Biogeochemical Transformations at Critical Interfaces

ORNL Mercury Science Focus Area (SFA) 2016 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 mercury contamination in natural environments results mostly from 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. On the Oak Ridge Reservation (ORR), for example, mercury pollution in the East Fork Poplar Creek (EFPC) watershed is caused by historical mercury use at the Y-12 National Security Complex where large quantities of mercury were lost to the environment during the 1950s and 1960s.

The Oak Ridge National Laboratory (ORNL) Critical Interfaces Science Focus Area (CI-SFA) program—formally known as the *Biogeochemical Transformations at Critical Interfaces* project—has and continues to make new transformational advances in Hg research and, more broadly, subsurface biogeochemistry. This next phase of the program seeks to address the following:

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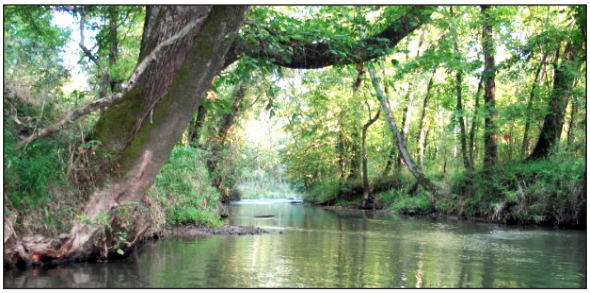


Fig. 1: Low-Order Streams. Streams are ranked based on a hierarchical network of channels within a watershed. Low-order streams (i.e., first- through fourth-order streams) are located in the headwater areas and typically convey small volumes of water. Such streams play a dominant role in the flow, biogeochemistry, 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.

Nine-Year Science Challenge: Determine the coupled hydro-biogeochemical processes that control Hg fate and transformation in low-order freshwater stream systems (See Fig. 1, this page).

Nine-Year Science Goal: Establish 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.

Developing predictive understanding of Hg and trace metal transport and fate in environmental systems, such as terrestrial surface and subsurface ecosystems, is a formidable challenge because it requires deciphering complex processes (i.e., physical, chemical, and biological), deconvoluting how these processes interact with one another, and understanding the factors that control system response over broad spatiotemporal scales.

Exchange and feedback processes at critical interfaces are central for determining fluxes, stocks, and transformation rates of key constituents such as oxygen, nutrients, and dissolved organic matter (DOM) that control Hg speciation, distribution, and bioavailability (See Fig. 2, Page 2). Therefore, over the next three years (**Phase I, FY 2016–18**), the ORNL CI-SFA program will focus on:

Determining the fundamental mechanisms and environmental factors that control Hg biogeochemical transformations at key interfaces in terrestrial and aquatic ecosystems.

The research outlined in **Phase I** of the ORNL CI-SFA plan comprises collaborative and complementary research activities that support four research thrusts:

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

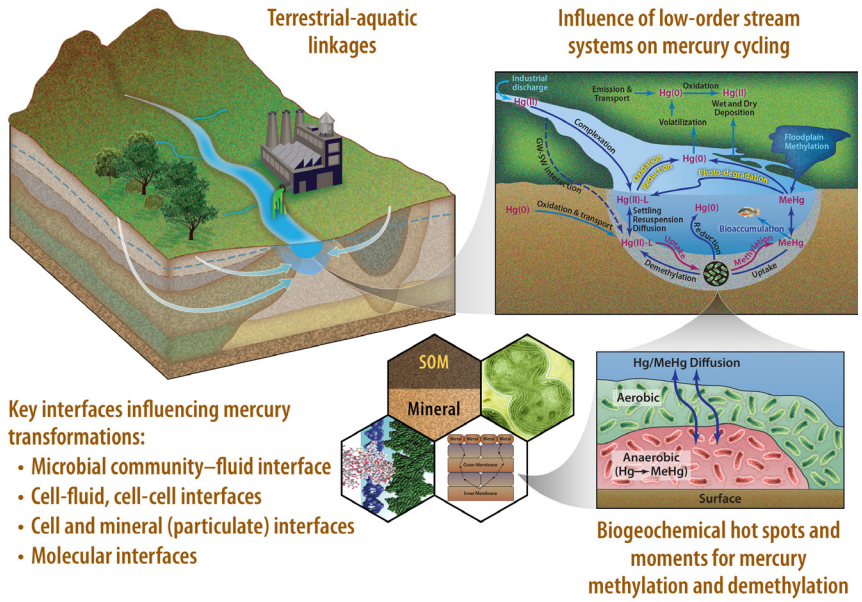


Fig. 2. Key Biogeochemical Interfaces. Interfaces are common boundaries between two or more system compartments or phases where steep gradients develop and govern the fate and transformation of material crossing those gradients.

This annual report summarizes the CI-SFA accomplishments from July 2015 to June 2016, a period representing the first year following the program's triennial peer review in April 2015 and proposal acceptance in August 2015 by the U.S. Department of Energy's (DOE) Office of Biological and Environmental Research (BER).

Snapshot of FY2016 Accomplishments

See Appendices A-D, pp. 20-25, for details on progress from July 2015 to June 2016, including:

- 9 papers published or in press
- 5 submitted manuscripts
- 29 presentations, abstracts, or posters delivered or accepted
- 11 invited talks

Scientific Progress

Task 1: Ecosystem Features Influencing Mercury Transformation

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.

The overarching objectives of Task 1 are to:

- Identify the ecosystem compartment(s) (e.g., channel margin, floodplain, periphyton) and hydro-biogeochemical conditions that govern net methylmercury concentration in EFPC.
- 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 and the development of a process-rich numerical model to challenge current understanding of watershed processes occurring over broad spatiotemporal scales.

FY15–FY16 Accomplishments

Over the past 12 months, Task 1 made significant progress toward milestones and published three papers. One paper provided in-depth analysis of detailed baseflow and stormflow sampling that was conducted over a 19-month period. The results suggest that MeHg is produced within the stream as opposed to originating from out-of-stream sources (e.g., adjacent wetlands). Further, results suggest that algal biofilms, also known as periphyton, are sources of MeHg to EFPC (Riscassi et al. 2016).

Mercury transformations are one of many element and nutrient transformation cycles in low-order, freshwater streams. It is likely that Hg 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 stream length). However, the uncertainty in these estimates is rarely evaluated; and, when it is, 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., in revision).

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 stream bed, and specifically, that periphyton may play an important role in MeHg production. This hypothesis is being tested by measuring the rate of Hg methylation and MeHg demethylation using periphyton samples collected from the field. Between-site differences in net methylation for samples collected from an upstream versus downstream location were driven by differences in the demethylation rate constant (k_d). In contrast, the within-site seasonal difference in net methylation was driven by changes in the methylation rate constant (k_m). Samples incubated in the dark had lower net methylation due to k_m values that were 60% less than those incubated in the light. Disrupting the biofilm structure decreased k_m by 50% and resulted in net demethylating conditions. Overall, the measured rates resulted in a net excess of MeHg generated and suggest intact, actively photosynthesizing periphyton biofilms harbor zones of MeHg production possibly making a substantial net positive contribution to the creek's MeHg budget (Olsen and Brooks, in revision; see Fig. 3, this page).

In conjunction with our methylation-demethylation assays, we are collaborating with Task 3 of the 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.

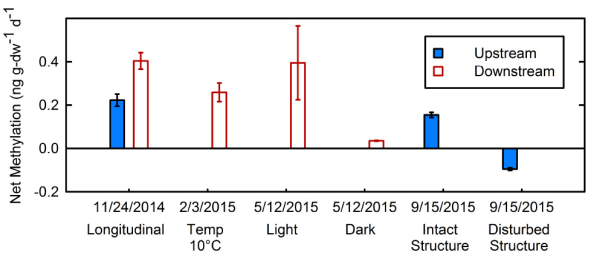


Fig. 3. Effect of treatments on net methylation (mean ± standard error) in periphyton samples from EFPC. Dates represent the day samples were taken and assays began. All assays were held at 20°C with full light exposure except the 2/3/2015 assay, which was held at 10°C, and the 5/12/2015 dark assay, which was wrapped in foil to exclude light.

Modeling Framework for Mercury Transport, Transformation

In FY16, the Task 1 team began working on a data-informed modeling framework for mercury 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. After evaluating the range of existing approaches, including detailed three-dimensional simulations, we developed a new hybrid modeling approach, which starts with 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 are extending that approach to incorporate representations of biogeochemical processes including aqueous speciation, complexation with dissolved organic material, sorption to particulate matter and sediments, and methylation/demethylation reactions. The key idea is to use unstructured computational meshes to construct flow networks that are consistent with the inferred travel-time distributions. In contrast to the existing travel-time approaches, hyporheic and surface storage zones are explicitly represented, which makes it possible to use existing reactive transport codes such as PFLOTRAN to represent mass transfer, biogeochemical speciation, and kinetic reactions. The resulting approach has the advantages of being extensible and tractable given practical constraints on stream corridor characterization. Although development is focused on mercury, the approach is broadly applicable to metal and nutrient transport in streams.

Manuscripts

Published or In Press

Riscassi, A., C., Miller, and S. C. Brooks. 2016. "Seasonal and Flow-driven Dynamics of Particulate and Dissolved Mercury and Methylmercury in a Stream Impacted by an Industrial Mercury Source." *Environmental Toxicology and Chemistry* **35**(6): 1386–1400. DOI: 10.1002/etc.3310

In Preparation or Submitted

Brooks, S. C., Brandt, C. C., and Griffiths, N. A. In revision. "Estimating Uncertainty in Ambient and Saturation Nutrient Uptake Metrics from Nutrient Pulse Releases in Stream Ecosystems." *Limnology and Oceanography: Methods*.

Olsen, T. A., and S.C. Brooks. In revision. "Periphyton biofilms Influence Net Methylmercury Production in an Industrially Contaminated System." *Environmental Science & Technology.*

Task 2: Biogeochemical Mechanisms Controlling Mercury Uptake and Methylation

The primary objective of Task 2 is to provide a fundamental understanding of the key geochemical and biochemical mechanisms controlling Hg sorption, uptake, and transformation at the interfaces between microbes, fluids, and organic and particulate-minerals. We are addressing the following specific scientific questions:

- What are the key geochemical and biochemical variables and interactions affecting Hg transformation and methylation?
- What are the molecular mechanisms controlling Hg and DOM interactions and its uptake by microorganisms?
- How does photo-redox transformation of Hg influence Hg reactivity and bioavailability?
- Under what conditions does Hg become more or less bioavailable in EFPC?

FY15–FY16 Accomplishments

Our previous research found that microbial methylation is strongly influenced by specific thiol ligands and that the uptake process is energy dependent (Schaefer et al. 2011; Lin et al. 2014). In particular, certain thiol compounds such as cysteine are found to greatly enhance Hg(II) methylation, but others (such as glutathione and penicillamine) completely inhibit the methylation by *G. sulfurreducens* PCA. However, the mechanism of thiol-enhanced or inhibited Hg methylation and the prevalence of cysteine-enhanced methylation in other strains remains poorly understood. We systematically examined the influence of cysteine concentration on time-dependent Hg(II) reduction, sorption, and methylation by *G. sulfurreducens* PCA wild type and its *c*-type cytochrome-deficient mutant Δ*omcBESTZ*. We found that without cysteine the Δ*omcBESTZ* mutant methylated twice as much Hg(II) as the wild type; whereas, addition of cysteine inhibited its methylation, regardless of the reaction time. The PCA wild type, however, exhibited both time-dependent and cysteine concentration–dependent methylation. At the low cysteine concentration (<1 μM), Hg methylation was inhibited. Relatively high concentrations of cysteine (up to 1 mM) enhanced Hg methylation, particularly with a longer incubation time. Our results indicate that the chemical speciation of Hg in the presence of cysteine and its competitive interactions with cells are important in controlling Hg uptake and methylation. Thiol-enhanced Hg methylation is bacterial species specific and time and cysteine concentration dependent (Lin et al. 2015a).

In parallel, we studied cell desorption and export of methylmercury since previous studies suggested that MeHg, once formed inside the cell, is rapidly exported out of the cell. We examined the factors affecting MeHg export and its distribution in cells, on cell surfaces, and in solution by both *Geobacter sulfurreducens* PCA and *Desulfovibrio desulfuricans* ND132. Contrary to previous findings, we found that a large percentage of MeHg (>90%) was associated with PCA cells in thiol-free assays, and <10% of the synthesized MeHg was found in solution. Thiols, such as cysteine, were found to greatly facilitate desorption and export of MeHg, particularly by PCA cells. This result explained previous observations of enhanced Hg methylation in which relatively high concentrations of cysteine were commonly used. However, these findings also are bacteria species specific. For the ND132 cells, about 77% MeHg was found in solution in the absence of thiols, leaving ~13% of the MeHg sorbed and ~10% inside the cells. We thus concluded that MeHg export is bacteria specific, time dependent, and is influenced by thiols—implicating important roles of ligands, such as natural organic matter, in MeHg production and mobilization within the environment (Lin et al. 2015b).

In addition to studies of the geochemical and biochemical mechanisms controlling Hg methylation, we investigated MeHg degradation or demethylation by a novel iron-reducing bacterium *Geobacter bemidjiensis* Bem (Lu et al. 2016). Microbial methylation and demethylation are two competing processes controlling the net production and bioaccumulation of MeHg in natural ecosystems. Although Hg methylation by anaerobic microorganisms

and demethylation by aerobic Hg-resistant bacteria have been extensively studied, little attention has been given to MeHg degradation by anaerobic bacteria such as *G. bemidjiensis* Bem. We report, for the first time, that the strain *G. bemidjiensis* Bem can mediate a suite of Hg transformations, including Hg(II) reduction, Hg(0) oxidation, and MeHg production, and degradation under anoxic conditions. Results suggest that *G. bemidjiensis* utilizes a reductive demethylation pathway to degrade MeHg, with elemental Hg(0) as the major reaction product, likely due to the presence of genes encoding homologs of a organomercurial lyase (MerB) and a mercuric reductase (MerA). In addition, the cells can strongly sorb Hg(II) and MeHg and reduce or oxidize Hg, resulting in both time- and concentration-dependent Hg species transformations. Moderate concentrations (10–500 μM) of Hg-binding ligands, such as cysteine, enhance Hg(II) methylation but inhibit MeHg degradation. These findings have significant environmental implications with respect to cycles of Hg methylation and demethylation among anaerobic bacteria and thus net MeHg production in anoxic water and sediments.

In collaboration with the Next Generation Ecosystem Experiments–Arctic (NGEE–Arctic) project examining soil organic matter degradation, we also investigated the temperature effect on the biosynthesis of MeHg in long-term incubation studies with a permafrost active layer soil (Yang et al. 2016). Little is known concerning the effects of rapid permafrost thaw on microbial methylation and how soil organic carbon degradation is coupled to MeHg biosynthesis. We describe the effects of warming on MeHg production in an Arctic soil during an 8-month anoxic incubation experiment. Net MeHg production increased >10 fold in both organic- and mineral-rich soil layers (8°C) compared to colder (–2°C) temperatures. The type and availability of labile soil organic carbon, such as reducing sugars and ethanol, were particularly important in fueling the rapid initial biosynthesis of MeHg. Freshly amended Hg was more readily methylated than preexisting mercury in the soil. Additionally, positive correlations between mercury methylation and methane and ferrous ion production indicate linkages between soil organic carbon degradation and MeHg production. These results show that climate warming and permafrost thaw could potentially enhance MeHg production by an order of magnitude, impacting Arctic terrestrial and aquatic ecosystems by increased exposure to mercury through bioaccumulation and biomagnification in the food web.

Manuscripts

Published or In Press

Lin, H., X. Lu, L. Y. Liang, and B. H. Gu. 2015. "Thiol-Facilitated Cell Export and Desorption of Methylmercury by Anaerobic Bacteria." *Environmental Science & Technology Letters* **2**(10): 292–296. DOI: 10.1021/acs.estlett.5b00209

Lu, X., Y. Liu, A. Johs, L. Zhao, T. Wang, Z. Yang, H. Lin, D. A. Elias, E.M. Pierce, L. Liang, T. Barkay, and B. Gu. 2016. "Anaerobic Mercury Methylation and Demethylation by *Geobacter bemidjiensis* Bem." *Environmental Science & Technology*. DOI: 10.1021/acs.est.6b00401

Yang, Z., W. Fang, X. Lu, G. Sheng, D. E. Graham, L. Liang, S. D. Wullschleger, and B. Gu. 2016. "Warming Increases Methylmercury Production in an Arctic Soil." *Environmental Pollution* **214**: 504–509. DOI: 10.1016/j.envpol.2016.04.069

In Preparation or Submitted

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. Jubb, L. Zhao, E. M. Pierce, P. Gobbi, S. Papa, and R. Coccioni. In review. "Mercury-Pollution Induction of Intracellular Lipid Accumulation and Lysosomal Compartment Amplification in the Benthic Foraminifer *Ammonia parkinsoniana*." *PLoS One.*

Luo, H., X. Yin, H. Chen, X. Lu, A. M. Jubb, W. Zhang, Lin, H., Y. Li, H. Yu, M. P. Paranthaman, L. Liang, G. Sheng, and B. Gu. In review. "Photochemically Driven Mercury Sulfide Formation and Decreased Methylmercury Production in Water." *Environmental Pollution.*

Qian, C., A. Johs, H. Chen, B. F. Mann, X. Lu, P. E. Abraham, R. L. Hettich, B. Gu. In review. "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.*

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

The overarching goals of Task 3 are two-fold: (1) Determine the breadth and depth of Hg-methylating species in a range of contexts and interfaces, 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?

FY15–FY16 Accomplishments

In the past year, we have made considerable progress in understanding the processes governing microbially mediated Hg transformations and the physicochemical factors that influence these processes across a range of scales. A summary of this progress is presented in the following two sections.

Microbial Cellular Mercury Methylation

Based on the discovery of the Hg-methylation genes *hgcAB* and their ability to be used as biomarkers for Hg-methylation, we have collaborated with Task 4 on two milestones. At the molecular level, we determined the cysteine residues that are essential to methylation to understand how Hg(II) is transferred and bound to the methyl group to form MeHg (Smith et al. 2015). Through targeted codon substitutions, we found that many residues in and out of the active site are essential for Hg methylation. At the biochemical level, we are determining HgcAB native function (i.e., what these proteins do in the absence of Hg) and are mapping the path of the methyl group for MeHg (i.e., identifying the methyl donor).

As an independent task that will benefit the project overall, we completed development of degenerate primers for the detection, identification, and quantification of *hgcAB* from all environments (Christensen et al. 2016) Three other reports exist but are incomplete. We tested all three methods using their protocols and materials and found that our primers provide a significant advantage and advancement. We now have a completed universal set of degenerate primers that amplify *hgcAB* from 28 of the 28 tested *hgcAB*$^{+}$ organisms and have clade-specific quantitative polymerase chain reaction (qPCR) primers for the *Deltaproteobacteria, Firmicutes,* and methanogenic *Archaea*. This allows for the overall capture and identification of *hgcAB*-containing organisms while yielding quantitative *hgcA* values that are clade specific—thus providing data tying community metabolism to Hg-methylation.

Our efforts to understand the native function of HgcAB (biochemical function in the absence of Hg) are yielding results in that we have determined that HgcAB is essential for up to 50% of the acetate generated from lactate oxidation and substantial CO_2 generation as well. We have deleted several genes and shown that many affect Hg-methylation while others do not, allowing us to narrow the biochemical pathways that feed into HgcAB.

We have collaborated with Task 2 on several efforts, some of which have yielded publications this year (Lu et al. 2016). In addition, we are collaborating with Task 1 to determine the status of biological Hg-methylation throughout EFPC and in specific compartments. One of these compartments is the periphyton biofilms growing on surfaces in the creek. As part of this collaborative effort, Task 3 is supporting the development of periphyton incubations and the determination of prokaryotic and eukaryotic diversity within periphyton samples tested under different regimes. Early results show a significant portion of the MeHg observed in the creek may be due to Hg-methylation within these periphyton.

Microbial Ecology of Mercury Methylation

Investigations into the microbial ecology and microbial physiology of Hg-methylation have yielded an increased understanding of MeHg production at the community level, as well as the geochemical influences on its generation. We have investigated the role of *Deltaproteobacteria* in Hg-methylation on the Oak Ridge Reservation (ORR) and found they are likely the major methylators. While working with Task 2 to understand cell surface interactions, Task 3 researchers have been determining methylation kinetics and the role of DOM in Hg-methylation during sulfate reduction. These findings will be tested in the proposed work using model (synthetic) microbial communities with results that can be extrapolated to and tested in the field.

We have taken this knowledge a step further and surveyed all metagenomes in various databases (> 3,500) to yield a global picture of Hg-methylation potential in many environments (Podar et al. 2015). We determined that while *hgcAB* was absent from mammalian microbiomes and the open oceans, it was prevalent in coastal dead zones, contaminated sites, engineered sites, and Arctic permafrost. We also discovered associations of methylating and nonmethylating organisms that co-occur in nature. These findings are significant to understanding the metabolic associations of key types of organisms and their effect on Hg-methylation. For example, we have determined that some fermenters (*Clostridium spp.*) frequently associate with selected sulfate reducers and methanogens. These symbiotic relationships are known to increase nutrient sharing, thus creating more efficient lifestyles. Similar

relationships may transfer to more efficient Hg-methylation potentials than have been observed in monocultures in the laboratory. This will guide the types of organisms used in synthetic communities, in planktonic and in biofilm cultures.

Manuscripts

Published or In Press

Podar, M., C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A. V. Palumbo, A. C. Somenahally, and D. A. Elias. 2015. "Global Prevalence and Distribution of Genes and Microorganisms Involved in Mercury Methylation." *Science Advances.* **1**(9): e1500675. DOI:10.1126/sciadv.1500675

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 Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment. *Applied Environmental Microbiology.* Accepted manuscript posted online 15 July 2016. DOI:10.1128/AEM.01271-16

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

The overarching objective of Task 4 is to understand at the molecular scale how mercury interacts with and is transformed by the species it encounters in natural and contaminated environments. Since our discovery of the Hg methylation genes, we have focused primarily on characterizing the structure and function of HgcA, but we have also continued work on Hg-ligand interactions and bacterial Hg resistance.

FY15–FY16 Accomplishments

Since July 2015, we have published three manuscripts. In collaboration with the University of South Carolina, we investigated mercury transformations in microbiomes with the aim of identifying potential correlations between biomarkers for mercury methylation, mercury resistance (demethylation), and human exposure to toxic MeHg. Metagenomic whole genome shotgun sequencing was used to identify genes implicated with mercury methylation and mercury resistance in human gut microbiota. Metagenomes of human gut microbiome samples did not contain homologs of *hgcA* or *merB*. Only *merA* encoding mercuric reductase was detected at low levels. The results show that the human gut microbiome contributes little to mercury transformations. High levels of methylmercury can affect the relative abundance of major phyla in human gut microbiomes (Rothenberg et al. 2016).

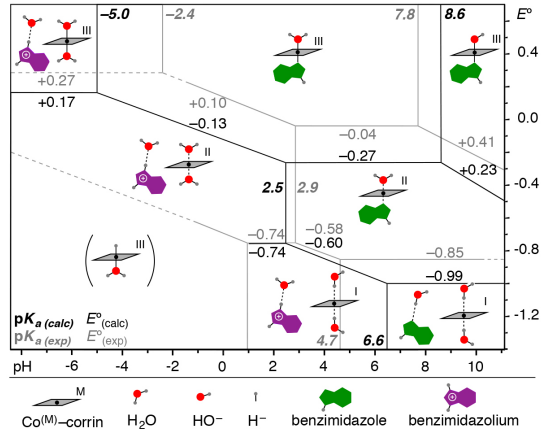


Fig. 4. Experimental (gray) and computed (black) Pourbaix (E-pH) diagram for aquacobalamin.

HgcA uses a corrinoid cofactor to accomplish the methylation of mercury. Corrinoids such as cobalamin exhibit complex redox, acid/base, and ligand dissociation behavior. All of these factors must be thoroughly understood to uncover the mechanism of mercury methylation. We have recently completed a computational study in which we calculated Co(III/II) and Co(II/I) reduction potentials and pK_as and $K_{on/off}$ values for aquacobalamin and diaquacobinamide (Johnston et al. 2016; see Fig. 4, this page).

We recently were invited to contribute an article for a volume of *Methods in Enzymology* on "Computational Approaches for Studying Enzyme Mechanism." In that manuscript, we describe our computational studies on mercury over the past eight years and its interactions with various proteins and enzymes (Parks and Smith, in press).

During the past year, we have made significant progress toward characterizing HgcA (*Milestones 4a and 4b*). In order to obtain sufficient material for the characterization of HgcA by spectroscopic techniques, we expressed the cobalamin-binding domain (CBD) of HgcA heterologously in *E. coli* either as an isolated domain or as an N-terminal maltose binding protein (MBP) fusion, the latter of which provides improved solubility and production yields. We have been working closely with the Ragsdale lab (University of Michigan, unfunded collaborator) to generate a series of CBD-MBP constructs and to characterize the samples with electron paramagnetic resonance (EPR) spectroscopy.

We have recorded reference UV-visible spectra of aquacobalamin under controlled redox conditions and

in the presence of relevant ligands to guide the reconstitution of HgcA with a cobalamin cofactor. Although all HgcA constructs bind one equivalent of cobalamin *in vitro* with a typical cofactor occupancy of >90%, the spectral characteristics of the reconstituted product are highly sensitive to the configuration of the respective construct. EPR spectroscopy is currently applied to gain further insight into the cobalt (Co) coordination environment after reconstitution.

A major goal of this fiscal year was to develop protocols to assay the mercury methylation activity of HgcA and HgcB *in vitro*. The methylation of mercury by HgcAB requires an electron donor, a methyl donor, and a mercury substrate (see Fig. 5, this page). Thus the biological process of mercury methylation comprises the following steps:

- Reduction of HgcB by an electron donor and transfer of low-potential electrons to the corrinoid cofactor on HgcA.
- Transfer of a methyl group to the cofactor (HgcA).
- Transfer of the methyl group to a mercury substrate.

We have demonstrated that the reconstituted MBP-HgcA-CBD fusion can be methylated with CH_3-H_4folate as the methyl donor in a reaction catalyzed by methyltransferase (MeTr) from *M. thermoacetica*. We also conducted experiments to evaluate the abiotic methylation of Hg(II) by methylcobalamin under physiologically relevant conditions. The rate of this reaction depends on pH and mercury speciation in solution. Finally, we are expanding the work by Choi et al. (1994) with *Desulfuvibrio desulfuricans* LS from the 1990s. The goal of these experiments is to demonstrate mercury methylation by a cell lysate of *Desulfovibrio desulfuricans* ND132 at environmentally relevant Hg(II) concentrations and to identify intracellular factors controlling the rate of mercury methylation.

In all cases, experimental characterization is being complemented with computational approaches. We have made key progress in using quantum chemical calculations to probe the mechanism of abiotic Hg methylation by methylcobalamin, a process that is poorly understood. Uncovering details of this reaction will inform mechanistic studies of enzymatic methylation by HgcA.

A nuclear magnetic resonance (NMR) structure would provide direct evidence for the Rossmann fold, cap helix motif, and "Cys-on" coordination proposed for $HgcA_{CBD}$. We have prepared ^{15}N-labeled protein and preliminary NMR data through a user proposal with the DOE Environmental Molecular Sciences Laboratory (EMSL). HgcA from *Desulfovibrio desulfuricans* ND132 contains two nonconserved cysteine residues, Cys47 and Cys142, which can form intermolecular disulfides. Dynamic light scattering SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel

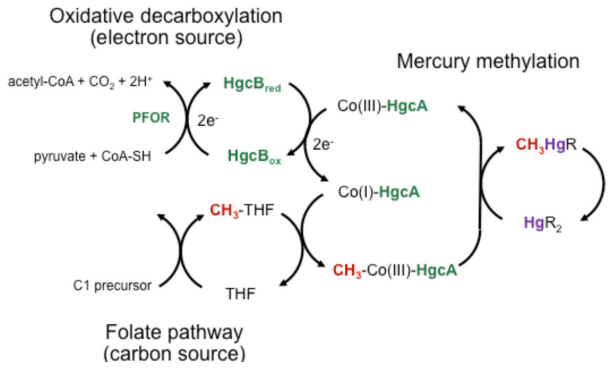


Fig. 5: Proposed Hg methylation pathway involving methyl transfer from CH_3-H_4folate to Cbl(I)-HgcA, methylation of HgR_2 by CH_3-Cbl(III)-HgcA to form CH_3HgR, and reduction back to Cbl(I)-HgcA by HgcB.

electrophoresis) under reducing and nonreducing conditions revealed disulfide bond formation through Cys crosslinking, which results in high molecular weight oligomers. To reduce the risk for crosslinking of HgcA-CBD during NMR data collection, we have constructed an expression vector encoding a C47S/C142S double mutant in which only Cys93 is retained. The ^{15}N-HSQC spectrum revealed a limited number of well-dispersed but broad resonances that indicate sample polydispersity, which may be caused by partial sample aggregation induced by the relatively high concentration required for these measurements.

Manuscripts

Published or In Press

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*, 2016. In press. DOI: 10.1021/acs.jpcb.6b02701

Parks, J. M., and J. C. Smith. 2016. "Modeling Mercury in Proteins." *Methods in Enzymology*: *Computational Approaches for Studying Enzyme Mechanism*. Ed. Gregory Voth. Elsevier, Inc. Amsterdam. In press.

Rothenberg, S. E., S. Keiser, N. J. Ajami, M. C. Wong, J. Gesell, J. F. Petrosino, and A. Johs. 2016. "The role of gut microbiota in fetal methylmercury exposure: Insights from a pilot study." *Toxicology Letters* 242:60-67. DOI: 10.1016/j.toxlet.2015.11.022

Select Research Highlights

In FY2016, a total of 14 manuscripts have been submitted by the CI-SFA. Of these publication, seven are published or in press, bringing the total to 67 for the CI-SFA since its inception. Of these 67 publications, 52 are the result of new mercury research; 15 represent DOE Environmental Remediation Sciences Program projects that were completed with partial SFA funding. In this section, we highlight 10 of the 14 manuscripts.

Research Highlight

Mercury and Methylmercury Dynamics in an Industrially Contaminated Stream

The Science

Detailed monitoring of changing mercury and methylmercury concentration in a creek during baseflow and flood events indicates MeHg is produced within the stream and further suggests that algal biofilms, also called periphyton, are major sources of MeHg generation.

The Impact

These findings may explain why past improvements in overall stream water quality have not resulted in concomitant improvements in MeHg concentration in water and in fish. Additionally, future alterations to stream management practices or climate that alter the abundance, activity, or composition of periphyton may have unintended negative consequences as they have the potential to influence MeHg production within the creek.

Summary

Sediments and floodplain soils in the East Fork Poplar Creek (EFPC) watershed in Oak Ridge, Tennessee, are contaminated with high levels of mercury from an industrial source at the headwaters. While baseflow conditions have been monitored, concentrations of Hg and MeHg during high-flow storm events—when the stream is more hydrologically connected to the floodplain—have yet to be assessed. The present study evaluates baseflow and event-driven Hg and MeHg dynamics in EFPC, five kilometers upstream of the confluence with Poplar Creek to determine the importance of hydrology to in-stream concentrations and downstream loads and ascertain if dynamics are comparable to systems without an industrial Hg source. Particulate Hg and MeHg were positively correlated with discharge (r2=0.64 and 0.58, respectively) and total suspended sediment (r2=0.97 and 0.89, respectively). Dissolved Hg (HgD) also increased with increasing flow (r2=0.18) and was associated with increases in dissolved organic carbon (DOC) (r2=0.65) similar to dynamics observed in uncontaminated systems. Dissolved MeHg (MeHgD) decreased with increases in discharge (r2=0.23) and was not related to dissolved organic carbon concentrations (p=0.56), dynamics comparable to relatively uncontaminated watersheds with a small percentage of wetlands (<10%). While stormflows exert a dominant control on HgP, MeHgP, and HgD concentrations and loads, baseflows were associated with the highest MeHgD concentration (0.38 ng/L) and represented the majority of the annual MeHgD load.

Publication

Riscassi, A., C. Miller, and S.Brooks. 2016. "Seasonal and Flow-driven Dynamics of Particulate and Dissolved Mercury and Methylmercury in a Stream Impacted by an Industrial Mercury Source." *Environmental Toxicology and Chemistry* **35**(6): 1386–1400. DOI: 10.1002/etc.3310

Research Highlight

Estimating Uncertainty in Ambient and Saturation Nutrient Uptake Metrics from Nutrient Pulse Releases in Stream Ecosystems

The Science

A new method to quantify uncertainty in stream nutrient uptake metrics and saturation kinetics has been developed. The method is robust, free of the simplifying assumptions and violations of statistical theory that characterized previous methods, and it can be applied to other stream ecosystem metrics (e.g., whole-stream metabolism, secondary production).

The Impact

The importance of uncertainty propagation and quantification in ecological systems has been identified but is rarely practiced or conducted under simplifying assumptions, some of which violate the underlying statistical theory. The new Monte Carlo (MC)–based method enables robust, assumption-free uncertainty quantification of stream ecosystem metrics.

Summary

Nutrient spiraling is an important ecosystem process characterizing nutrient transport and uptake in streams. Various nutrient addition methods are used to estimate uptake metrics; however, uncertainty in the metrics is not often evaluated. A method was developed to quantify uncertainty in ambient and saturation nutrient uptake metrics estimated from saturating pulse nutrient additions (Tracer Additions for Spiraling Curve Characterization; TASCC). Using an MC approach, the 95% confidence interval (CI) was estimated for ambient uptake lengths ($S_{w\text{-}amb}$) and maximum areal uptake rates (U_{max}) based on 100,000 datasets generated from each of four nitrogen and five phosphorous TASCC experiments conducted seasonally in a forest stream in eastern Tennessee. Uncertainty estimates from the MC approach were compared to the CIs estimated from ordinary least squares (OLS) and nonlinear least squares (NLS) models used to calculate $S_{w\text{-}amb}$ and U_{max}, respectively, from the TASCC method. The CIs for $S_{w\text{-}amb}$ and U_{max} were large but were not consistently larger using the MC method. Despite the large CIs, significant differences (based on nonoverlapping CIs) in nutrient metrics among seasons were found with more significant differences using the OLS/NLS versus the MC method. We suggest that the MC approach is a robust way to estimate uncertainty, as the calculation of $S_{w\text{-}amb}$ and U_{max} violates assumptions of OLS/NLS while the MC approach is free of these assumptions. The MC approach can be applied to other ecosystem metrics that are calculated from multiple parameters, providing a more robust estimate of these metrics and their associated uncertainties.

Publication

Brooks, S. C., C. C. Brandt, and N. A. Griffiths. In revision. "Estimating Uncertainty in Ambient and Saturation Nutrient Uptake Metrics from Nutrient Pulse Releases in Stream Ecosystems." *Limnology and Oceanography: Methods.*

Research Highlight

Periphyton Biofilms Influence Net Methylmercury Production in an Industrially Contaminated Systems

The Science

Working under the hypothesis that periphyton biofilms within a freshwater creek are a significant source of MeHg, researchers used enriched stable isotopes of Hg to quantify the rates of Hg methylation and MeHg demethylation for samples collected from the field and incubated in the lab. Results indicate that periphyton biofilms can make a substantial net positive contribution to the MeHg budget in the creek.

The Impact

Results demonstrate that biofilms at the water-streambed interface play a significant role in Hg transformations in the creek. Controlled manipulations in the lab (e.g., changing temperature and light exposure) yielded results consistent with field-scale observations. These results provide important updates to our conceptual and numerical models.

Summary

Mercury methylation and methylmercury demethylation activity of periphyton biofilms from East Fork Poplar Creek, in Oak Ridge, Tennessee, were measured during 2014–15 using stable Hg isotopic rate assays. $^{201}Hg^{II}$ and $Me^{202}Hg$ were added to intact periphyton samples in ambient streamwater and the formation of $Me^{201}Hg$ and loss of $Me^{202}Hg$ were monitored over time and used to calculate first-order rate constants for methylation and demethylation, respectively. The influence of location, temperature, season, light exposure, and biofilm structure on methylation and demethylation 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 constant (k_d). In contrast, the within-site seasonal difference in net methylation was driven by changes in the methylation rate constant (k_m). Samples incubated in the dark had lower net methylation due to k_m values that were 60% less than those incubated in the light. Disrupting the biofilm structure decreased k_m by 50% and resulted in net demethylating conditions. 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 that intact, actively photosynthesizing periphyton biofilms harbor zones of MeHg production.

Publication

Olsen, Todd A., and Scott C. Brooks. In revision. "Periphyton Biofilms Influence Net Methylmercury Production in an Industrially Contaminated System." *Environmental Science & Technolology.*

Research Highlight

Cell Methylmercury Export is More Complicated than Previously Thought

The Science

Methylmercury toxin, formed by certain anaerobic bacteria, is thought to be rapidly excreted from cells, but this process has been poorly studied and its mechanism remains unclear. We found that MeHg export is bacteria specific and thiol-concentration dependent. In the absence of thiol ligands, MeHg is strongly sorbed by *Geobacter sulfurreducens* PCA, but not by *Desulfovibrio desulfuricans* ND132 cells. The presence of thiols such as cysteine can greatly facilitate MeHg desorption and export by cells.

The Impact

Our results provide fundamental understandings on how MeHg is transported out of bacterial cells and what environmental factors may control desorption and cell export of MeHg. We suggest important roles of complexing ligands, such as natural organic matter, in MeHg production and mobilization in the environment.

Summary

Researchers studied factors affecting MeHg export and its distribution in cells, on cell surfaces, and in solution by two known mercury methylators, *Geobacter sulfurreducens* PCA and *Desulfovibrio desulfuricans* ND132. Thiols, such as cysteine, were found to greatly facilitate desorption and export of MeHg, particularly by PCA cells. In cysteine-free assays (4 h), <10% of the synthesized MeHg was found in solution, >90% was associated with PCA, of which ~73% was sorbed on the cell surface and 19% remained inside the cells. In comparison, 77% MeHg was in solution, leaving ~13% of the MeHg sorbed and ~10% inside the ND132 cells. Results demonstrate that MeHg export is bacteria specific, time dependent, and influenced by thiols—implicating important roles of ligands, such as natural organic matter, in MeHg production and mobilization in the environment.

Publication

Lin, H., X. Lu, L. Y. Liang, and B. H. Gu. 2015. "Thiol-Facilitated Cell Export and Desorption of Methylmercury by Anaerobic Bacteria." *Environmental Science & Technology Letters* **2**(10): 292–296. DOI: 10.1021/acs.estlett.5b00209.

Research Highlight

Simultaneous Mercury Methylation and Demethylation by *Geobacter bemidjiensis* Bem Discovered

The Science

For the first time, we found that the strain *Geobacter bemidjiensis* Bem can methylate mercury and degrade methylmercury toxin concurrently under anoxic conditions. A reductive demethylation pathway is utilized by Bem to degrade methylmercury, possibly due to its genes encoding homologs of a organomercurial lyase (MerB) and a mercuric reductase (MerA).

The Impact

Geobacter bemidjiensis bacteria widely occur in sediments and water, including permafrost soils, and may thus play an important role in controlling net methylmercury production and bioaccumulation in biota in natural aquatic environments.

Summary

Microbial methylation and demethylation are two competing processes controlling the net production and bioaccumulation of neurotoxic methylmercury in natural aquatic ecosystems. Although mercury methylation by anaerobic microorganisms and demethylation by aerobic Hg-resistant bacteria have both been extensively studied, little attention has been given to MeHg degradation by anaerobic bacteria, particularly the iron-reducing bacterium *Geobacter bemidjiensis* Bem. We report, for the first time, that the strain *G. bemidjiensis* Bem can mediate a suite of Hg transformations, including Hg(II) reduction, Hg(0) oxidation, and MeHg production and degradation under anoxic conditions. Results suggest that *G. bemidjiensis* utilizes a reductive demethylation pathway to degrade MeHg, with elemental Hg(0) as the major reaction product, possibly due to the presence of genes encoding homologs of a organomercurial lyase (MerB) and a mercuric reductase (MerA). In addition, the cells can strongly sorb Hg(II) and MeHg, and reduce or oxidize Hg, resulting in both time- and concentration-dependent Hg species transformations. Moderate concentrations of Hg-binding ligands, such as cysteine, enhance Hg(II) methylation but inhibit MeHg degradation. These findings

indicate a cycle of Hg methylation and demethylation among anaerobic bacteria, thereby influencing net MeHg production in anoxic water and sediments.

Publication

Lu, Xia, Yurong Liu, Alexander Johs, Linduo Zhao, Tieshan Wang, Ziming Yang, Hui Lin, Dwayne A. Elias, Eric M. Pierce, Liyuan Liang, Tamar Barkay, and Baohua Gu. 2016. "Anaerobic Mercury Methylation and Demethylation by *Geobacter bemidjiensis* Bem." *Environmental Science & Technology*. DOI: 10.1021/acs.est.6b00401

Research Highlight

Warming Increases Methylmercury Production in Arctic Soils

The Science

Climate warming not only influences the degradation of stored soil organic carbon and climate feedback but also the production and bioaccumulation of methylmercury toxins in natural ecosystems. Researchers determined the effects of warming on methylmercury production in an Arctic soil during an eight-month anoxic incubation experiment and found that net methylmercury production increased >10 fold in a permafrost active layer soil of 8°C compared to colder (–2°C) temperatures. More importantly, it was found that the biosynthesis of methylmercury is strongly coupled with processes such as methanogenesis and iron reduction during soil organic carbon degradation.

The Impact

Results show that climate warming and permafrost thaw could potentially enhance methylmercury toxin production by an order of magnitude, impacting Arctic terrestrial and aquatic ecosystems by increased exposure to mercury through bioaccumulation and biomagnification in the food web.

Summary

Rapid temperature rise in Arctic permafrost impacts not only the degradation of stored soil organic carbon (SOC) and climate feedback, but also the production and bioaccumulation of methylmercury (MeHg) toxin that can endanger humans, as well as wildlife in terrestrial and aquatic ecosystems. Little is known concerning the effects of rapid permafrost thaw on microbial methylation and how SOC degradation is coupled to MeHg biosynthesis. Here we describe the effects of warming on MeHg production in an Arctic soil during an eight-month anoxic incubation experiment. Net MeHg production increased >10 fold in both organic- and mineral-rich soil layers at 8°C compared to colder (–2°C) temperatures. The type and availability of labile soil organic carbon (SOC), such as reducing sugars and ethanol, were particularly important in fueling the rapid initial biosynthesis of MeHg. Freshly amended mercury was more readily methylated than preexisting mercury in the soil. Additionally, positive correlations between mercury methylation and methane and ferrous ion production indicate linkages between SOC degradation and MeHg production. These results show that climate warming and permafrost thaw could potentially enhance MeHg production by an order of magnitude, impacting Arctic terrestrial and aquatic ecosystems by increased exposure to mercury through bioaccumulation and biomagnification in the food web.

Publication

Yang, Z., W. Fang, X. Lu, G. Sheng, D. E. Graham, L. Liang, S. D. Wullschleger, and B. Gu. 2016. "Warming Increases Methylmercury Production in an Arctic Soil." *Environmental Pollution* **214**: 504–509. DOI: 10.1016/j.envpol.2016.04.069

Metadata

Organic Carbon Transformation and Mercury Methylation in Tundra Soils from Barrow Alaska. DOI:10.5440/1235032

Research Highlight

The Role of Gut Microbiota in Fetal Methylmercury Exposure

The Science

Microorganisms can modulate the toxicity of mercury. Methylation increases toxicity, while demethylation (resistance) decreases toxicity. The role of the gut microbiome in mercury transformations and human exposure to toxic methylmercury was investigated. The human gastrointestinal tract is a densely populated ecosystem, and thus may have an important role in mercury and methylmercury cycling and related fetal exposure.

The Impact

Results allow the identification of risk factors for methylmercury exposure associated with the gut microbiome. Findings showed the human microbiome contributes little to mercury transformations in the gut.

Summary

The mechanisms by which gut microbiota contribute to methylmercury exposure in humans remain unclear. The main objective of this pilot study was to investigate potential correlations between biomarkers for mercury methylation and mercury resistance in the human gut microbiome with methylmercury exposure. Metagenomic whole genome shotgun sequencing, which enables rapid identification of microorganisms and genes, was used to identify genes implicated with mercury methylation and mercury resistance in human gut microbiota. The diversity of gut microbiota from a cohort of pregnant women was determined using 16S rRNA gene profiling. Six samples with highest and lowest methylmercury concentrations were searched for homologs of the primary gene implicated with mercury methylation (*hgcA*) and two genes involved in mercury resistance (merA and merB). Seventeen bacterial genera were significantly correlated (increasing or decreasing) with gut methylmercury, gut inorganic (unmethylated) mercury, or hair total mercury. However, aside from one genus, there was no correlation with biomarker results derived from metagenomic whole genome shotgun sequencing. There were no definitive matches for *hgcA* or merB, while merA was detected at low concentrations in all six samples. Proportional differences in gut methylmercury levels cannot be attributed to methylation/demethylation by gut microbiota. Exposure to toxic mercury species appears to be unrelated to the presence of *hgcA* or genes in the mer operon in the gut microbiome.

Publication

Rothenberg, S. E., S. Keiser, N. J. Ajami, M. C. Wong, J. Gesell, J. F. Petrosino, and A. Johs. 2016. "The Role of Gut Microbiota in Fetal Methylmercury Exposure: Insights from a Pilot Study." *Toxicology Letters* **242**: 60–67. DOI: 10.1016/j.toxlet.2015.11.022

Research Highlight

Development and Validation Qualitative and Quantitative Molecular Probes for the Hg-methylating Genes *hgc*AB

The Science

Universal qualitative and clade specific quantitative (*Deltaproteobacteria*, *Firmicutes*, or methanogenic *Archaea*) DNA and mRNA primers were developed to determine the species involved in Hg-methylation and to quantify the genes for each clade, respectively, since each has shown a different level of methylation in the lab. All primers were validated on pure bacterial strains that methylate as positive controls and those that do not methylate as negative controls. Environmental samples were further used to validate the primers and to determine corrective calculations for DNA extraction and polymerase chain reaction (PCR) amplification efficiencies.

The Impact

These primers are a substantial improvement over earlier reports in that both qualitative and quantitative primers were developed by the same team, and the quantitative primers take into consideration the different degrees of methylation potential for each clade which ranges from ~10% in the *Archaea* to ~90% in some *Deltaproteobacterial* species. These findings, combined with the determination of the corrective factors for DNA extraction and PCR amplification will now allow for a more realistic picture to be generated regarding the possible levels of methylmercury generation that may occur in a given environment. These data can now be used for more accurate risk management assessments and can be more confidently used in the generation of hydrobiogeochemical models of methylmercury generation.

Summary

Two genes, *hgc*A and *hgc*B, are essential for microbial mercury (Hg)-methylation. Detection and estimation of their abundance in conjunction with quantification of Hg species and other geochemical factors is critical in determining potential hot spots of

methylmercury (MeHg) generation in at-risk environments. We developed broad-range degenerate PCR primers spanning known *hgcAB* genes to determine the presence of both genes in diverse environments. These broad-range primers were tested against an extensive set of pure cultures with published genomes, including 13 *Deltaproteobacteria*, nine *Firmicutes*, and nine methanogenic *Archaea*. A distinct PCR product at the expected ~950 base pair size was confirmed for all *hgcAB*+ strains tested and validated via Sanger sequencing. Additionally, clade-specific, degenerate quantitative primers (qPCR) targeted *hgcA* in the three dominant Hg-methylating clades (*Deltaproteobacteria*, *Firmicutes* and *Archaea*) using the same cultures and resulted in amplification of *hgcA* from 64%, 88%, and 86% of the tested species, respectively. Amplification was specific for the designed clade using the tested conditions. Sensitivity was lower in the methanogens due to a lack of sequence conservation. The two matrices were amended with representative strains from the three clades in equimolar ratios to demonstrate the qPCR primer specificity and sensitivity in complex environments.

Publication

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 Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment. *Applied Environmental Microbiology.* Accepted manuscript posted online 15 July 2016. DOI:10.1128/AEM.01271-16

Research Highlight

Toward Quantitatively Accurate Calculation of the Redox-Associated Acid-Base and Ligand Binding Equilibria of Aquacobalamin

The Science

By combining density functional theory with continuum solvation, researchers developed a consistent computational approach for computing pH-dependent redox and ligand dissociation properties of a complex corrinoid cofactor. Specifically, the approach provides balanced accuracy for three thermochemical properties, yielding RMS errors of 80 mV for seven reduction potentials, 2.0 log units for five pKas, and 2.3 log units for two log Kon/off values for the aquacobalamin system.

The Impact

These findings provide insight into the mechanism of Hg methylation by HgcA, which uses a corrinoid cofactor. The approach is more broadly applicable to modeling and simulation of other subsurface redox processes, which are known to play important roles in mercury speciation and transformation.

Summary

Redox processes in complex transition metal-containing species are often intimately associated with changes in ligand protonation states and metal coordination number. A major challenge is therefore to develop consistent computational approaches for computing pH-dependent redox and ligand dissociation properties of organometallic species. Reduction of the cobalt (Co) center in the vitamin B12 derivative aquacobalamin can be accompanied by ligand dissociation, protonation, or both, making these properties difficult to compute accurately. We examine this challenge here by using density functional theory and continuum solvation to compute cobalt–ligand binding equilibrium constants (Kon/off), pKas and reduction potentials for models of aquacobalamin in aqueous solution. Two models for cobalamin ligand coordination were considered: the first follows the hexa, penta, tetra coordination scheme for CoIII, CoII, and CoI species, respectively. The second model features saturation of each vacant axial coordination site on CoII and CoI species with a single, explicit water molecule to maintain six directly interacting ligands or water molecules in each oxidation state. Comparing these two coordination schemes in combination with five dispersion-corrected density functionals, findings showed that the accuracy of the computed properties is largely independent of the scheme used, and that varying the Co coordination number yields marginally better results than saturating the first solvation shell around Co throughout. PBE performs best, displaying balanced accuracy and superior performance overall, with RMS errors of 80 mV for seven reduction potentials, 2.0 log units for five pKas, and 2.3 log units for two log Kon/off values for the aquacobalamin system. Furthermore, the BP86 functional commonly used in corrinoid studies suffered from erratic behavior and inaccurate descriptions of Co–axial ligand binding, leading to substantial errors in predicted pKas and Kon/off values. These findings demonstrate the effectiveness of this approach for computing electrochemical and thermodynamic properties of a complex transition metal-containing cofactor.

Publication

Johnston, R.C., J. Zhou, J.C. Smith, and J.M. Parks. In revision. "Toward Quantitatively Accurate Calculation of the Redox-associated Acid-Base and Ligand Binding Equilibria of Aquacobalamin." *Journal of Physical Chemistry B.*

Research Highlight

Modeling Mercury in Proteins

The Science

Understanding the toxic effects of mercury and its cycling in the environment requires detailed characterization of its interaction with proteins. Computational approaches are ideally suited to studies of mercury in proteins because they provide a detailed picture and circumvent issues associated with toxicity. This work highlights eight years of combined computational and experimental studies on proteins and enzymes involved in mercury methylation, demethylation, and reduction.

The Impact

This work has greatly expanded the molecular understanding of biological transformations of mercury. Additionally, this work on mercury in proteins is placed in the context of what is required for comprehensive multi-scale modeling of environmental mercury cycling.

Summary

Mercury is a naturally occurring element released into the biosphere both by natural processes and anthropogenic activities. Its reduced, elemental form Hg(0) is relatively nontoxic. But, other forms such as Hg2+ and, in particular, its methylated form, methylmercury, are toxic with deleterious effects on both ecosystems and humans. Microorganisms play important roles in the transformation of mercury in the environment. Inorganic Hg2+ can be methylated by certain bacteria and archaea to form methylmercury. Conversely, bacteria also demethylate methylmercury and reduce Hg2+ to relatively inert Hg(0). Transformations and toxicity occur as a result of mercury interacting with various proteins. Understanding the toxic effects of mercury and its cycling in the environment requires characterization of these interactions. Computational approaches are ideally suited to studies of mercury in proteins because they can provide a detailed picture and circumvent issues associated with toxicity. This work describes computational methods for investigating and characterizing how mercury binds to proteins, how inter- and intra-protein transfer of mercury is orchestrated in biological systems, and how chemical reactions in proteins transform the metal. Also described are quantum chemical analyses of aqueous Hg(II), which reveal critical factors that determine ligand binding propensities. A perspective is provided on how chemical reasoning was used to discover how microorganisms methylate mercury. Combined computational and experimental studies of the proteins and enzymes of the mer operon, a suite of genes that confers mercury resistance in many bacteria, also are highlighted. Lastly, work on mercury in proteins is placed in the context of what is needed for a comprehensive multi-scale model of environmental mercury cycling.

Publication

Parks, J.M., and Smith, J.C. In press. "Modeling Mercury in Proteins." In *Methods in Enzymology*: *Computational Approaches for Studying Enzyme Mechanism*, edited by Gregory Voth. Elsevier, Inc., 2016.

Table 1. Top Cited Publications

Title	Year	Citations
The Genetic Basis for Bacterial Mercury Methylation, *Science*	2013	100
Mercury Reduction and Complexation by Natural Organic Matter in Anoxic Environments, *Proceedings of the National Academy of Sciences of the United States of America*	2011	64
Sulfate-Reducing Bacterium *Desulfovibrio desulfuricans* ND132 as a Model for Understanding Bacterial Mercury Methylation, *Applied and Environmental Microbiology*	2011	60
Mercury Methylation by Novel Microorganisms from New Environments, *Environmental Science & Technology*	2013	54
Kinetic Controls on the Complexation between Mercury and Dissolved Organic Matter in a Contaminated Environment, *Environmental Science & Technology*	2009	42

Media Mentions

- **Bacterial Genes that Turn Mercury Lethal Mapped Across the World** — *New Scientist,* October 9, 2015
- **Scientists Find a Toxic Threat Lurking Under the Melting Arctic** — *Takepart.com,* October 9, 2015
- **Survey Points Where Toxic Mercury Accumulates in World Environments** — *Smithsonian Insider,* October 9, 2015
- **Arctic Mercury Pollution to Increase as Permafrost Thaws** — *Barents Observer,* October 13, 2015
- **Methylmercury: A Dangerous Environmental Toxin** — *Laboratory Equipment,* December 1, 2015
- **Methylmercury May Arise Anywhere** — *Deutschlandfunk,* December 10, 2015
- **Horizons Spotlights ORNL Mercury Research** — *BBC,* Last aired Feb. 14, 2016
- **Key Elements of Proteins Involved in the Bacterial Production of the Toxin Methylmercury** — *Atlas of Science,* February 17, 2016
- **As Heard on Insight: Fighting an Almost Invisible Pest: *Longtime biochemistry professor teams up with national laboratory to get a better genetic grasp on methylmercury*** — *CAFNR News,* February 25, 2016
- **ORNL Scientists Solve the Mystery of Mercury** — *ORNL Review,* Spring 2016

Fig. 6. ORNL Review, Spring 2016.

Staff Award

Baohua Gu has been selected by the Geological Society of America (GSA) Council as a GSA Fellow, an honor bestowed annually in recognition of distinguished contributions to the geosciences. Baohua was chosen for seminal work on elucidating key molecular-scale mechanisms that govern biogeochemical cycling of contaminants, trace metals, and natural organic matter that have made significant contributions to our understanding of soil organic and metal cycling in terrestrial ecosystems and contaminated sites.

National and International Impact

ORNL Mercury SFA team members attend strategic conferences in the United States and abroad to gain insights into the state of the science, share project findings and strategies with the broader mercury research community, and identify collaborative opportunities. From July 2015 to June 2016, SFA scientists delivered or published 29 presentations, abstracts, or posters and gave two invited talks (see Appendix C, page 24, for details). Described below are team members' contributions to Environmental System Science Principal Investigators Meeting , AGU Fall Meeting, and 26th Goldschmidt Conference.

Environmental System Science Principal Investigators (PI) Meeting

Subsurface Biogeochemical Research staff attended the PI meeting April 26-27, 2016, at the Bolger Center in Potomac, Md., and gave nine poster presentations. Additionally, Scott Brooks gave an invited presentation titled "Field and Laboratory Scale Investigations of Biogeochemical Gradients Across the Surface Water Groundwater Interface."

American Geophysical Union

AGU American Geophysical Union

Elias and Gilmour convened a session at the 2015 AGU Fall Meeting—B13I: Mercury Biogeochemistry, Genomics, and Environmental Change II—on December 14, 2015, in San Francisco, Calif. Additional organizers included Michael Banks from the University of Massachusetts and James Shanley from the U.S. Geological Survey (USGS).

Goldschmidt 2016

Mercury SFA team members hosted a session titled "Microbiological and Geochemical Controls on Trace Metal Speciation, Transformation, and Transport" at the 26th Goldschmidt Conference in Yokohama, Japan, June 26–July 1, 2016. The Goldschmidt Conference is the premier international geochemistry conference. Session organizers include Eric Pierce and Baohua Gu from ORNL along with a number of co-organizers from around the world: Christian Mikutta, Swiss Federal Institute of Technology in Zürich, Switzerland; Adrien Mestrot, University of Bern in Bern, Switzerland; Marco Keiluweit, University of Massachusetts, Amherst; and Samantha Ying, University of California, Riverside. See session description (goldschmidt.info/2016/program/programViewThemes/).

In addition to hosting a session, several presentations were given by SFA team members, two of which were invited talks. These include:

- Anaerobic Mercury Methylation and Demethylation by *Geobacter bemidjiensis* Bem, presented by Xia Lu, a former graduate student who contributed to the SFA.
- Mercury Methylation by Methylcobalamin: Kinetics and Mechanisms Revisited, presented by Liyuan Liang.
- Coupled Interactions between (Hg), Organic Ligands, and Microorganisms on Hg Reduction, Oxidation, and Methylation, presented by Baohua Gu.

Program Structure and Advisory Committee

Organization and Leadership

The scientific objectives of the Mercury SFA 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 SFA as an integrated team effort. Eric Pierce is the Laboratory Research Manager (LRM) and the point of contact with DOE Subsurface Biogeochemical Research program managers. He speaks to Paul Bayer biweekly on SFA progress and potential issues. Task leaders are Scott Brooks, Baohua Gu, Dwayne Elias, and Jerry Parks, who lead Tasks 1–4, respectively (See Fig. 7, this page). These leaders meet triweekly to discuss research directions, staffing, budget, and cost issues. SFA staff also meets triweekly to discuss task progress. See website for a complete organization chart (www.esd.ornl.gov/programs/rsfa/contacts.shtml).

Scientific Advisory Committee

The FY16 Scientific Advisory Committee (SAC) meeting was held May 16-18, 2016, at ORNL. Carl Lamborg from the University of California, Santa Cruz, joined our distinguished committee as a new member. Members currently include committee chair Dave Krabbenhoft, U.S. Geological Servey; Alex MacKerell, University of Maryland; Richard Sparling, University of Manitoba; and Elizabeth Phillips, DOE Oak Ridge Office of Environmental Management. All currently funded SFA participants attended to highlight recent progress toward objectives and goals.

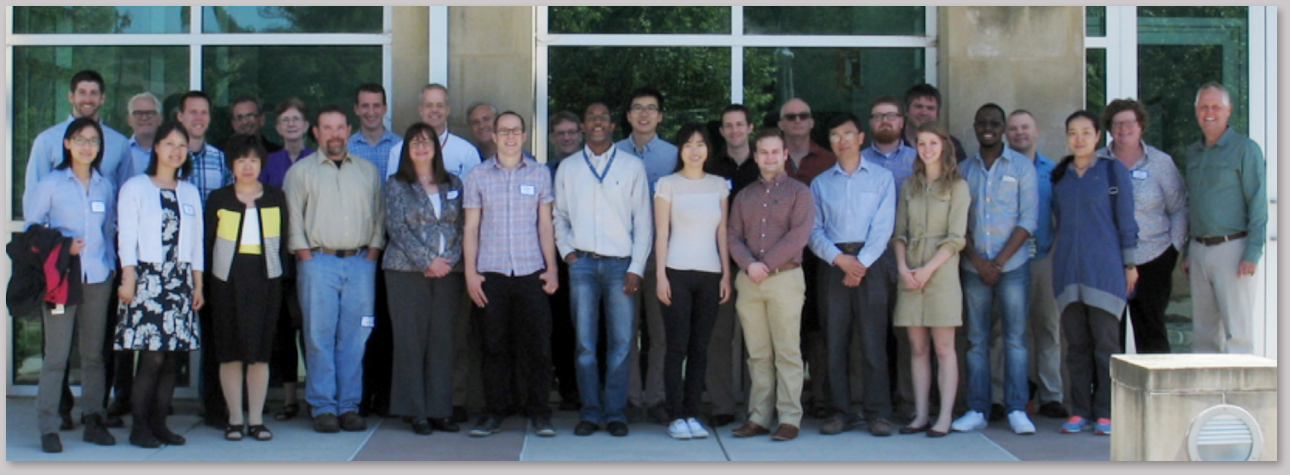


Fig. 7. 2016 ORNL SFA staff and SAC members.

Ongoing Collaborative Research Activities

The ORNL Mercury SFA continues to engage a number of key collaborators in the project. In FY16, Task 1 collaborated with the DuPont-sponsored South River Science Team, enabling us to compare similar mercury cycling studies in South River, Virginia. Tasks 2 and 4 continue to collaborate with Environmental Molecular Sciences Laboratory (EMSL) staff (i.e., Rosalie Chu, Nikola Tolic, and Patrick Reardon) to identify the organic molecules responsible for mercury complexation in dissolved organic matter isolated from EFPC using fourier transform ion cyclotron resonance spectroscopy (proposal #48386) and to determine the structure of HgcA using nuclear magnetic resonance spectroscopy (proposal #48184), respectively. External collaborators Cynthia Gilmour (Smithsonian Research Institute) and Judy Wall (University of Missouri) continue to collaborate with Tasks 3 and 4 to ascertain native functions of the genes hgcA and hgcB and to characterize the proteins and enzymes relevant for mercury methylation. Nonfunded collaborators include Graham George at the Canadian Light Source to investigate cobalt-ligand binding environments and Steve Ragsdale at the University of Michigan for his expertise in corrinoid protein structure and function.

Although the SFA's primary objective is fundamental science, it is important that project personnel have the opportunity to translate scientific discovery into information relevant to the DOE Office of Environmental Management (EM) and the broader DOE complex. Currently, Eric Pierce serves as ORNLs' point of contact for the DOE Office of Environmental Management (EM) headquarters and the 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 and Laura Wilkerson), EM headquarter staff (Karen Skubal and Kurt Gerdes), and the site specific advisory panels. These interactions provide the forum to inform DOE EM on how mercury is transformed in environmental systems, which is a need recently outlined in the DOE EM report titled, Technology Plan to Address EM Mercury Challenge.

Fig. 8. Key ORNL SFA partners.

Postgraduate Spotlight

A key goal of the CI-SFA and ORNL is to train the next generation of scientists and engineers. To this end, the SFA has maintained a number of outstanding graduate and postgraduate researchers since its inception seven years ago. As part of this year's report, we highlight three outstanding postgraduate researchers—Bryan Crable, Steve Smith, and Hongmei Chen—who have contributed significantly to the overall SFA goals and objectives. See website for complete list of past graduate and postgraduates (www.esd.ornl.gov/programs/rsfa/alumni.shtml).

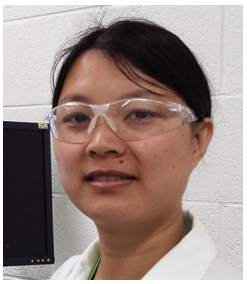
Bryan Crable

Bryan Crable received his master's degree in Biology from Duquesne University and Ph.D. in Microbiology from the University of Oklahoma. Crable specializes in microbial physiology because he recognized the essential role bacteria play in solving the world's toughest environmental challenges. His doctoral dissertation was titled "Enzyme Systems Involved in Interspecies Hydrogen and Formate Transfer Between Syntrophic Fatty and Aromatic Acid Degraders and *Methanospirillum-hungatei*." This work demonstrated the role of a novel iron-sulfur (FeS) oxidoreductase complex in catalyzing reverse electron transfer. He was appointed in 2014 as an ORNL postdoctoral research associate under the mentorship of Dwayne Elias to study the native physiological function of HgcA. Crable is first author on a comprehensive invited review on mercury methylation for the journal *Trends in Microbiology*, first author on a paper published in *Enzyme Research*, and coauthor on seven papers including a publication in *Science Advances*. In FY15, Crable gave an invited talk at the 2015 American Society for Microbiology General Meeting and participated in the Applied and Environmental Microbiology Gordon Research Conference. Most recently, Crable was awarded a $130,000 FY16 grant from the DOE-funded Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) program to study the impact of phages on subsurface microbial communities. Outside the laboratory, Crable is an internationally competitive Highland bagpiper and has won awards in six countries.

Steve Smith

Steve Smith received his undergraduate degree from the University of Missouri, Columbia. His first job in science was working in a perinatal biology laboratory at Washington University School of Medicine in St. Louis. The lab focused on hypoxic regulation of transport and cell death in the human placenta. Smith returned to Missouri and obtained his Ph.D. in biochemistry in the laboratory of Judy D. Wall. Smith's doctoral research focused on the genetics and enzymes responsible for microbial mercury methylation. His dissertation was titled "Protein Components of the Microbial Mercury Methylation Pathway." His work employed mutational analysis to characterize functional domains of the newly identified HgcA and HgcB proteins integral for mercury methylation and led to his first author publication "Site-Directed Mutagenesis of HgcA and HgcB Reveals Amino Acid Residues Important for Mercury Methylation" in *Applied and Environmental Microbiology*. Smith presented this research at the International Conference on Mercury as a Global Pollutant in 2015 in Jeju, Korea. He is a co-author on numerous perinatal biology papers along with the *Science* paper that initially identified the *hgcA* and *hgcB* genes. Smith is currently working in the Wall lab finishing a publication to identify methyl donors to the mercury methylation reaction. In his free time, he is an avid smallmouth bass fisherman on many of Missouri's small rivers and streams.

Hongmei Chen



Hongmei Chen earned her Ph.D. in Chemistry from Old Dominion University in 2014. Her doctoral research was focused on "Molecular Characterization and Photochemical Transformation of Dissolved Organic Matter from Land to Ocean" using state-of-the-art high resolution mass spectrometry (FTICR-MS). She is currently a postdoctoral research associate with Baohua Gu at Oak Ridge National Laboratory, and her research is focused on molecular scale understanding of mercury-dissolved organic matter complexes affecting mercury biogeochemical transformations in natural water and sediments.

Appendix A. Cited References

Brooks, S. C., C. C. Brandt, and N. A. Griffiths. In revision. "Estimating uncertainty in ambient and saturation nutrient uptake metrics from nutrient pulse releases in stream ecosystems." *Limnology and Oceanography: Methods*.

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 Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment. A*pplied Environmental Microbiology.* Accepted manuscript posted online 15 July 2016. DOI:10.1128/AEM.01271-16

Choi, S.C., Chase, T. Jr., and R. Bartha. 1994. "Enzymatic Catalysis of Mercury Methylation by *Desulfovibrio desulfuricans LS*." *Applied and Environmental Microbiology* **60:**1342–1346.

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, 107–148. San Diego. Academic Press.

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*, 2016. In press. DOI: 10.1021/acs.jpcb.6b02701

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.

Lin, H., J. L. Morrell-Falvey, B. Rao, L. Liang, and B. Gu. 2014. "Coupled mercury-cell sorption, reduction, and oxidation affecting methylmercury production by *Geobacter sulfurreducens* PCA." *Environmental Science & Technology* **48** (20):11969–11976. DOI:10.1021/es502537a

Lin, H., X. Lu, L. Liang, and B. Gu. 2015a. "Cysteine inhibits mercury methylation by *Geobacter Sulfurreducens* PCA mutant ΔomcBESTZ . *"Environmental Science & Technology Letters* **2**: 144–148. DOI: 10.1021/acs.estlett.5b00068

Lin, H., X. Lu, L. Y. Liang, and B. H. Gu. 2015b. "Thiol-Facilitated Cell Export and Desorption of Methylmercury by Anaerobic Bacteria." *Environmental Science & Technology Letters* **2**(10): 292–296. DOI: 10.1021/acs.estlett.5b00209

Lu, X., Y. Liu, A. Johs, L. Zhao, T. Wang, Z. Yang, H. Lin, D. A. Elias, E. M. Pierce, L. Liang, T. Barkay, and B. Gu. 2016. "Anaerobic Mercury Methylation and Demethylation by *Geobacter bemidjiensis* Bem." *Environmental Science & Technology*. DOI: 10.1021/acs.est.6b00401

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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.

Olsen, T. A., and S. C. Brooks. In revision. "Periphyton biofilms influence net methylmercury production in an industrially contaminated system." *Environmental Science & Technology.*

Parks, J. M., and J. C. Smith. In press. 2016. "Modeling Mercury in Proteins." In *Methods in Enzymology*: *Computational Approaches for Studying Enzyme Mechanism*. Ed. Gregory Voth. Elsevier, Inc. Amsterdam.

Podar, M., C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A.V. Palumbo, A. C. Somenahally, and D. A. Elias. 2015. "Global Prevalence and Distribution of Genes and Microorganisms Involved in Mercury Methylation." *Science Advances* **1**(9): e1500675. DOI:10.1126/sciadv.1500675

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Rothenberg, S. E., S. Keiser, N. J. Ajami, M. C. Wong, J. Gesell, J. F. Petrosino, and A. Johs. 2016. "The role of gut microbiota in fetal methylmercury exposure: Insights from a pilot study." *Toxicology Letters* **242**: 60–67. DOI: 10.1016/j.toxlet.2015.11.022

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Smith, S. D., R. Bridou, A. Johs, J. M. Parks, D. A. Elias, R. A. Hurt, S. D. Brown, M. Podar, and J. D. Wall. 2015. "Site-Directed Mutagenesis of HgcA and HgcB Reveals Amino Acid Residues Important for Mercury Methylation." *Applied and Environmental Microbiology* **81**(9): 3205–3217. DOI: 10.1128/aem.00217-15

Yang, Z., W. Fang, X. Lu, G. Sheng, D. E. Graham, L. Liang, S. D. Wullschleger, and B. Gu. 2016. "Warming increases methylmercury production in an Arctic soil." *Environmental Pollution* **214**: 504–509. DOI: 10.1016/j.envpol.2016.04.069

Appendix B. SFA Publications

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

2016

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 Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment. A*pplied Environmental Microbiology.* Accepted manuscript posted online 15 July 2016. DOI:10.1128/AEM.01271-16

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*, 2016. In press. DOI: 10.1021/acs.jpcb.6b02701

Lin, H., X. Lu, L. Y. Liang, and B. H. Gu. 2015. "Thiol-Facilitated Cell Export and Desorption of Methylmercury by Anaerobic Bacteria." *Environmental Science & Technology Letters* **2** (10): 292–296. DOI: 10.1021/acs.estlett.5b00209

Lu, X., Y. Liu, A. Johs, L. Zhao, T. Wang, Z. Yang, H. Lin, D. A. Elias, E. M. Pierce, L. Liang, T. Barkay, and B. Gu. 2016. "Anaerobic Mercury Methylation and Demethylation by *Geobacter bemidjiensis* Bem." *Environmental Science & Technology* DOI: 10.1021/acs.est.6b00401

Parks, J. M., and J. C. Smith. In press. 2016. "Modeling Mercury in Proteins." In *Methods in Enzymology*: *Computational Approaches for Studying Enzyme Mechanism*. Ed. Gregory Voth. Elsevier, Inc. Amsterdam.

Podar, M., C. C. Gilmour, C. C. Brandt, A. Soren, S. D. Brown, B. R. Crable, A.V. Palumbo, A. C. Somenahally, and D. A. Elias. "Global Prevalence and Distribution of Genes and Microorganisms Involved in Mercury Methylation." *Science Advances* **1**(9): e1500675. DOI:10.1126/sciadv.1500675

Riscassi, A., C. Miller, and S. Brooks. 2016. "Seasonal and flow-driven dynamics of particulate and dissolved mercury and methylmercury in a stream impacted by an industrial mercury source." *Environmental Toxicology and Chemistry* **35**(6): 1386–1400. DOI: 10.1002/etc.3310

Rothenberg, S. E., S. Keiser, N. J. Ajami, M. C. Wong, J. Gesell, J. F. Petrosino, and A. Johs. 2016. "The role of gut microbiota in fetal methylmercury exposure: Insights from a pilot study." *Toxicology Letters* **242**: 60–67. DOI: 10.1016/j.toxlet.2015.11.022

Yang, Z., W. Fang, X. Lu, G. Sheng, D. E. Graham, L. Liang, S. D. Wullschleger, and B. Gu. 2016. "Warming increases methylmercury production in an Arctic soil." *Environmental Pollution* **214**: 504–509. DOI: 10.1016/j.envpol.2016.04.069

Submitted Manuscripts

Brooks, S. C., C. C. Brandt, and N. A. Griffiths. In revision. "Estimating uncertainty in ambient and saturation nutrient uptake metrics from nutrient pulse releases in stream ecosystems." *Limnology and Oceanography: Methods*.

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. Jubb, L. Zhao, E. M. Pierce, P. Gobbi, S. Papa, and R. Coccioni. In review. "Mercury-Pollution Induction of Intracellular Lipid Accumulation and Lysosomal Compartment Amplification in the Benthic Foraminifer *Ammonia parkinsoniana*." *PLoS One.*

Luo, H., X. Yin, H. Chen, X. Lu, A. M. Jubb, W. Zhang, Lin, H., Y. Li, H. Yu, M. P. Paranthaman, L. Liang, G. Sheng, and B. Gu. In review. "Photochemically driven mercury sulfide formation and decreased methylmercury production in water." *Environmental Science & Technology Letters.*

Olsen, Todd A., and Scott C. Brooks. In revision. "Periphyton biofilms influence net methylmercury production in an industrially contaminated system." *Environmental Science & Technology.*

Qian, C., A. Johs, H. Chen, B. F. Mann, X. Lu, P. E. Abraham, R. L. Hettich, B. Gu. In review. "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.*

Ph.D. Thesis

Smith, S. D. 2015. *Protein components of the microbial mercury methylation pathway*. Dissertation in partial fulfillment of requirements of a Ph.D. degree in Biochemistry at the University of Missouri, Columbia.

Appendix C. Presentations and Conferences

Published or Accepted Conference Abstracts or Presentations

Chen, H., B. Mann, C. Qian, A. Johs, X. Lu, L. Liang, E. M. Pierce, R. Hettich, and B. Gu. "High-Resolution Mass Spectrometric Analyses of Mercury-DOM Complexation and Cellular Response of *Geobacter sulfurreducens* PCA following its *hgcAB* Gene Deletion." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Christensen, G. A., A. M. Wymore, A. J. King, M. Podar, R. A. Hurt, E. U. Santillan, C. C. Gilmour, C. C. Brandt, S. D. Brown, A. V. Palumbo, and D. A. Elias. "A Study of Mercury Methylation Genetics: Qualitative and Quantitative Analysis of *hgcAB* in Pure Culture." 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

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. "Development and Validation of Broad-Range Qualitative and Clade-Specific Quantitative Molecular Probes for Assessing Mercury Methylation in the Environment." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Crable, B.R., R. Harvey, S. D. Smith, R. Bridou, S. Brown, A. V. Palumbo, M. Podar, J. D. Wall, C. C. Gilmour, and D. A. Elias. "Investigation in to the Native Function of the Mercury Methylation Genes *hgcAB*.." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Demers, J. D., J. D. Blum, S. C. Brooks, P. M. Donovan, B. Gu, C. L. Miller, and A. L. Riscassi. "Hg Isotopes Reveal Importance of In-stream Processing and Legacy Inputs in East Fork Poplar Creek, Oak Ridge, Tennessee, USA." 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

Dickson, J. O., M.A. Mayes, S.C. Brooks, D.B. Watson, T.L. Mehlhorn, M.J. Peterson, and E.M. Pierce. "Mercury Loading from Floodplain Soils in a Southern Appalachian Watershed." Society of Environmental Toxicology and Chemistry North America 36th Annual Meeting North America 36th Annual Meeting. November 1–5, 2015. Salt Lake City, Utah.

Elias, D. A., A. C. Somenahally, J. G. Moberly, R. A. Hurt, S. D. Brown, M. Podar, A. V. Palumbo, and C. C. Gilmour. "Microbial Community Response to Carbon Substrate Amendment in Mercury Impacted Sediments: Implications on Microbial Methylation of Mercury." 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

Gilmour, C. C., E. U. Santillan, D. A. Elias, G. A. Christensen, A. J. King, M. Podar, A. M. Wymore, A. Soren, and A. McBurney. "Method Development and Early Testing of Molecular Probes for the Microbial Mercury Methylation gene pair *hgcAB*." 2015 Geological Society of America Annual Meeting, November 1–4, 2015. Baltimore, Md.

Gu, B., X. Lu, H. Lin, A. Johs, A. Jubb, L. Liang, and E. Pierce. "Anaerobic Mercury Methylation and Demethylation by *Geobacter bemidjiensis* Bem, and factors affecting methylmercury export and distribution." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Gu, B., X. Lu, Y. Liu, and H. Lin. "Facilitated Cell Export and Desorption of Methylmercury by Anaerobic Bacteria." 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

Hsu-Kim, H., C. Johnson, M. Deshusses, and D. A. Elias. "Quantification of Mercury Bioavailability and Methylation Potential in Contaminated Aquatic Ecosystems." 2015 Geological Society of America Annual Meeting, November 1–4, 2015. Baltimore, Md.

Johnson, C., E. Hung, D. A. Elias, M. Deshusses, and H. Hsu-Kim. "Tracking the Methylation of Stable-Isotope-labeled Inorganic Forms of Mercury and the Effect of Activated Carbon on Their Bioavailabilities." 2015 Geological Society of America Annual Meeting, November 1–4, 2015. Baltimore, Md.

Johs, A., R.C. Johnston, L. Liang, K. Neupane, J.M. Parks, S.W. Ragsdale, K.W. Rush, S.J. Tomanicek, A.M. Whited, and J. Zhou "Insights into the Biochemistry of Microbial Mercury Methylation." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Kemner, K. M., M. Boyanov, T. Flynn, E. J. O'Loughlin, D. A. Antonopoulos, S. Kelly, K. Skinner, B. Mishra, S. C. Brooks, D. B. Watson, and W. Wu. "Investigating Redox Processes Under Diffusive and Advective Flow Conditions Using a Coupled Omics and Synchrotron Approach." Abstracts of the 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

King, A. J., G. A. Christensen, A. M. Wymore, M. Podar, R. A. Hurt, S. D. Brown, A. V. Palumbo, K. S. Bender, M. W. Fields, C. C. Gilmour, E. U. Santillan, C. C. Brandt, and D. A. Elias . "Investigating the Connection Between hgcA and Mercury Methylation Rates in the Environment." 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

Olsen, T. A., and S. C. Brooks. "Periphyton Communities Methylate Mercury in an Industrially Contaminated Creek." Society of Environmental Toxicology and Chemistry North America 36th Annual Meeting, November 1–5, 2015. Salt Lake City, Utah.

Olsen, T. A., A. J. King, D. A. Elias, S. C. Brooks. "Periphyton Biofilms Influence Net Methylmercury Production in an Industrially Contaminated System." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Painter, S., S. Brooks, G. Tang. "Exposure Time-based Approach for Modeling Mercury Transport and Transformation in Low-order Streams." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Parks, J.M., R.C. Johnston, A. Johs, L. Liang, J. C. Smith, S.J. Tomanicek, and J. Zhou "Probing Electrochemistry and Chemical Equilibria with Computational Chemistry: Corrinoids Related to Mercury Methylation." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Pierce, E. M., S. C. Brooks, B. Gu, D. Elias, J. Parks, A. Johs, C. Gilmour, and J. Wall. "Biogeochemical Transformations at Critical Interfaces Science Focus Area: An Overview." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Qian, C., A. Johs, H. Chen, B.F. Mann, X. Lu, P.E. Abraham, B. Gu, and R.L. Hettich. "Global Proteome Response to Deletion of Genes Related to Mercury Methylation and Dissimilatory Metal Reduction Reveals Changes in Respiratory Metabolism in *Geobacter sulfurreducens* PCA." The 64th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics. June 5–9, 2016. San Antonio, Texas.

Santillan, E. F., C. C. Gilmour, G. Schwartz, G. A. Christensen, A. J. King, and D. A. Elias. "The Distribution and Abundance of Mercury Methylating Microorganisms in Mid-Atlantic Wetlands." 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

Wall, J. D., R. Bridou, S. D. Smith, K. Mok, F. Widner, A. Johs, J. Parks, E. M. Pierce, D. A. Elias, C. C. Gilmour, and M. Taga. "Metabolic Interfaces of Mercury Methylation Proteins in *Desulfovibrio* sp. ND132." 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

Watson, D., B. Lester, T. Mehlhorn, K. Lowe, S. Brooks, M. Peterson, and C. Miller. "The Impacts of Water Chlorination, Dechlorination and Other Chemical Use Practices on Mercury Mobility at a Mercury Spill site." Society of Environmental Toxicology and Chemistry North America 36th Annual Meeting, November 1–5, 2015. Salt Lake City, Utah.

Invited Presentations

Brooks, S. C. "Field and Laboratory Scale Investigations of Biogeochemical Gradients Across the Surface Water Groundwater Interface." Environmental System Science Principal Investigator Meeting. April 26–27, 2016. Potomac, Md.

Elias, D. A. "New Developments and Novel Approaches for Understanding Mercury and Methylmercury." Invited talk at Missouri University of Science and Technology. December 2015.

Hsu-Kim, H. M. Deshusses, and D. A. Elias. "Strategies to Quantify and Decrease Mercury Bioavailability and Methylation Potential in the Aquatic Environment." 2015 American Geophysical Union Fall Meeting. December 14–18, 2015. San Francisco, Calif.

Johs, A. "Global Response of Gene Deletions on the Proteome of *Geobacter sulfurreducens* PCA: Implications for Dissimilatory Respiration and Mercury Methylation." Joint Institute for Neutron Sciences Seminar. March 31, 2016. Shull Wollan Center, Oak Ridge, Tenn.

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

Liang, L. "Role of Coordination Chemistry in Mercury Transformation." 252nd American Chemical Society National Meeting and Exposition. August 21–25, 2016. Philadelphia, Pa.

Liang, L. "From Chemistry to Mercury Methylation Gene Discovery." Joint Institute for Neutron Sciences seminar Shull Wollan Center, Joint Institute for Neutron Sciences Seminar. March 31, 2016. Shull Wollan Center, Oak Ridge, Tenn.

Parks, J.M. "Discovery and Biophysical Characterization of HgcA, a Corrinoid-dependent Methyltransferase." Department of Chemistry and Biochemistry, University of Oklahoma. September 2015. Norman, Okla.

Parks, J.M. "Discovery and Biophysical Characterization of HgcA, a Corrinoid-dependent Methyltransferase." Biochemistry & Cellular and Molecular Biology Departmental Seminar, University of Tennessee. September 2015. Knoxville, Tenn.

Pierce, E. "Legacy Mercury: A Watershed Scale Scientific and Technical Conundrum." US Army Corp of Engineers, Engineering and Research Development Center. Nov. 9, 2015. Vicksburg, Miss.

Pierce, E.M. "DOE Sponsored Mercury-related Research: DOE SC Science Focus Area and DOE EM Applied Field Research Initiative." Presented at A Strategic Approach for Addressing DOE's Mercury Contamination Challenges in Waste Processing Systems and the Environment hosted by DOE Office of Environmental Management. Sept. 1, 2015. Germantown, Md.

Appendix D. Leadership Activities, Outreach, and User Proposals

Leadership Activities

Baohua Gu

- Geological Society of America Fellow
- Regional Editor for *Environmental Engineering Science*
- Organized Session at 2016 26th Goldschmidt Conference session entitled: Microbiological and Geochemical Controls on Trace Metal Speciation, Transformation, and Transport.

Dwayne Elias

- Academic Editor for *PLoS One*
- Associated Editor for *Frontiers in Microbiology Journal*
- Organized Session at 2015 AGU Fall Meeting: Mercury Biogeochemistry, Genomics, and Environmental Change II.

Eric Pierce

- Associate Editor for *Applied Geochemistry Journal*
- Organized Session at 2016 26th Goldschmidt Conference entitled "Microbiological and Geochemical Controls on Trace Metal Speciation, Transformation, and Transport"
- Participant in Local Organizing Committee for the 2017 International Conference on Mercury as a Global Pollutant
- Organized and presented at DOE's Office of Environmental Management (EM) meeting focused on mercury contamination at EM sites, which was held in September 2015. This meeting was a forum to provide an overview of SFA accomplishments and findings to date.
- Gave an invited oral presentation to the U.S. Army Corps of Engineers Engineer Research and Development Center (ERDC) in Vicksburg, Miss., in November 2015.
- Particpated in the Environmental System Science Cyber Infrastructure meeting. "Building a Cyber Infrastructure for Environmental System Science: Modeling Frameworks, Data Management, and Scientific Workflows."
- Member of CESD–ESS Cyber Infrastructure Executive Committee.

Jerry Parks

- Served on the Scientific Advisory Committee for the Biosciences Division of SLAC National Accelerator Laboratory in October 2015.
- Gave two invited presentations in September 2015 on the discovery and characterization of HgcA. One was presented at the University of Oklahoma (Department of Chemistry and Biochemistry), and the other was presented at the University of Tennessee, Knoxville (Department of Biochemistry and Cellular and Molecular Biology).

Scott Brooks

- Task 1 staff are also involved in an U.S. DOE EM-funded technology development (TD) project whose aim is 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.
- Scott Brooks remains engaged with the South River Science Team, which is led by DuPont to address legacy mercury contamination in the South River, Va.
- Task 1 is cooperating with a USGS-led continental-scale air sampling study by deploying air samplers at one location along upper EFPC. Results of the study may lend important insights into the aqueous reaction path followed by Hg resulting in the formation of volatile Hg(0).

Outreach

- In FY16 we redesigned the SFA website.

User Proposals Submitted

- In a user proposal to the Advanced Light Source at LBNL we have successfully obtained synchrotron beamtime for high-throughput small-angle X-ray scattering (ALS beamline 12.3.1) to characterize the oligomeric states and molecular envelopes of heterologously expressed HgcA and HgcB.
- In a user proposal to EMSL, Task 4 aims to determine molecular structures of HgcA and HgcB by nuclear magnetic resonance spectroscopy. EMSL proposal 48393: "Nuclear magnetic resonance (NMR) structure determination of the cobalamin binding protein HgcA and the ferredoxin-like protein HgcB which are required for bacterial mercury methylation" (EMSL contact: Patrick Reardon).

Appendix E. Agenda for 2016 Scientific Advisory Committee Visit

2016 Oak Ridge National Laboratory SFA Advisory Committee Site Visit

May 17-18, 2016

Building 1520, Rm. 202, Beech River Conference 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, Xiangping Lisa Yin

Post-Doctoral Researchers/Graduates: Hongmei Chen, Geoffrey Christensen, Bryan Crable, Jeremy Eskelsen, Ryan Harvey (University of Missouri), Allison Holt, Ryne Johnston, Aaron Jubb*, Andrew King, Xia Lu*, Todd Olsen, Steve Tomanicek, Ziming Yang, Linduo Zhao, Jing Zhou

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

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

Tuesday, May 17th		
Schedule	**Topic**	**Participants**
7:30 a.m.–8:00 a.m.	Badging	SAC Members
8:30 a.m.–9:00 a.m.	Reviewers Orientation	All
9:00 a.m.–9:30 a.m.	Start ORNL SFA Overview Presentation	Eric Pierce
9:30 a.m.–9:40 a.m.	Questions / Discussion	All
9:45 a.m.–10:15 a.m.	Task 1 Overview Presentation / Q&A	Scott Brooks
10:15 a.m.–10:30 a.m.	Break	All
10:30 a.m.–11:00 a.m.	Modeling Presentation / Q&A	Scott Painter
11:05 a.m.–11:35 a.m.	Task 2 Overview Presentation / Q&A	Baohua Gu
11:40 a.m.–12:10 p.m.	Task 3 Overview Presentation / Q&A	Dwayne Elias
12:10 p.m.–2:00 p.m.	Lunch / Poster Session	All
2:00 p.m.–2:15 p.m.	Break	All
2:15 p.m.–2:45 p.m.	Task 4 Overview Presentation / Q&A	Jerry Parks, Alex Johs
2:45 p.m.–3:15 p.m.	Group Picture	All
3:15 p.m.–5:00 p.m.	Final Questions / Discussions	All
5:00pm	No-host Dinner at *Calhoun's – Turkey Creek*	All
Wednesday, May 18th		
Schedule	**Topic**	**Participants**
8:30 a.m.–11:00 a.m.	Committee Sequester / Discussion	SAC Members
11:00 a.m.–11:45 a.m.	Committee Debriefing to SFA Task Leaders	SAC Members, Task Leaders
11:45 a.m.–12:00 p.m.	Executive Briefing to Jay Gulledge, Division Director–Environmental Sciences Division / Closing Comments to SAC Members	SAC Members, Jay Gulledge
12:00 p.m.–12:05 p.m.	Wrap-up / Meeting Adjourned	SAC Members, Eric Pierce

*Remote participation or not in attendance

Acronyms and Abbreviations

BBC	British Broadcasting Company
BER	Office of Biological and Environmental Research
CBD	cobalamin-binding domain
CbI	hydroxocobalamin
CI	confidence interval
CI-SFA	Critical Interfaces Science Focus Area
Co	cobalt
CO_2	carbon dioxide
Cys	cysteine
DOC	dissolved organic carbon
DOE	U.S. Department of Energy
DOM	dissolved organic matter
EFPC	East Fork Poplar Creek
EM	DOE Office of Environmental Management
EMSL	DOE Environmental Molecular Sciences Laboratory
EPR	electron paramagnetic resonance
EXAFS	extended X-ray absorption fine structure spectroscopy
FTICR-MS	Fourier transform ion cyclotron resonance–mass spectrometry
Hg	mercury
HgD	dissolved mercury
HgP	particulate mercury
HSQC	heteronuclear single quantum coherence
LBNL	Lawrence Berkeley National Laboratory
LRM	Laboratory Research Manager
MBP	maltose binding protein
MC	Monte Carlo
MeHg	methylmercury
MeHgD	dissolved methylmercury
MeHgP	particulate methylmercury
MerA	mercuric reductase
MerB	organomercurial lyase
Met	methionine
MS	mass spectrometry
NMR	nuclear magnetic resonance
ORNL	Oak Ridge National Laboratory
OREM	Oak Ridge Office of Environmental Management
ORR	Oak Ridge Reservation
PCR	polymerasc chain reaction
PI	Principal Invesitagor
qPCR	quantitative polymerase chain reaction
SAC	Scientific Advisory Committee
SBR	DOE Subsurface Biogeochemical Research program
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SFA	Science Focus Area
SOC	soil organic carbon
TASCC	tracer additions for spiraling curve characterization
UV/Vis	ultraviolet–visible spectroscopy

SFA Contact and Sponsor

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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.