transport and Coupled Biogeochemical Transformations in Low-Order Streams Using a Stochastic Travel-time Approach." Environmental System Science Principal Investigator Meeting. April 25–26, 2017. Potomac, Md.

Parks, J.M., J. Zhou, R.C. Johnston, A. Johs, T.A. Olsen, M.D. Smith, X. Cheng, S.C. Brooks and J.C. Smith. "Biomolecular Insights into the Transport and Transformations of Mercury." Environmental System Science Principal Investigator Meeting. April 25–26, 2017. Potomac, Md.

Peterson, M.J., S.C. Brooks, T. Mathews, M.A. Mayes, D. Watson, A. Johs, T. Mehlhorn, J.O. Dickson, C. Mansfield, E. Phillips, and E.M. Pierce. "An Integrated, Systems-based Approach to Mercury Research and Technology Development." Waste Management Symposium 2017. March 5–9, 2017. Phoenix, Ariz.

Pierce, E.M., S.C. Brooks, B. Gu, D.A. Elias, J.M. Parks, A. Johs, C.C. Gilmour, and J. Wall. "Biogeochemical Transformations at Critical Interfaces Science Focus Area: An Overview." Environmental System Science Principal Investigator Meeting. April 25–26, 2017. Potomac, Md.

Zhao, L., X. Lu, H. Chen, and B. Gu. "Effects of Natural Organic Matter on Microbial Methylation of Mercury (Hg) Under Anaerobic Conditions." International Conference on Mercury as a Global Pollutant. July 16–21, 2017. Providence, R.I.

Invited Presentations

Christensen, G.A. "Validation of Cost-Effective Molecular Probes to Assess Mercury Methylation in the Environment: an Effort to Link *hgcA* Abundance to Methyl- and Total Mercury Concentrations." 2017 International Conference on Mercury as a Global Pollutant. July 16–21, 2017. Providence, R.I.

Christensen, G.A. "Validation of Cost-Effective Molecular Probes to Assess Mercury Methylation in the Environment: an Effort to Link *hgcA* Abundance to Methyl- and Total Mercury Concentrations." Society for Industrial Microbiology and Biotechnology Annual Meeting. July 30-August 3, 2017. Denver Colo.

Gu, B. "Natural Organic Matter on the Redox Transformation and Cycling of Metals and Radionuclides in the Environment." The Geological Society of America Annual Meeting. September 25–28, 2016. Denver, Colo.

Gu, B. "Mercury Photo-Redox Transformation and Its Reactivity and Bioavailability in Freshwater Systems." The 252st American Chemical Society National Meeting. August 21–25, 2016. Philadelphia, Pa.

Gu, B. "Microbial Cell Surface-Mediated Mercury Reduction, Oxidation, and Sorption on Methylmercury Biosynthesis." The 252st American Chemical Society National Meeting. August 21–25, 2016. Philadelphia, Pa.

Appendix D. Leadership Activities, Outreach, and User Proposals

Leadership Activities

Scott Brooks

- Participates in U.S. DOE EM-funded technology development (TD) project that aims to identify remedial actions that will decrease Hg concentrations, Hg flux, and Hg levels in fish. Natural synergies exist between the SFA and TD projects while maintaining uniqueness of effort within each project.
- Engaged with the South River Science Team, which is led by DuPont to address legacy mercury contamination in the South River, Va.
- Participates in a USGS-led continental-scale air sampling study by deploying air samplers at one location along upper EFPC. Study results may lend important insights into the aqueous reaction path followed by Hg resulting in the formation of volatile Hg(0).

Dwayne Elias

- Academic Editor for *PLoS One.*
- Associated Editor for *Frontiers in Microbiology Journal.*

Baohua Gu

- American Association for the Advancement of Science Fellow.
- Regional Editor for *Environmental Engineering Science.*
- Organized Session at 2017 International Conference on Mercury as a Global Pollutant.

Eric Pierce

- Associate Editor for *Applied Geochemistry Journal*
- Participant in the Local Organizing Committee for the 2017 International Conference on Mercury as a Global Pollutant.
- Supports the Oak Ridge Office of Environmental Management in revising the Mercury Remediation Strategy. This effort represents a forum to help site managers translate SFA accomplishments and findings collected to date into actionable decisions.
- Member of CESD–ESS Cyber Infrastructure Executive Committee and participated in the 2017 CESD–ESS Working Groups Meeting.

User Proposals Submitted

- Johs, A., S. Date, K.W. Rush, and S.W. Ragsdale. Identification of Protein Interaction Networks, Cofactors and Protein Structures Essential for Mercury Methylation in Anaerobic Bacteria. EMSL user proposal. *Submitted.*
- Parks, J.M. and J.C. Smith. Key Roles of Relativity in Hg Biogeochemical Transformations. EMSL user proposal. *Submitted.*
- Gu., B. and H. Chen. Molecular Identification of Mercury Complexes with Natural Organic Matter. EMSL user proposal #49344.
- Gu, B., M. Philben, and H. Chen. Tracking the production, degradation, and stabilization of microbial soil organic carbon in arctic soils. EMSL user proposal. *Submitted.*

Outreach

Scott Brooks visited Philadelphia Elementary School in Loudon County, Tenn., for their "I Love Math" day.

Appendix E. 2017 Scientific Advisory Committee Visit

March 30–31, 2017

Oak Ridge National Laboratory - Clinch River Cabin, Gallaher Bend Room
Meeting Host/Point of Contact: Eric Pierce

ORNL Team: Craig Brandt, Scott Brooks, Steven Brown, Dwayne Elias, Baohua Gu, Alex Johs, Frank Loeffler*, Kenneth Lowe, Scott Painter, Anthony Palumbo, Jerry Parks, Eric Pierce, Mircea Podar, Jeremy Smith*, Ann Wymore, and Xiangping Lisa Yin

Post-Doctoral Researchers/Graduates: Jing An**, Hongmei Chen, Geoffrey Christensen, Swapneeta Date, Jeremy Eskelsen, Ryan Harvey (University of Missouri), Allison Holt, Ryne Johnston, Mackenzie Lynes, Xia Lu, Katie Muller**, Todd Olsen, Shan Wu**, Linduo Zhao, and Jing Zhou

Collaborators: Cindy Gilmour (Smithsonian) and Judy Wall (University of Missouri)

Scientific Advisory Committee Members: David Krabbenhoft, Alex MacKerrell*, Elizabeth Phillips, Richard Sparling, and Carl Lamborg

**Remote participation or not in attendance*
***Visiting/guest faculty, scholar, or postdoctoral researcher*

Thursday, March 30th		
Schedule	**Topic**	**Lead**
8:00–8:30 am	Check-in at ORNL Visitors Center / Badging Transit to Clinch River Cabin	SAC Members, Eric Pierce
8:30–9:00 am	Welcome / Reviewers Orientation	Eric Pierce
9:00–9:30 am	ORNL SFA Overview Presentation	Eric Pierce
9:30–9:45 am	Questions / Discussion	All
9:45–10:30 am	Task 1 Overview Presentation (30 min.), Q&A (10 min.)	Scott Brooks
10:30–10:45 am	Break	All
10:45–11:30 am	Modeling Presentation (30 min.), Q&A (10 min.)	Scott Painter
11:30 am–12:15 pm	Task 2 Overview Presentation (30 min.), Q&A (10 min.)	Baohua Gu
12:15–1:15 pm	Working Lunch: 12:15–12:30 Morning Summary / Discussion 12:30–1:15 Task 3 Overview Presentation (30 min.), Q&A (10 min.)	All Eric Pierce Dwayne Elias
1:15–1:45 pm	University of Missouri Overview (15 min.), Q&A (10 min.)	Judy Wall
1:45–2:15 pm	Smithsonian Research Institution Overview (15 min.), Q&A (10 min.)	Cindy Gilmour
2:15–2:30 pm	Break	
2:30–3:00 pm	Group Picture	All
3:00–3:45 pm	Task 4 Overview Presentation (30 min.), Q&A (10 min.)	Jerry Parks and Alex Johs
3:45–4:30 pm	Final Questions / Discussions	All
4:30–5:00 pm	Transit to Lakeside Tavern for Poster Session and Setup	All
5:00–8:00 pm	Poster Session and Working Dinner	All
Friday, March 31th		
Schedule	**Topic**	**Participants**
8:00–11:00 am	Working Breakfast: Committee Sequester to Discuss Issues and Questions for SFA Team	Eric Pierce (kick-off) and SAC Members
11:00–11:50 am	Committee Debriefing to SFA Task Leaders	SAC Members and Task Leaders
11:50 am–12:00 pm	Wrap-up / Meeting Adjourned	SAC Members and Task Leaders

Acronyms and Abbreviations

AAAS	American Association for the Advancement of Science
BER	DOE Office of Biological and Environmental Research
ATS	Advanced Terrestrial Simulator
C1	one-carbon
CI-SFA	Critical Interfaces Science Focus Area
Cys	cysteine
DFT	density functional theory
DOM	dissolved organic matter
DOE	U.S. Department of Energy
EFPC	East Fork Poplar Creek
EM	DOE Office of Environmental Management
EMSL	DOE Environmental Molecular Sciences Laboratory
FTICR-MS	Fourier transform ion cyclotron resonance–mass spectrometry
Hg	mercury
hgcAB	Hg-methylation gene pair
HgcAB	protein
HgS	mercury sulfide
IHg	inorganic mercury
IMGP	International Conference on Mercury as a Global Pollutant
MD	molecular dynamics
MeHg	methylmercury
MerB	organomercurial lyase
NOM	natural organic matter
ORNL	Oak Ridge National Laboratory
OREM	Oak Ridge Office of Environmental Management
ORR	Oak Ridge Reservation
PCR	polymerase chain reaction
PFLOTRAN	massively parallel subsurface flow and reactive transport code
PI	principal investigator
qPCR	quantitative polymerase chain reaction
SAC	scientific advisory committee
SBR	DOE Subsurface Biogeochemical Research program
SFA	Science Focus Area
SPRUCE	Spruce and Peatland Responses Under Changing Environments
TD	technology development
TnLE-seq	transposon liquid enrichment sequencing
UNEP	United Nations Environment Programme

SFA Contact and Sponsor

Contact: Eric Pierce, ORNL, pierceem@ornl.gov

Sponsor: The ORNL Mercury SFA is sponsored by the Subsurface Biogeochemical Research (SBR) program within the U.S. Department of Energy's Office of Biological and Environmental Research. Contact Paul Bayer, SBR Program Manager, at paul.bayer@science.doe.gov.