

## **Final Report**

**Department of Energy Office of Science  
Office of Biological and Environmental Research  
Subsurface Biogeochemical Research Program**

### **Isotopic Characterization of Biogeochemical Pools of Mercury and Determination of Reaction Pathways for Mercury Methylation.**

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## Executive Summary

This project titled “Isotopic Characterization of Biogeochemical Pools of Mercury and Determination of Reaction Pathways for Mercury Methylation” represents a collaborative project between the University of Michigan (Joel Blum, PI) and Oak Ridge National Laboratory (Baohua Gu, lead Co-I). The objective of this proposed project was to utilize natural mercury (Hg) stable isotope composition and fractionation to reveal the locations of Hg methylation in the East Fork Poplar Creek (EFPC) watershed in Oak Ridge, TN and place new constraints on the chemical and biological processes that lead to methyl mercury (MeHg) production and transport to aquatic ecosystems. This methodology has the potential to elucidate the factors and mechanisms leading to the apparent decoupling of inorganic Hg (iHg) and MeHg concentrations in the contaminated EFPC ecosystem and other contaminated stream ecosystems.

To achieve these objectives we proposed a two-pronged research approach that involved 1) an ecosystem level study of sediment, water, and biota within the East Fork Poplar Creek and adjacent riparian wetlands, and 2) a series of experimental studies designed to fill knowledge gaps in Hg isotope fractionation that would help inform the interpretation of ecosystem observations. Experimental studies of isotope fractionation were designed to allow prediction of fractionation associated with major biogeochemical Hg transformations under conditions that are relevant to the EFPC site.

A regional survey of Hg isotope composition in stream sediments showed that 38% to 78% of the Hg in downstream Clinch River sediments could be attributed to Y12 derived sediment-bound Hg exported from EFPC. Another source, likely regional non-Y12 Hg emissions to the atmosphere, also appeared to be an important additional source of Hg to sediments within the region.

Within the East Fork Poplar Creek ecosystem, Hg isotope techniques revealed that in-stream processing and legacy inputs of mercury from hyporheic and adjacent riparian wetlands may be more important than recognized from previous research efforts within the EFPC. Variations in Hg isotopic composition of the dissolved and suspended fractions along the flow path of EFPC suggest that: (1) physical processes such as dilution and sedimentation cannot fully explain decreases in total mercury concentrations along the flow path; (2) in-stream processes include photochemical transformations (~20%), but microbial reduction is likely more dominant (~80%); and (3) additional sources of mercury inputs to EFPC at base flow may predominantly arise from the hyporheic zone during the growing season, with adjacent riparian wetlands and non-point-source impacted tributaries increasing in importance during the dormant season when the stream channel is more hydrologically connected to the watershed.

The isotopic composition of sediment, periphyton, and fish from the EFPC ecosystem were all distinctly different from the sediment, periphyton, and fish from the non-point-source impacted reference site. At the reference site, methylmercury (MeHg) and inorganic mercury (iHg) in fish appeared to be derived from different sources, with MeHg arising from mercury within the sediments, and inorganic mercury possibly being more influenced by precipitation. Within the EFPC, both MeHg and iHg sources appeared to be derived from the streambed sediment or streambed periphyton coatings. This suggests differences in the bioavailability of various sources of iHg for transformation to MeHg in natural environments. Also, differences in the relationship between fish and sediment mercury isotopic composition at various sites along the EFPC suggests differences in the balance between methylation and demethylation pathways that are the source of MeHg to aquatic ecosystems.

Broadly, the mercury isotopic assessment in this study suggests that the decoupling of inorganic mercury and MeHg in the Lower EFPC could, in part, be derived from legacy Hg sources. This suggests that mitigation strategies that focus only on reducing present-day loadings of inorganic mercury from Y12 may not entirely meet the intended goal of reducing MeHg in downstream waters, biota, and fish of the Lower EFPC ecosystem.

In order to manage Hg contaminated sites and make informed decisions about Hg remediation strategies additional tools are needed to trace the sources, pathways, and bioavailability of Hg. This study demonstrates that Hg stable isotope techniques can be used to gain new insight into the processes underlying Hg dynamics in complex aquatic environments, and to reveal the sources of legacy Hg contributed to stream water and accumulated in biota.

## Overall Project Objectives

The overarching objectives of this project were to utilize natural mercury (Hg) stable isotope composition and fractionation to reveal the locations of Hg methylation in the East Fork Poplar Creek (EFPC) watershed in Oak Ridge, TN and place new constraints on the chemical and biological processes that lead to methylmercury (MeHg) production and transport to aquatic ecosystems. This methodology provided the opportunity to elucidate the factors and mechanisms leading to the apparent decoupling of inorganic Hg (iHg) and MeHg concentrations in the contaminated EFPC ecosystem, thus illustrating an approach that could be utilized widely across other contaminated stream ecosystems.

To achieve these research objectives, researchers from the University of Michigan (lead by Joel Blum, PI) and Oak Ridge National Laboratory (lead by co-PI Baohua Gu) carried out a collaborative two-pronged research approach that involved (1) an ecosystem level study of natural samples, and (2) a series of experimental studies designed to fill knowledge gaps in Hg isotope fractionation. Natural samples were collected from stream surface water and hyporheic pore water, wetland surface water and pore water, streambed sediment and periphyton, and as a monitor of MeHg in the ecosystem, young-of-year herbivore/detritivore stoneroller minnows (*Camptostoma anomalum*) and omnivore/piscivore redbreast sunfish (*Lepomis auritus*). Experimental studies of isotope fractionation focused on pathways considered to be important in East Fork Poplar Creek, including photochemical oxidation of mercury and dark oxidation of mercury under natural EFPC stream water conditions. The results of experimental studies were utilized to inform the interpretation of fractionation observed within natural samples collected from the East Fork Poplar Creek Ecosystem.

Our research was organized around a series of five objectives which were addressed through a series of associated hypotheses, tasks, and specific sub-tasks. Below, we summarize project activities for the entire period of funding, including hypotheses, approaches, and findings. A separate subsequent section lists publications that have resulted from this project, and additional manuscripts currently in preparation.

## Summary of Activities and Findings

**Objective 1:** *Characterize the spatial and temporal variability of the concentration and isotopic composition of total mercury and methyl mercury in sediments and waters of streams and wetlands of the EFPC watershed in order to differentiate sources of mercury that may contribute to elevated stream water total Hg and MeHg loads.*

A critical first step in determining the ultimate fate of legacy Hg stored in the watershed of the EFPC was to characterize the isotopic composition of in-stream and riparian wetland Hg pools. Once characterized, it becomes possible to use this information to track when and how different sources of Hg are contributed to the stream channel, and the extent to which legacy Hg stored in the streambed and in riparian wetlands contributes to waterborne Hg and MeHg loads within the EFPC. Associated with this objective, were the following hypotheses:

**Hypothesis 1A:** Legacy Hg stored in riparian wetlands adjacent to the EFPC stream will have an isotopic composition distinct from both in-stream Hg sources (streambed sediments and hyporheic interstitial water), and new Hg currently released to the headwater of EFPC at Y-12 National Security Complex.

**Hypothesis 1B:** Net MeHg inventories (expressed as %MeHg) will be the highest in reduced wetland sediments at the end of the growing season when water table levels are the lowest, water/sediment temperatures are warmest, and there has been a lack of hydrologic flushing of riparian soils.

**Hypothesis 1C:** iHg and MeHg are transported disproportionately from riparian wetlands to the stream during high flow events.

### Task-Specific Results for Objective 1.

**Task 1: Measure the isotopic composition of potential inorganic Hg and MeHg sources within the EFPC watershed.**

### **Subtask 1A. Site Selection and Approach to Ecosystem Sampling.**

Our goal was to place our Hg isotope research efforts within the framework of established long-term sampling sites and ongoing complementary research efforts [e.g., *Southworth et al.*, 2000; *Southworth et al.*, 2010, and currently within the Subsurface Science Group and Ecological Assessment Group within ORNL Environmental Sciences Division]. After consultation with ORNL researchers including Baohua Gu, Scott Brooks, Carrie Miller, and Teresa Mathews, we implemented a study design that included longitudinal stream water sampling of the EFPC, two intensive sampling sites on the EFPC, and one intensive sampling site on the Hinds Creek reference stream; all of these sites overlapped with recent and ongoing research being conducted by Scott Brooks, Carrie Miller, and Ami Riscassi within the Subsurface Science Group, and by Teresa Mathews within the Ecological Assessment Group.

We employed both synoptic and intensive site sampling strategies during three seasonal sampling campaigns (October 2011, April 2012, and August 2012), in a reasonable effort to assess both spatial and temporal variation in Hg isotopic composition within the natural ecosystem across a single water year. The headwaters of East Fork Poplar Creek originate within the US Department of Energy (DOE) Y-12 National Security Complex (hereafter, Y12), and flow 26 km downstream to its confluence with Poplar Creek (Figure 1) [*Loar et al.*, 2011]. Locations along the EFPC are denoted by their distance upstream from this confluence, in kilometers. All samples were collected during base flow conditions.

Synoptic sampling consisted of eight stream sampling locations along the flow path of the EFPC, ranging from EFK5.0 to EFK25.4, two of which were within the Y12 boundary along the Upper EFPC (Figure 1). At each location, we characterized the isotopic composition of waterborne mercury in both the particulate suspended fraction and the dissolved fraction, as well as within streambed sediment. Synoptic samples were collected to assess changes in the isotopic composition of waterborne and sediment associated mercury along the flow path of EFPC. Sediment mercury isotopic composition was also set into a broader regional context using additional sediment samples collected from the Clinch River, Poplar Creek, and EFPC by the Tennessee Department of Environmental Conservation (TDEC) [*Donovan et al.*, 2014].

Intensive site sampling was completed during each season at two sites along the EFPC (EFK5.0, EFK22.3) and at a regional reference stream, Hinds Creek, located ~25 km northeast of Y12 (Figure 1). These intensive sampling efforts were designed to assess differences in mercury isotopic composition within various components of the stream ecosystem. At each intensive site, we collected surface water, hyporheic pore water from both the center and side of the main channel, and streambed biofilm coatings. At EFK22.3, we also collected surface water and pore water from an Hg-contaminated riparian floodplain.

**Subtask 1B, 1C, 1D, and 1E. Characterize isotopic composition of mercury in stream water and streambed sediments, riparian wetland pore water and sediments, and riparian overland flow.**

#### ***Identification of Multiple Mercury Sources to Stream Sediments near Oak Ridge, Tennessee, USA.***

***[Donovan et al., 2014] (OSTI ID: 1239629)***

We measured the THg concentration and Hg isotope composition of streambed sediments from EFPC, EFPC tributaries, Hinds Creek (reference stream), and upstream and downstream locations in Poplar Creek and the Clinch River [*Donovan et al.*, 2014].

Sediments directly downstream of Y12 in EFPC and Poplar Creek had a unique Hg isotope composition compared to the uncontaminated, upstream sediments in the region [*Donovan et al.*, 2014]. Sediment THg concentrations in EFPC and downstream reaches of Poplar Creek ranged from 2.16 to 60.1  $\mu\text{g/g}$  with  $\delta^{202}\text{Hg}$  of  $0.02 \pm 0.14\text{‰}$  and  $\Delta^{199}\text{Hg}$  of  $-0.08 \pm 0.03\text{‰}$  (mean  $\pm$  1SD; n=16) [*Donovan et al.*, 2014]. At sampling sites far from Y12 or other point source Hg discharges (Hinds Creek and upstream Clinch River), sediment THg concentrations were between 0.01 and 0.2  $\mu\text{g/g}$  and their Hg isotope composition was significantly different, with much lower  $\delta^{202}\text{Hg}$  ( $-1.40 \pm 0.06\text{‰}$ ) and  $\Delta^{199}\text{Hg}$  ( $-0.26 \pm 0.03\text{‰}$ ; mean  $\pm$  1SD; n=6) [*Donovan et al.*, 2014]. Thus, sediments in the region have at least two sources of Hg with distinct Hg isotope compositions. The first source is the Hg integrated and accumulated within

a large regional watershed whereas the second Hg source, with elevated THg concentrations (2.16 to 60.1 µg/g), is the result of historical releases of Hg from Y12 to EFPC [Donovan *et al.*, 2014].

Sediments in the Clinch River downstream of EFPC had elevated THg concentrations (0.24 to 0.76 µg/g), and intermediate Hg isotopic composition ( $\delta^{202}\text{Hg}$  values of -0.28‰ to -0.85‰;  $\Delta^{199}\text{Hg}$  values of -0.07‰ to -0.10‰) that can be explained by the binary mixing of the two Hg sources below the Poplar Creek/Clinch River confluence [Donovan *et al.*, 2014]. Between 38% and 78% of the Hg in downstream Clinch River sediments could be attributed to Y12 derived, sediment bound Hg that is likely exported from EFPC. However, intermediate THg concentration sediments (0.062 to 0.212 µg/g) in upstream Poplar Creek and EFPC tributaries cannot be directly attributed to simple binary mixing between Y12 and regional background Hg (see Figure 4 in Donovan *et al.*, 2014). Instead these sediments were characterized by anomalously low  $\delta^{202}\text{Hg}$  (as low as -5.07) and  $\Delta^{199}\text{Hg}$  that was near zero. Although somewhat speculative, Donovan *et al.* [2014] hypothesize that the isotopic composition of these sediments could be derived from local Hg emissions in the region and represent a third potentially important source to of Hg to sediments within the region.

***Hg isotopes reveal in-stream processing and legacy inputs in East Fork Poplar Creek, Oak Ridge, Tennessee, USA. [Demers *et al.*, Submitting to PNAS Plus]***

Concentrations of mercury associated with suspended particulates (THg<sub>p</sub>, >0.45 µm) and the dissolved aqueous phase (THg<sub>d</sub>, <0.45 µm) decreased rapidly along the 26 km flow path from the headwaters of EFPC to its confluence with Poplar Creek (Figure 2), consistent with previous observations [Southworth *et al.*, 2000; Southworth *et al.*, 2004]. A limited number of measurements suggest that dissolved gaseous mercury (DGM) follows a similar pattern. In contrast with inorganic mercury fractions, methylmercury (MeHg) concentrations increased downstream of Y12 (Figure 2). This is also consistent with previous observations that have demonstrated a decoupling of inorganic and methylmercury dynamics along the flow path of the EFPC [Southworth *et al.*, 2000; Southworth *et al.*, 2004].

Mercury isotope ratio measurements spanning three seasonal sampling periods (October, April, and August) suggest a temporal consistency in the biogeochemical transformations influencing the suspended phase along the flow path of the EFPC. Both odd-mass-number MIF and MDF signatures associated with THg<sub>p</sub> can be explained by a combination of photochemical reduction (~20%) and microbial reduction (~80%). The isotopic composition of the suspended particulate phase was indistinguishable from the biofilm associated with streambed surfaces. Both suspended particulate and biofilm mercury isotopic composition converged with the isotopic composition of the fine fraction of streambed sediment (<125 µm) along the ~25 km study reach [Donovan *et al.*, 2014]. Overall, these observations suggest that the suspended load during base flow is mainly derived from organic and inorganic components of the biofilm layer, that photochemical and microbial reduction likely occur within this surficial layer of the streambed, and that this load spirals downstream, being repeatedly deposited and re-suspended.

The odd-mass-number MIF signature of the dissolved phase was small in magnitude and transient, likely reflecting release of DGM to the water column from photochemical reduction within the suspended particulate phase or streambed biofilm that was subsequently volatilized to the atmosphere. In contrast, the MDF signature of the dissolved phase could not be explained by in-stream biogeochemical processes, but rather appeared to be influenced by inputs from the hyporheic zone and riparian wetlands. Hyporheic exchange appeared to have greater influence on the isotopic composition of dissolved mercury in stream water during August and October when the EFPC was less hydrologically connected to the watershed; whereas, riparian wetlands appeared to increase in influence during April, when the EFPC was more hydrologically connected to the watershed.

Mercury isotopic measurements suggest that stream water dissolved inorganic mercury concentrations are not entirely sustained by export from Y12 during base flow, but are augmented by hyporheic exchange and inputs from riparian wetlands, depending on seasonal hydrologic connectivity. Southworth *et al.* (2013) suggested that mercury associated with streambed sediment and floodplain soils

is in highly insoluble fractions, and thus the ongoing dissolved inorganic Hg loading from Y12 should be the predominant source of bioavailable Hg that is methylated within the Lower EFPC ecosystem. However, mercury isotope systematics revealed that the hyporheic zone and riparian wetlands contribute to dissolved mercury dynamics in the Lower EFPC. Given that the hyporheic zone and riparian wetlands are well known to be anoxic sites that support high rates of MeHg production (e.g., pore water mercury ranged up to 70% MeHg during this study), the mercury isotopic assessment in this study suggests that the decoupling of inorganic mercury and MeHg in the Lower EFPC could, in part, be derived from these legacy sources. This suggests that mitigation strategies that focus only on reducing present-day loading of inorganic mercury from Y12 may not entirely meet the intended goal of reducing MeHg in downstream waters, biota, and fish of the Lower EFPC ecosystem. Future research should focus on better refining flux estimates along the EFPC corridor, quantifying the influence of hyporheic exchange and riparian wetlands, identifying the processes that lead to the release of these legacy sources to the water column, and assessing the sources of mercury that are methylated and accumulated within biota.

Broadly, this study demonstrates that Hg stable isotope techniques can be used to gain new insights into the processes underlying Hg dynamics in complex aquatic ecosystems, and to reveal the sources of legacy Hg contributing to stream water mercury concentrations. As methods for the extraction and pre-concentration of mercury for isotopic analysis continue to be refined, it should become possible to also use mercury isotope techniques to assess the influence of non-point-source derived legacy mercury in ecosystems with background concentrations of soil and water that result in the bioaccumulation of mercury to levels that are harmful to fish and wildlife. This study suggests that various sources of legacy mercury in the environment have isotopic signatures that can in some cases be differentiated, and thus it should also be possible to track MeHg generated from these various sources into biota.

**Objective 2:** *Characterize the Hg isotopic composition of creek bottom periphyton, stoneroller minnows (*Campostoma anomalum*) and redbreast sunfish (*Lepomis auritus*) from biological monitoring sites in order to identify the source(s) of MeHg that is (are) available for bioaccumulation at various sites in EFPC.*

In order to identify the source of available MeHg within the EFPC ecosystem, we characterized the isotopic composition of MeHg in biological materials that are natural bioaccumulators. Not all MeHg delivered to the stream channel may come from the same sources; that is, the timing of delivery (base flow vs. high-flow) and the form of MeHg (dissolved vs. particulate-bound) mobilized from various pools likely effects its transport and fate. Thus, this approach will allow us to distinguish differences in the sources of various MeHg pools being contributed to surface waters of the EFPC. Associated with this objective, we tested the following hypotheses:

**Hypothesis 2A:** The Hg isotopic composition of creek bottom periphyton, stoneroller minnows and redbreast sunfish will vary systematically at each sampling site due to a mixture of isotopically distinct MeHg (produced largely in riparian wetlands) and iHg released to the stream from Y-12 NSC.

**Hypothesis 2B:** MeHg produced in wetlands will have an isotopic composition closest to the value inferred for MeHg in biota after correction for fractionation during transport, partial MeHg degradation and trophic transfer.

### **Task-Specific Results for Objective 2.**

#### **Task 2: Characterize isotopic composition of Hg in periphyton and EFPC biota.**

*Linking the Hg isotopic composition of Hg in the aquatic food web back to the ecosystem source(s) of methylmercury production in East Fork Poplar Creek, Oak Ridge, TN. [Demers et al. In prep]*

We collected periphyton (streambed biofilm) from the intensive sampling site locations (EFK22.3, EFK5.0, and HCK20.6) during all three seasonal sampling periods (October 2011, April 2012, and August 2012) for the analysis of total mercury (THg) concentration and isotope composition. In conjunction with Teresa Matthews (Ecological Assessment Program, Environmental Sciences Division),

we obtained archived fish samples that were collected from the EFPC in 2010 and 2011. We were able to obtain stone roller minnows, red breast sunfish, and rock bass caught near each intensive site (EFK22.3, EFK5.0, and HCK20.6) for the analysis of THg concentration and isotopic composition, and MeHg concentration.

The isotopic composition of sediment, periphyton, and fish from the EFPC ecosystem were all distinctly different from the sediment, periphyton, and fish from the non-point-source impacted reference site (Hinds Creek). Mercury concentration and isotopic composition of periphyton samples appear to be similar to THg associated with TSS in stream water at the point of collection. The isotopic composition of the periphyton in the EFPC ( $\delta^{202}\text{Hg} = 0.19\text{‰}$  to  $-0.40\text{‰}$ ;  $\Delta^{199}\text{Hg} = 0.01\text{‰}$  to  $-0.13\text{‰}$ ) was distinctly different from the periphyton from the non-point-source impacted reference site (HCK20.6,  $\delta^{202}\text{Hg} = -1.06\text{‰}$ ,  $\Delta^{199}\text{Hg} = -0.30\text{‰}$ ). Fish Hg isotopic composition was also distinctly different in the EFPC as compared to the reference site, with Y12 influenced samples having more positive  $\delta^{202}\text{Hg}$  values ( $-1.01$  to  $-0.13\text{‰}$ ) than background samples ( $\delta^{202}\text{Hg} = -1.16\text{‰}$  to  $-1.06\text{‰}$ ) (Figure 3).

Using regression techniques, we utilized the spatial and species specific variations in %MeHg in fish to estimate the isotopic composition of MeHg entering the aquatic ecosystem of the EFPC and the Hinds Creek reference site. The estimated isotopic composition of inorganic mercury (iHg) and MeHg sources accumulating in fish was distinctly different between contaminated sites of EFPC and the Hinds Creek reference site. The estimated  $\Delta^{199}\text{Hg}$  values of iHg in the upper and lower reaches of the EFPC were  $-0.11\text{‰}$  and  $+0.01\text{‰}$ , respectively;  $\Delta^{199}\text{Hg}$  values for MeHg were  $+0.22\text{‰}$  and  $+0.20\text{‰}$ , respectively (Figure 3). We could not use linear regression to estimate  $\delta^{202}\text{Hg}$  of iHg and MeHg in EFPC fish. Whereas MIF signatures of fish mercury are likely driven by photochemical demethylation alone, and apparently result in iHg and MeHg sources with relatively uniform  $\Delta^{199}\text{Hg}$  values, MDF signatures likely result from multiple processes (e.g., microbial methylation and demethylation) that combine to produce iHg and MeHg sources with variable  $\delta^{202}\text{Hg}$  values in the EFPC.

The estimated isotopic composition of iHg in Hinds Creek reference site fish was  $+0.22\text{‰}$   $\Delta^{199}\text{Hg}$ , and  $-0.99\text{‰}$   $\delta^{202}\text{Hg}$ ; estimated MeHg isotopic composition was  $-0.13\text{‰}$   $\Delta^{199}\text{Hg}$ , and  $-1.21\text{‰}$   $\delta^{202}\text{Hg}$  (Figure 3). This suggests that MeHg and iHg accumulating in fish are derived from different sources, with MeHg likely arising from mercury associated with organic matter in sediments, and iHg possibly being more influenced by precipitation. Thus, this suggests differences in the bioavailability of various sources of iHg for transformation to MeHg in natural environments. Also, differences in the relationship between fish and sediments at each site suggests differences in the balance between methylation and demethylation pathways that are the source of MeHg to aquatic ecosystems.

**Objective 3:** *Evaluate various sediment Hg extraction schemes for isotopic measurement of the “bioavailable” sediment Hg pool and measure the isotopic composition of this reactive pool to evaluate whether it is the same or different from total Hg in sediment samples.*

This experimental study was designed to aid the field study described in Objective 1, by identifying which pool of Hg in sediment can be mobilized and thus transported from wetlands to the creek. Experimental studies also allow the determination of the isotope signature of the specific fraction of Hg that is most relevant to being a source of MeHg rather than the bulk sediment, whose isotope signature may be obscured by “refractory” Hg that is not involved in methylation and transport. Associated with this objective, were the following hypotheses:

**Hypothesis 3A:** Reactive Hg pools (for instance Hg reduced by  $\text{SnCl}_2$  or complexed by DOM) and nonreactive Hg complexed with sulfide or other minerals will yield different rates of Hg methylation by sediment incubation.

**Hypothesis 3B:** The isotopic composition of the pools of Hg described above will contrast that of THg in sediments demonstrating that bulk sediment isotope values cannot simply be used to estimate the isotopic composition of the Hg pool that is available for methylation.

### **Task-Specific Results for Objective 3.**

#### **Task 3: Compare isotopic composition of reactive and total Hg.**

**Subtask 3A and B. Extraction of “reactive” Hg from sediments, and measurement of isotope composition of bioavailable and total Hg in sediments.**

We addressed this task by measuring the isotopic composition of THg in two size fractions of sediments sampled from EFPC and its tributaries: sub-125  $\mu\text{m}$  sediments (“fines”) and sub-2mm sediments (“bulk” sediment) [Donovan *et al.*, 2014]. There were interesting differences in  $\delta^{202}\text{Hg}$  between fine and bulk sediment, leading to a  $\delta^{202}\text{Hg}_{\text{offset}}$  ( $\delta^{202}\text{Hg}_{\text{offset}} = \delta^{202}\text{Hg}_{\text{fines}} - \delta^{202}\text{Hg}_{\text{bulk}}$ ) that varied among locations within the stream (see Figure S2 in Donovan *et al.*, 2014). The  $\delta^{202}\text{Hg}_{\text{offset}}$  was significant at the uppermost and lowermost sites in EFPC (+0.45‰ and +0.33‰, respectively) and in EFPC tributary sediments with intermediate THg concentrations ( $\delta^{202}\text{Hg}_{\text{offset}}$  between -0.42 and -1.51‰). We hypothesize that this variability could be explained by differences in Hg binding forms that are preferentially retained within certain size classes (e.g., sorbed to clays vs. Hg bound in sulfides) or differences in the mass contribution of isotopically distinct Hg sources (e.g., floodplain soils vs. upstream sediments) at each location.

These differences in Hg isotope composition of sediment size classes provide motivation for future investigation of the isotopic composition of “reactive” Hg in sediments. In particular, the isotopic composition of the most reactive Hg will help evaluate the relative bioavailability of Hg and possible differences in Hg binding forms (e.g., Hg-sulfides, organic bound Hg, easily soluble Hg) between locations. Moreover, understanding the fraction of Hg in sediment that is available to be contributed to surface waters will improve our understanding of the role of legacy Hg sources in hyporheic zone and riparian wetland sediments. The addition of reactive Hg isotopic composition to our completed sediment THg isotope data enhance our understanding of the link between inorganic Hg and MeHg in the EFPC Ecosystem.

**Objective 4:** *Measure the isotope fractionation during sorption/complexation of Hg(II) and MeHg to DOM using a range of DOM compositions including DOM from the EFPC to determine the effect of sorption on the isotope signature of MeHg.*

This objective was designed to aid in the interpretation of the field results described in Objective 2. The isotope signatures obtained from food webs may have been fractionated by various transformations such as the sorption/complexation of Hg with DOM and minerals. Therefore, laboratory studies on the isotope systematics of these transformations are necessary to properly link isotope signatures in biota to those of sediment and wetlands. Complexation to DOM is the most common reaction of Hg in natural waters because DOM is ubiquitous and it has high affinity for Hg [Morel *et al.*, 1998; Dong *et al.*, 2010]. This is particularly important in wetlands with high DOM. Associated with this objective, are the following hypotheses:

**Hypothesis 4A:** Complexation of Hg(II) or MeHg with EFPC DOM and minerals results in mass independent Hg isotope fractionation, and the fractionation will vary depending on types of binding sites of DOM or specific functional groups on DOM (e.g., the thiols vs carboxylic functional groups).

**Hypothesis 4B:** Hg isotope fractionation will vary among different DOM isolates (e.g., EFPC DOM vs Suwannee River fulvic acid) and the ratio of DOM to Hg.

### **Task-Specific Results for Objective 4.**

#### **Task 4. Isotope Fractionation during Complexation of Hg(II) and MeHg to Various DOM.**

The transformation and transport of Hg in natural sediments and waters are likely facilitated by adsorption or complexation to DOM. A recent study by Wiederhold *et al.* [2010] demonstrated that equilibrium between Hg(II) in solution and those bound to a thiol resin resulted in a mass dependent fractionation of up to 0.6 ‰ ( $\delta^{202}\text{Hg}$ ). In natural waters where the complexation between Hg and DOM is



kinetically controlled, even greater isotope fractionation is expected. Determining fractionation during this process is thus necessary to properly interpret the isotope signature of Hg in sediment/water columns. It is also helpful in tracking the transport of Hg facilitated by the complexation to DOM.

B. Gu and W. Zheng focused on a series of laboratory experiments investigating aqueous dark oxidation of Hg(0) in the presence of natural organic matter (NOM). This was also potentially an important Hg transformation pathway within the East Fork Poplar Creek Ecosystem. Results from this work helped inform the interpretation Hg isotope signatures observed along the flow path of the EFPC (see Objective 1).

***Fractionation of mercury isotopes during aqueous dark oxidation. [Zheng et al. In prep.]***

Natural compositions of Hg stable isotopes have been increasingly used as a tracer of Hg sources and transformations in the environment. However, the isotope fractionation pattern and mechanism in many transformation processes have not been well studied. One of these processes is dark oxidation of elemental Hg(0), which plays a critical role in Hg speciation, bioavailability and mobility. Here we report experimental results on Hg isotope fractionation during abiotic dark oxidation of dissolved Hg(0) in both anoxic and oxic conditions by different oxidants. In both oxidation processes, we observed enrichment of heavier or larger isotopes in oxidized Hg species. Anoxic oxidation in the presence of thiol compounds followed equilibrium isotope fractionation with a mass dependent enrichment factor ( $\epsilon^{202}\text{Hg}_{\text{oxidized-reduced}}$ ) ranging from 0.99‰ to 1.50‰. Oxidation by  $\text{Cl}^-$  + benzoquinone and  $\text{H}_2\text{O}_2$  produced more variable enrichment factors ranging from ~1.12‰ to 2.40‰. In addition, small negative mass independent fractionation (MIF) (less than 0.4‰) was also observed in oxidized Hg in both oxidation experiments. Based on the comparison with similar MIF observed in previous experimental and theoretical studies, we conclude that the MIF observed in dark oxidation is likely caused by nuclear volume effect (NVE). The experimental enrichment factors (particularly for anoxic oxidation) showed good agreement with previous theoretical calculations, suggesting that a combination of mass dependent fractionation and NVE was responsible for the observed Hg isotope fractionation. Overall, our results provide additional experimental constraints on Hg isotope signatures found in redox active environments, and thus improve the application of Hg isotope fractionation as a tracer of redox transformations.

**Objective 5:** *Measure the natural isotope fractionation during photochemical reduction of Hg(II) and photochemical degradation of MeHg using a range of DOM compositions, including DOM from the EFPC site, and varying Hg/DOM ratios to determine the extent to which abiotic reactions may shift the isotopic composition of MeHg.*

Similar to Objective 4, this objective was designed to address another critical transformation of Hg to aid the understanding of isotope signatures obtained in field studies. Photochemical reduction of Hg(II) and MeHg degradation are among the most important reactions of Hg in the EFPC water column, and we consider this process as another major modifier of isotope composition of Hg besides the sorption/complexation reactions. Associated with this objective, are the following hypotheses:

**Hypothesis 5A:** Photochemical reduction of Hg(II) or Hg-DOM complexes produces mass-independent Hg isotope fractionation that varies with a range of DOM isolates with varying compositions and functional groups.

**Hypothesis 5B:** Photochemical MeHg degradation also results in mass-independent Hg isotope fractionation that varies with different DOM isolates with varying compositions or DOM/Hg ratios.

**Task-Specific Results for Objective 5.**

**Task 5. Measure Hg isotope fractionation during photochemical reduction of Hg(II) and photochemical degradation of MeHg.**

**Subtask 5A and 5B. Isotope fractionation during photochemical reduction of Hg(II) and**

### **photodegradation of MeHg by various DOM and by low-molecular weight organic ligands.**

B. Gu and F. He focused on a series of laboratory experiments investigating photochemical oxidation of Hg(0) in the presence of natural organic matter (NOM). This was also potentially an important Hg transformation pathway within the East Fork Poplar Creek Ecosystem. Results from this work helped inform the interpretation Hg isotope signatures observed along the flow path of the EFPC (see Objective 1).

#### ***Photochemical Oxidation of Dissolved Elemental Mercury by Carbonate Radicals in Water.***

**[He et al., 2014] (OSTI ID: 1239560)**

Photochemical oxidation of dissolved elemental mercury, Hg(0), affects mercury chemical speciation and its transfer at the water–air interface in the aquatic environment. The mechanisms and factors that control Hg(0) photooxidation, however, are not completely understood, especially concerning the role of dissolved organic matter (DOM) and carbonate ( $\text{CO}_3^{2-}$ ) in natural freshwaters. Here, we evaluate Hg(0) photooxidation rates affected by reactive ionic species (e.g., DOM,  $\text{CO}_3^{2-}$ , and  $\text{NO}_3^-$ ) and free radicals in creek water and a phosphate buffer solution (pH 8) under simulated solar irradiation. The Hg(0) photooxidation rate ( $k = 1.44 \text{ h}^{-1}$ ) is much higher in the presence of both  $\text{CO}_3^{2-}$  and  $\text{NO}_3^-$  than in the presence of  $\text{CO}_3^{2-}$ ,  $\text{NO}_3^-$ , or DOM alone ( $k = 0.1\text{--}0.17 \text{ h}^{-1}$ ). Using scavengers and enhancers for singlet oxygen ( $^1\text{O}_2$ ) and hydroxyl ( $\text{HO}\cdot$ ) radicals, as well as electron paramagnetic resonance spectroscopy, we found that carbonate radicals ( $\text{CO}_3^{\cdot-}$ ) primarily drive Hg(0) photooxidation. The addition of DOM to the solution of  $\text{CO}_3^{2-}$  and  $\text{NO}_3^-$  decreased the oxidation rate by half. This study identifies an unrecognized pathway of Hg(0) photooxidation by  $\text{CO}_3^{\cdot-}$  radicals and the inhibitory effect of DOM, which could be important in assessing Hg transformation and the fate of Hg in water containing carbonate such as hard water and seawater [He et al., 2014].

#### ***Mercury isotope fractionation during photochemical oxidation in natural waters of East Fork Poplar Creek, Oak Ridge, TN. [Demers et al. In prep].***

A subset of the experiments from He et al. [2014] have been analyzed for Hg isotopes in order to characterize fractionation during these processes. Photochemical oxidation of Hg(0) to Hg(II) resulted in –MDF and +MIF of the oxidized fraction:  $\delta^{202}\text{Hg}$  values shifted 1.84‰, and  $\Delta^{199}\text{Hg}$  values shifted +1.07‰. Thus fractionation during photochemical oxidation in the presence of EFPC NOM resulted in a  $\Delta^{199}\text{Hg} / \delta^{202}\text{Hg}$  ratio of -0.5; note that Hg isotope fractionation diagnostic of photochemical oxidation has not been previously reported, and thus this result both fills knowledge gaps regarding Hg isotope systematics and will promote the assessment of photochemical oxidation in natural ecosystems.

#### **Publications:**

Two manuscripts have been published from this work, and four additional publications are anticipated:

Donovan, P.M., J.D. Blum, J.D. Demers, B. Gu, S. Brooks, and T. Peryam. 2014. Identification of multiple mercury sources to stream sediments near Oak Ridge, TN, USA. *Environmental Science & Technology*, 48, 3666-3674, doi 10.1021/es4046549.

- Accepted Manuscript publicly available through the Office of Science and Technical Information. (OSTI ID: 1239629)

He, F., W. Zhao, L. Liang, B. Gu. 2014. Photochemical oxidation of dissolved elemental mercury by carbonate radicals in water. *Environmental Science & Technology Letters*, 1, 499-503, doi 10.1021/ez500322f.

- Accepted Manuscript publicly available through the Office of Science and Technical Information. (OSTI ID: 1239560)

Demers, J.D., J.D. Blum, S.C. Brooks, P.M. Donovan, C.L. Miller, A.L. Riscassi, W. Zheng, B. Gu.

Mercury isotopes reveal in-stream processing and legacy inputs in East Fork Poplar Creek, Oak Ridge, TN., USA. *Submitting to PNAS Plus*.

- This manuscript is complete, and is currently being formatted for submission to *PNAS Plus*. This unpublished manuscript is available to Program Managers upon request.

Zheng, W., S. Ghosh, J.D. Demers, B. Gu, J.D. Blum, B.A. Bergquist, Fractionation of mercury isotopes during aqueous dark oxidation. *In prep*.

- This manuscript is complete, and is currently with coauthors for review.

Demers, J.D., J.D. Blum, P.M. Donovan, T. Mathews, and B. Gu. Linking the Hg isotopic composition of mercury in the aquatic food web back to the ecosystem source(s) of methylmercury production in East Fork Poplar Creek, Oak Ridge, TN. *In prep*.

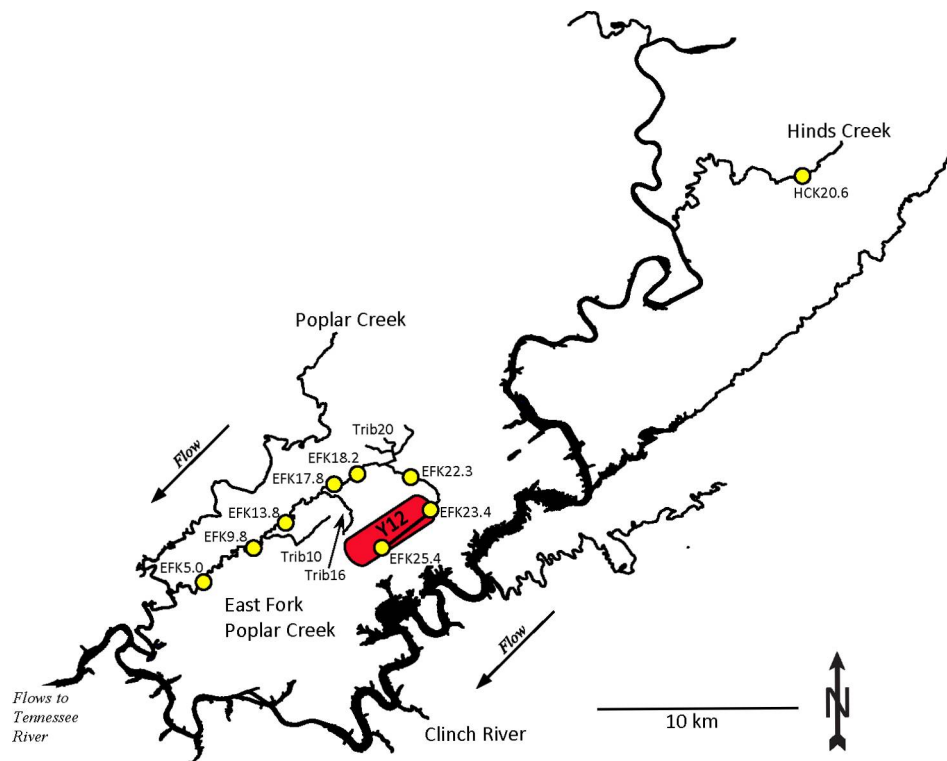
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Equilibrium Mercury Isotope Fractionation between Dissolved Hg(II) Species and Thiol-Bound  
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**Figure 1.** Map of sampling sites along the flow path of East Fork Poplar Creek (EFPC) in Oak Ridge, TN, USA. Sampling sites are identified by a three letter code corresponding to the stream, followed by the kilometers upstream from its confluence with Poplar Creek.



**Figure 2.** Concentration of (A) total particulate-bound mercury ( $\text{THg}_p$ ) associated with total suspended solids (TSS), expressed on both a volume ( $\text{ng/L}$ ) and a mass ( $\mu\text{g/g}$ ) basis; (B) total dissolved mercury ( $\text{THg}_d$ ); and (C) total dissolved methylmercury ( $\text{MeHg}_d$ ) in stream water during seasonal synoptic sampling of East Fork Poplar Creek, Oak Ridge, TN, USA.

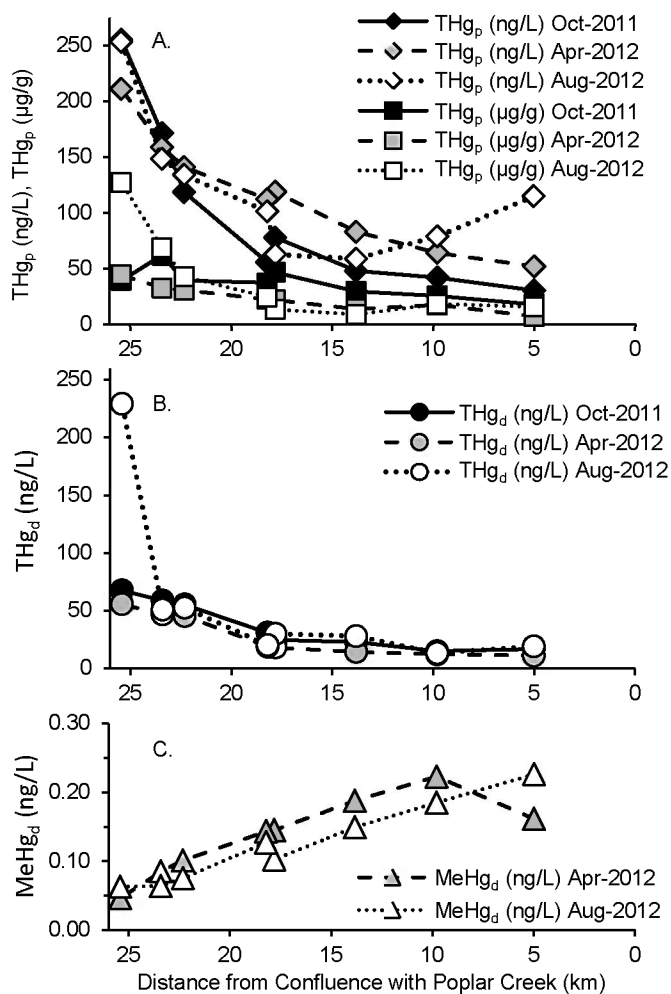


Figure 3. The isotopic composition of inorganic mercury (iHg) and methylmercury (MeHg) in fish tissue from Hg contaminated sites of East Fork Poplar Creek.

