Illinois-Indiana Sea Grant Final Report

A Spatio-Temporal Study of Methylmercury Biogeochemistry in Wetlands of the Southern Lake Michigan Watershed

Completion Date: August 31, 2008

Robert J.M. Hudson

Department of Natural Resources and Environmental Sciences
University of Illinois at Urbana-Champaign

W-503 Turner Hall
1102 S. Goodwin Avenue, Urbana, IL 61801
Phone: 217-333-7641
Fax: 217-244-3219
Email: rjhudson@illinois.edu

Robert A. Sanford

Department of Geology
University of Illinois at Urbana-Champaign

245 Natural History Building
1301 W. Green St.
Urbana, Illinois 61801
Phone: 217-244-7250
Fax: 217-
Email: rsanford@illinois.edu

Abstract

Mercury pollution in aquatic ecosystems occurs from a combination of atmospheric deposition, a process that tends to deposit Hg relatively evenly across a region the size of the Grand Calumet waershed, and legacy contamination, which can be distributed very unevenly due to geological processes or to point discharges. In addition, the variability in biological and geochemical factors between particular aquatic ecosystems can lead to their having very different levels of methylmercury (MeHg) in the water even with comparable rates of Hg inputs from deposition and legacy contamination. Since MeHg is the only form of Hg that biomagnifies in food webs, MeHg levels in biota can also exhibit a high degree of spatial variability. Consequently, predicting what ecosystems within a landscape will have the greatest problems is challenging for environmental managers, who are simultaneously seeking to maximize the human use of natural resources and protect humans and wildlife. The goal of this project has been to apply state-of-the-art methods in Hg analysis in support of this management objective.

A significant part of this study has involved analytical method development. The most important advance made was the development of a new method of isolating MeHg from freshwater samples. An inter-comparison of the new and standard methods that was also conducted in this study shows that the new method has a significantly greater ability to extract MeHg in about two-thirds of the samples examined. This result should have important implications for the field of MeHg analysis. In addition, we refined an existing sediment digestion method for measuring MeHg in sediments using our new analytical system. We are able to obtain very precise measurements with the refined method.

Since it is well established that wetlands can be major sources of MeHg to streams and lakes, we performed a field investigation of the distribution of MeHg in wetland surface waters of the Grand Calumet region. A survey of 29 sites was conducted in July 2006 and nine of these sites were revisited 3 times in a seasonal study conducted during 2007. The survey results are consistent with previous geochemical studies indicating that *p*H and DOC are the water quality variables that exert the main control over dissolved MeHg. We also found that MeHg levels in fish do not exceed EPA standards in most wetlands. However, analysis of our data in combination with the National Descriptive Model of Mercury in Fish suggests that two sites – one industrial and one in a natural area in Gary – are likely to have fish with Hg levels that exceed EPA standards.

Goals and Objectives

In a previous Sea Grant project, the Principal Investigator's research group developed a novel system for analyzing methylmercury (MeHg) in environmental samples. The goals of this project were to further develop new sample preparative chemistries for isolating MeHg from environmental samples for analysis using our new analytical system and to apply them to study the impacts of Hg pollution in a region known to be affected by a century of elevated mercury inputs from the atmosphere. Our aim has been to broaden our understanding of Hg biogeochemistry in the region so that we can:

a) Measure the levels of total Hg and MeHg in the surface waters of wetlands across the Grand Calumet region of northwestern Indiana.
b) Measure the concentrations of total and MeHg in wetland sediments – the main repositories for legacy Hg contamination in these wetlands.

c) Measure the concentrations of total and MeHg in porewaters of wetland sediments – the main route for MeHg transport from the sediments to the overlying waters – in the region.

d) Assess the MeHg levels in fish from wetlands with permanent open water.

In addition to the measurements implied by the above goals, our aim is to synthesize the various types of data obtained here using simple models as a basis for extrapolating to other wetlands in the region. Our goal is to use this information to assess the biogeochemical factors that govern the impacts of Hg contamination of wetland ecosystems.

Narrative Report

Managing the effects of Hg pollution in watersheds depends on knowing the distribution of locations with problematic levels of methylmercury (MeHg) and being able to quantify (model) the relationship between i) legacy Hg in sediments and ii) Hg in atmospheric depositional and dissolved MeHg, the form of mercury that accumulates in aquatic food webs. At the landscape scale, it is well established that wetlands can be major sources of MeHg to streams and lakes. Thus, the distribution of MeHg in wetland waters of the Grand Calumet region, and its relationship to legacy Hg in sediments is vital for evaluating the health of aquatic ecosystems in the region.

Note that the emphasis on MeHg in water in this project represents a change from our proposed study plan, which emphasized MeHg in sediments. This shift was made possible by our technological breakthrough in measuring MeHg in water. As dissolved MeHg is more directly relevant for predicting MeHg accumulation in fish, this new approach is actually preferable for

the applications this project was intended to support, namely evaluating factors that control the accumulation of Hg in biota in the Grand Calumet.

The work performed over the project period can be divided into three main tasks. First, we continued obtaining samples for our seasonal study of nine wetland sites in the region and analyzed the water samples (plus some from the previous year) for their contents of total and methylmercury (THg and MeHg), dissolved organic carbon, and other constituents. Second, we conducted a comparative study of methods for extracting MeHg from sediments. Finally, we conducted a detailed inter-comparison of our new method for analyzing MeHg in water with the current standard method, distillation/ethylation. The results of these efforts are summarized here.

1. Methylmercury Analysis

Accurate measurements of methylmercury (MeHg) distributions and cycling rates in watersheds and aquatic ecosystems are essential for quantitatively assessing/modeling the impacts of anthropogenic Hg deposition. Analyzing methylmercury (MeHg) in samples from freshwater ecosystems is extremely challenging since it typically comprises a small fraction of the total Hg and occurs at such low concentrations, e.g., at parts per billion levels in sediments and sub-parts per trillion levels in water. The analytical methods capable of meeting this challenge all involve two main steps: sample preparation (where one isolates MeHg from the sample matrix and preconcentrates it) and instrumental analysis (where one quantifies the MeHg in the prepared sample using a particular combination of analytical instruments). Since the chemistry of any preparative method must be compatible with the analytical system used, the two tend to evolve together.

The Standard Method

The analytical approach that currently underlies nearly all analysis of MeHg in environmental samples was first published in 1989 by Bloom (*1*). It relies on ethylation of the $MeHg^+$ ion in aqueous solution, pre-concentration of the MeHgEt by purge and trap, separation of MeHgEt from ethylated Hg^{II} and Hg^0 by gas chromatography, and quantitation of the MeHgEt using CVAFS or in some cases ICP-MS (*2*). Although the analysis is complicated, ethylation-GC has made possible the recent explosion of research into the biogeochemistry and ecotoxicology of MeHg in aquatic systems.

Since the ethylation step is inhibited by ligands that strongly complex MeHg, such as the reduced-sulfur compounds found in samples from freshwater ecosystems, the MeHg in such samples is generally isolated from the sample matrix by combining either i) water vapor distillation or ii) acid leaching/solvent extraction with ethylation (*3*). Extracting MeHg from natural ligands, the strongest of which are generally thought to be thiolic moieties (RSH) in natural organic matter and therefore associated with sediment organic matter (SOM) or dissolved organic matter (DOM), is difficult because their high affinity makes ligand exchange reactions difficult and because one cannot oxidize the DOM without risking the same fate for the MeHg. Thus, most sample preparation protocols for extracting MeHg from environmental samples have employed a combination of proton-assisted dissociation from thiols and complexation by added ligands (X^-), as summarized in the reactions below:

$$\begin{aligned} MeHg-SR + H^+ &\longleftrightarrow MeHg^+ + HSR \\ MeHg^+ + X^- &\xleftrightarrow{K_X} MeHgX^0 \end{aligned} \qquad (1)$$

In distillation/ethylation, water vapor distillation of preserved samples amended with H_2SO_4 and KCl or KBr strips the $MeHgCl^0$ ($K_X = 10^{5.2}$) or $MeHgBr^0$ ($K_X = 10^{6.5}$) formed via the above reactions from the sample. The volatilized $MeHgX^0$ is then trapped in aqueous solution, ethylated, and pre-concentrated by purging to a Tenax trap. In acid leaching/solvent extraction, the $MeHgX^0$ is extracted to the organic solvent and then transferred into water for ethylation.

Hg-Thiourea Complex Ion Chromatography

In conjunction with a previous Sea Grant project, the PI's group developed a new analytical system for MeHg analysis – Hg-thiourea complex ion chromatography with CVAFS detection or HgTU/IC/CVAFS (*4*) – with capabilities that are comparable to ethylation/GC/CVAFS . The method separates thiourea-bound Hg^{2+} and $MeHg^+$ based on charge differences using ion chromatography (Fig. 1). Once separated, the two Hg species are oxidized, reduced to Hg^0, and transferred to a stream of Ar gas for quantification using CVAFS (Fig. 2).

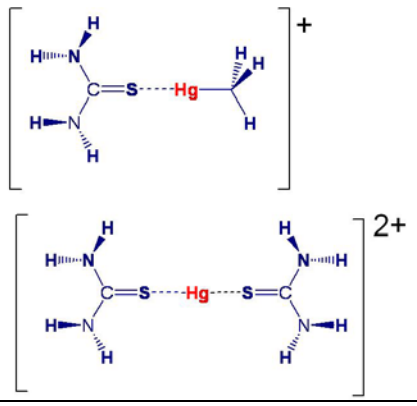


Figure 1. Molecular structure of Thiourea complexes of methylmercury (MeHg) and mercuric mercury (Hg^{II}).

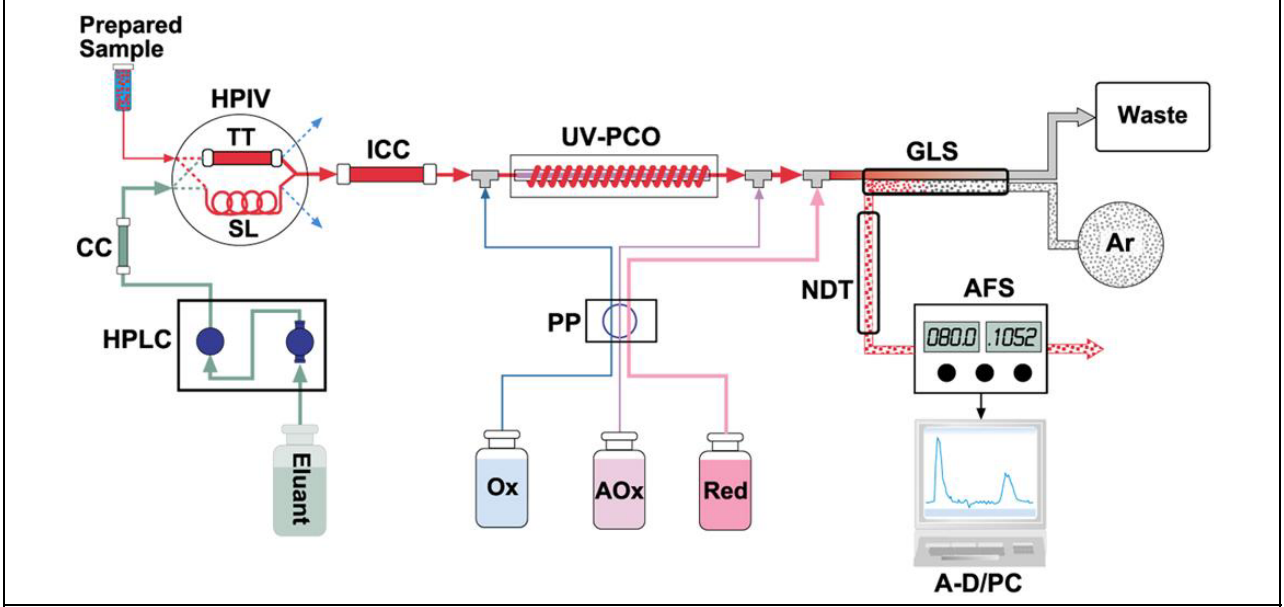


Figure 2. Schematic diagram of Hg-thiourea complex ion chromatography system (*4*).

From the perspective of environmental analysis, the most important difference in its chemistry from ethylation/GC is that *samples prepared for HgTU/IC can contain high levels of very strong ligands*, while ethylation of MeHg requires a aqueous solution chemistry with relatively weak MeHg ligands. Thus, nearly any sample preparation used with ethylation/GC is compatible with HgTU/IC, while the opposite is not true. Preparative methods for the HgTU/IC system have been developed and validated using certified reference materials for biological tissues (*5*) and sediments (*6*) and results obtained using the system have excellent precision. A new method for preparing water samples has been developed as well (*7*). These methods are described next.

2. Sample Preparation Procedures

New sample preparation methods for two environmental sample types – sediments/soils and water – that can be coupled with HgTU-IC were developed as a part of this study. A novel

method for a third essential sample type – biological tissues – was recently developed by a former student who was supported by a previous Sea Grant-funded project. Since these methods were used for the field study, we present a brief description of them and their validation here.

A. Sediment/Soil Methylmercury Determination by Hg-Thiourea Complex Ion Chromatography with On-line Cold Vapor Atomic Fluorescence Spectrometry

Brian R. Vermillion, Christopher W. Shade, and Robert J.M. Hudson

Submitted to *Analytica Chimica Acta* in Sept 2008.

For this project, it was necessary to develop a new method for analyzing MeHg in sediments. We chose to investigate two sulfuric acid-based leaching/solvent extraction procedures that have been conventionally combined with ethylation/GC by coupling them to our new system for Hg speciation analysis (Hg-TU/IC/CVAFS) and applying the protocols to analyzing sediment reference materials and samples taken from the field. The procedure's steps include: i) a simultaneous sediment leach using solutions of H_2SO_4 (0.75 – 0.95 *M*), KBr or KCl (1-2 *M*), and $CuSO_4$ (0.1 – 0.2 *M*) and solvent extraction using toluene, followed by ii) back-extraction into acidic thiourea solution, and iii) methylmercury quantification via direct injection of the backextract into the Hg-TU/IC/CVAFS system.

Method Details

Acid Leaching/Solvent Extraction Step: Two sulfuric acid leaching schemes were derived from standard methods that have been successfully coupled to ethylation/GC (Fig. 3). The first leaching solution, referred to as **H_2SO_4/KBr**, is prepared by mixing stocks of H_2SO_4/KBr (0.9 *M*/1.5 *M*) and $CuSO_4$ (1 *M*) in 5+1 proportions. The second leaching solution, referred to as

H_2SO_4/KCl, is prepared by mixing stocks of H_2SO_4 (9 *M*), KCl (2.4 *M*), and $CuSO_4$ (1 *M*) in 0.4+3.0+0.4 proportions. To leach a sediment, a defined volume of leaching solution – 6 mL of **H_2SO_4/KBr** or 3.8 mL of **H_2SO_4/KCl** – and 10 mL of toluene (Fisher Certified A.C.S.) are added to sediment (up to 1-g wet sediment or the specified amounts of sediment reference materials) in a 40-mL I-Chem vial with a PTFE lined lid and gently mixed on an orbital shaker for 2 hours.

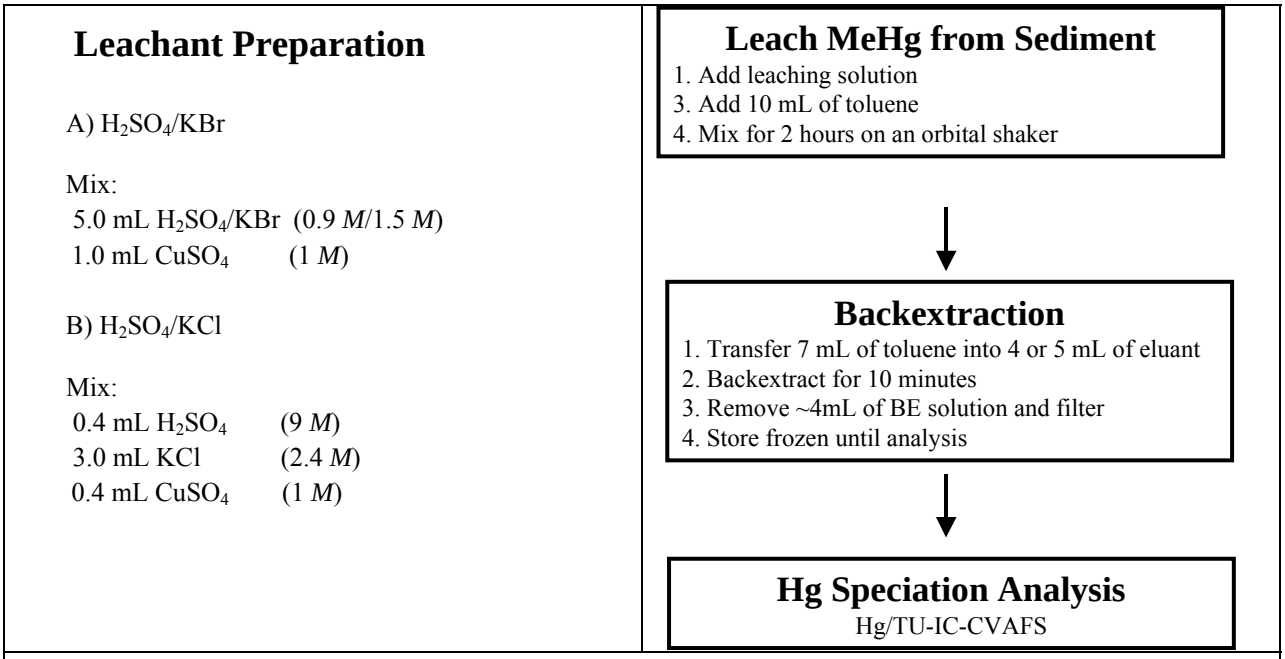


Figure 3. Acid Leaching/Solvent Extraction protocol for preparing sediment samples for analysis by HgTU-IC/CVAFS.

Back-extraction Step: After 2 hours of leaching, 7 mL of the toluene layer containing extracted MeHgX is transferred into 15-mL centrifuge tubes containing 5 mL of eluant – cleaned 0.2 *M* TU (Acros A.C.S.), 1 *M* HCl (TraceMetal Grade, Fisher), 1.75 *M* Acetic Acid (Fisher Certified A.C.S. – and gently mixed for ~10 minutes to back-extract the MeHg from the toluene. An aliquot, ~4 mL, of the back-extract is then filtered (0.45-µm PTFE syringe filter) to ensure

that no particles are transferred. The filtered solution is then ready for analysis either by direct sample loop injection or if desired pre-concentration onto a thiol-functionalized resin.

Analysis Step: Direct injection of sample limits the amount analyzed, but saves considerable time over on-line pre-concentration. To directly inject the prepared back-extract, one only needs to rinse the sample loop with ~2 mL of eluant to prevent carryover of MeHg between samples before injecting the sample. If it is neccessary to pre-concentrate onto the on-line thiol resin, the recommended loading procedures require buffering the sediment extract to *p*H 3.5 and then pumping eluant (2 *mL*), borate buffer (2.5 *mL*), and larger volumes of sample (up to 4 *mL*) through the online resin. All sample analysis was conducted in HEPA filtered laminar flow hoods in clean laboratories designated for Hg analysis (M-223 Turner Hall).

Tests of Artifact Formation and Matrix Spike Recovery: Tests to determine MeHg matrix spike recoveries were performed by adding MeHgOH standards directly onto the sediment at levels that would approximately double the expected ambient [MeHg] (0.2 – 40 ng). Tests to determine artifact formation were performed by adding $Hg(NO_3)_2$ (CertiPrep) standards directly onto the sediment at levels that would increase the $[Hg_T]$ by 1, 2, and 5 times. The added MeHg and Hg^{II} were delivered to sediments in a solution of 48 mM HCl (TraceMetal, Fisher) and allowed to equilibrate for 15 – 30 minutes before adding the leaching solutions.

Results

Processed Standards: MeHg in standard stocks added to leaching solutions were completely recovered. However, it should be noted that while the solvent extraction step is quantitative, only 70% of the toluene is bacextracted and 20% of the backextract is analyzed in the standard

procedure. Thus, injected extracts contain at most only 14% of any MeHg in the original samples.

In order to determine if any reagents used in the analytical process are capable of causing MeHg to form from Hg^{II} during the procedure, 1 μg of Hg^{2+} was spiked into three separate aliquots of leachant at various stages of preparation: i) before extraction with toluene, ii) after extraction with toluene but before backextraction, and iii) during the backextraction step. Production of MeHg was not observed as a response to the reagents used or the preparative steps employed for leaching sediments.

Reference Sediments: When applied to the IAEA-158 and IAEA-405 reference sediments, both leaching methods yielded precise results that were within the certified range (Table 1). Consistent with the good agreement, the matrix spike recoveries indicate that quantitative recoveries were obtained as well. Although the spike recoveries were also quantitative with BCR-580 reference sediment (Table 1), only the H_2SO_4/KCl leaching solution yielded results that were within the certified range. With H_2SO_4/KBr leaching, we found that BCR-580 had 60.0 ± 0.6 ng g^{-1} (Table 1).

Interestingly, the lower value observed with H_2SO_4/KBr leaching agrees relatively closely with results determined by Hintelmann (*8*) using the same acid leaching/solvent extraction protocol, but with species-specific isotope addition techniques (SSIA) and ICP-MS detection. The SSIA work included a correction for matrix spike recovery and a test for artifactual MeHg formation (none was observed upon addition up to 2 μg of isotopically labeled Hg^{II} before leaching/extraction) yet was even further outside the certified range than our value.

We also note that there was a marked difference in results generated using the two different leaching solutions. The difference suggests that either: i) MeHg is not quantitatively isolated

from BCR-580 when prepared by H_2SO_4/KBr leaching, or ii) gross amounts of artifactual MeHg are produced when preparing BCR-580 by H_2SO_4/KCl leaching. One hypothesis to explain the difference is that the methylation of ambient inorganic Hg^{II} may be less favorable in the H_2SO_4/KBr treatment since formation of Hg^{2+}-bromide complexes is more thermodynamically favorable than Hg^{2+}-chloride complexes. The lack of observable artifact formation in the H_2SO_4/KCl treatment would have to result from saturation of the methylating agents in the Cl^- treatment (a plausible suggestion since Hg_T is so high for this sediment).

Table 1: Results from analyses of sediment reference materials and matrix spike recovery and artifactual methylation tests using H_2SO_4/KBr and H_2SO_4/KCl acid leaching/toluene extraction method.

Reference Material	Certified MeHg (ng g-dw^{-1})	Measured MeHg (ng g-dw^{-1})		Matrix Spike Recovery (%)		Artifactual Methylation[b]	
		KBr	KCl	KBr	KCl	KBr	KCl
IAEA-158	1.38 ± 0.27[a]	1.37 ± 0.03	1.35 ± 0.02	97.7	94.0	ND	ND
IAEA-405	5.49 ± 0.53	4.96 ± 0.03	4.98 ± 0.12	102.5	109.6	ND	ND
BCR-580	75.5 ± 3.7	60.0 ± 0.6	76.5 ± 0.5	99.7	99.4	ND	ND

[a] Mean of reported measurements and is not yet a certified value.
[b] Result of adding $HgCl_2$ to raise total sediment Hg by factors of 1, 2, and 5 relative to ambient. ND: No increase in MeHg detected upon addition of $HgCl_2$.

Validation with Field Samples: A variety of sediments collected in July 2006 from wetlands located within the field study area were analyzed. The close agreement between results obtained using the two leaching methods (Figure 4), despite the differences in the affinities of the added ligands for MeHg (reaction 2), suggests that both methods completely extract MeHg. Although not a definitive proof, the essentially complete (97.8 – 109.0 %) recoveries of matrix spike additions for both leaching methods over a wide range of SOM contents (5.7 – 72.9 %) support this conclusion as well. Note that other workers using ethylation/GC have shown that the efficiency of H_2SO_4/KBr-toluene leaching protocol is independent of total organic carbon and sulfur (*9*).

The precision of the method was investigated by analyzing MeHg concentrations in a variety of wetland sediments from the Grand Calumet region. The observed concentrations ranged from 0.14 – 2.58 ng g-dw^{-1}, and were positively correlated with OM content and total mercury (Fig. 5). The relative standard deviation of sample replicates varied from 0.1 to 7.9 % (average 2.5%) regardless of the preparative method.

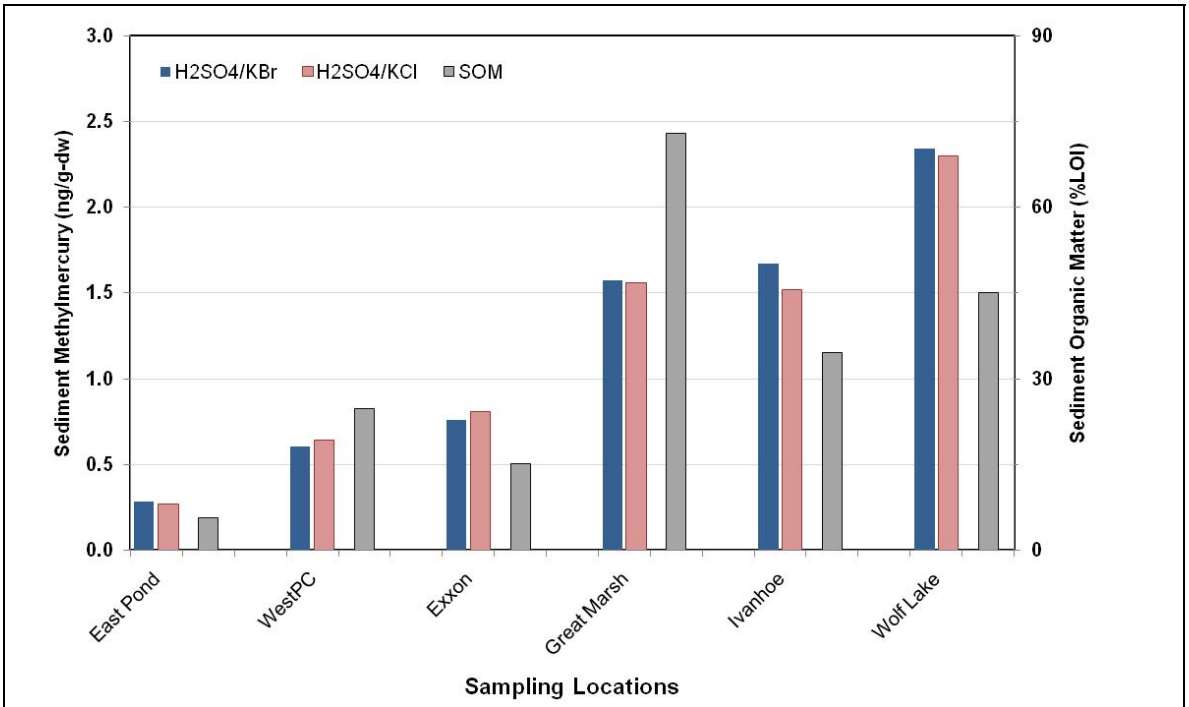


Figure 4. Comparison of sediment MeHg concentrations measured by the two different acid leaching/solvent extraction procedures to total sediment Hg at a variety of sites in the Grand Calumet area.

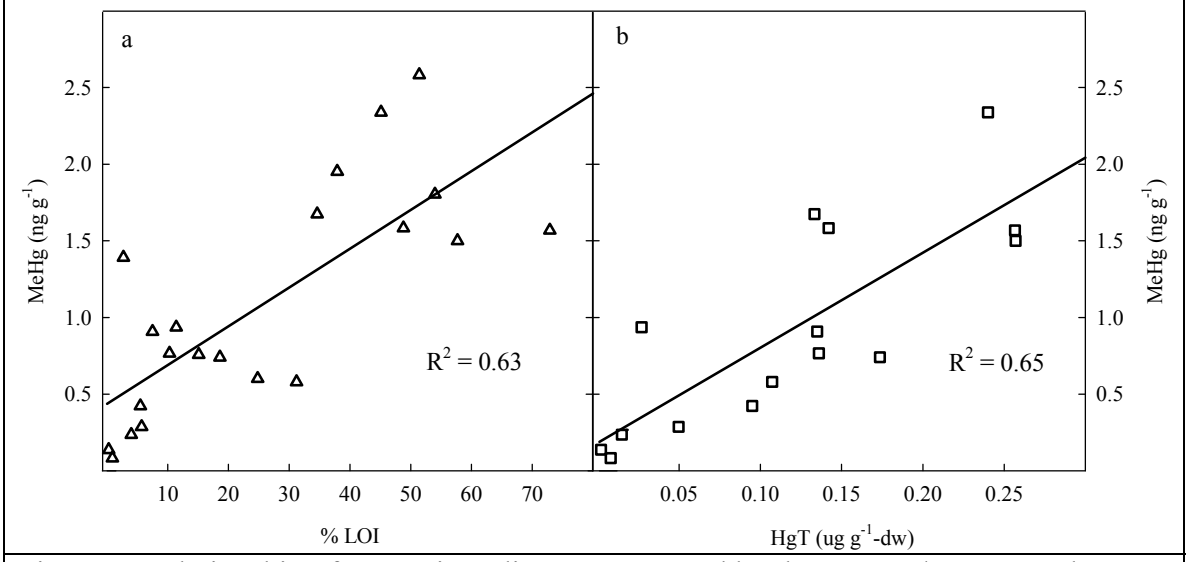


Figure 5. Relationship of MeHg in sediments measured by the H2SO4/KBr procedure to a) sediment organic matter content (%LOI) and b) total sediment Hg (µg g^{-1}).

B. Thiourea catalysis of MeHg ligand exchange between natural dissolved organic matter and a thiol-functionalized resin: a novel method of matrix removal and MeHg preconcentration for ultratrace Hg speciation analysis in freshwaters.

Brian R. Vermillion and Robert J.M. Hudson

Analytical and Bioanalytical Chemistry **2007**, *388*, 341–352.

In the most widely used analytical approach for measuring MeHg in water samples, codified as USEPA Draft Method 1630 (*10*), one isolates MeHg from the matrix by distillation and then pre-concentrates it via ethylation and purge/trap of the volatile MeHgEt species. Next, the species mass is quantified using gas chromatography with detection by CVAFS. This approach, referred to simply as distillation/ethylation (D/E), is now the standard for environmental work because i) its recoveries are the best of the available methods, ii) it has manageable levels of

artifact, and iii) it is highly sensitive, with method detection limits as low as 0.006 ng L^{-1} reported. However, the distillation step is still susceptible to matrix interferences, with spike recoveries of <80% reported for humic-rich and/or anoxic waters. The main alternative that has been coupled with ethylation/GC/CVAFS, acid leaching/solvent extraction, is not as potent as distillation at isolating MeHg from DOM-rich waters.

Solid phase extraction is an another alternative method that in principle should succeed due to the highly favorable equilibria for MeHg adsorption at the high concentrations of thiols that occur within resins. Law (*11*) developed the first approach to solid phase extraction of dissolved MeHg and Hg^{II} using a thiol-functionalized chelating resin, but neither this nor any of the succeeding resins that have been suggested for use in Hg analysis have achieved widespread use as a means of pre-concentrating dissolved MeHg. A few analysts use direct pre-concentration of MeHg from natural waters onto sulfhydryl cotton fibers (SCF) (*12*) and thiol-based DGT probes (*13*) are arguably forms of SPE, but these methods have limitations that prevent them from measuring total dissolved Mehg. Part of the difficultly may lie in leaching MeHg from the SPE resins into a solution that still permits ethylation of MeHg (see above).

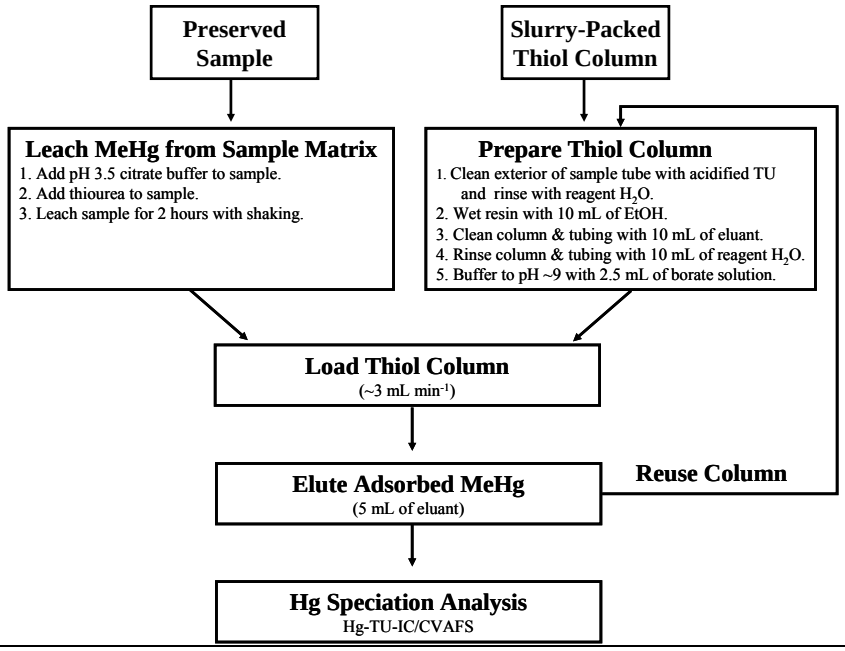
In practice pre-concentration with such sorbents has not proved to be consistently effective. With SCF, reported MeHg recoveries from natural water samples are diminished in samples with high DOC, with reported values ranging from 47-69% in lakewater containing 5 mg-DOC L^{-1} (*12*) to ~80% at 20 mg-DOC L^{-1} [20] depending on amounts of sorbent used. Since thermodynamics favors adsorption onto sufficiently strong resins, it stands to reason that the failure of these sorbents to satisfactorily isolate MeHg from the matrices of environmental samples results from slow kinetics of MeHg exchange from DOM complexes to the sorbents.

In this method of preparing samples for MeHg speciation analysis, thiourea serves as an aqueous chaperone for MeHg between natural dissolved organic matter (DOM) and thiolated resins via the following reactions. Thiourea, or TU (Fig. 1), is a small molecule with a high affinity for MeHg. Thus, when added to a water sample to initiate the reaction step, it rapidly diffuses into any colloidal aggregates present and reacts with MeHg to release it from the DOM. Then, because TU has a lower affinity for MeHg than a thiol resin does, it readily exchanges its MeHg to the thiol-resin during the SPE step of sample preparation. Since the reaction step can be given several hours rather than the few seconds normally allowed for dissolved MeHg to react with a resin during SPE, the thiourea facilitates the transfer of MeHg from DOM to resin. Below, we describe this process for MeHg pre-concentration in some detail.

Method Details

MeHg Analysis by Thiourea-Catalyzed SPE: The most relevant aspects of our method for preparing water samples are described in Fig. 6. Preserved samples are buffered to pH 3.5 using Na_3citrate (Fisher) and mixed with sufficient volumes of cleaned 260 mM thiourea (TU) (Acros) solution to attain a final TU concentration of ~42 mM. After equilibration overnight, solid-phase extraction (SPE) onto a custom, thiol-functionalized divinyl benzene (DVB) resin is effected by pumping treated sample at a rate of ~3 mL min^{-1} through a glass column containing the resin (Fig. 6B). The pre-concentrated MeHg is eluted from the resin using 4 mL of eluant (0.2 *M* TU, 1 *M* HCl, 1.75 *M* Acetic acid). The eluant containing the sample MeHg is then buffered to pH ~ 3.5 and loaded onto the on-line thiol column of the Hg-thiourea complex ion chromatography system.

A)



B)

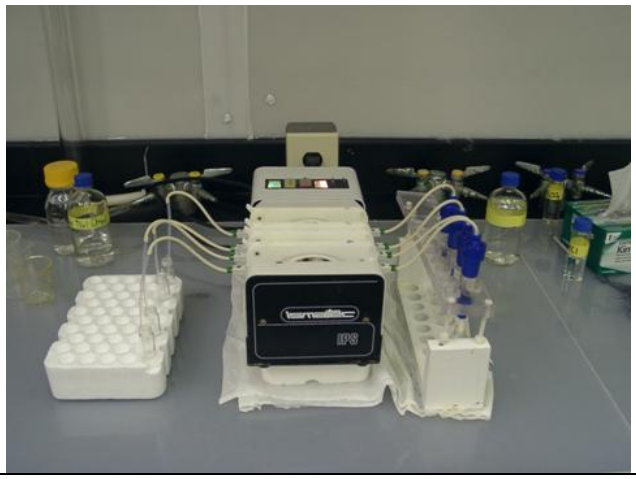


Figure 6: A) Procedure for MeHg pre-concentration by TU-SPE. B) Apparatus for Thiourea-catalyzed SPE. Peristaltic pump transfers solutions from the vials on the left to the tops of the SPE columns on the right.

Reagent Preparation: All reagent solutions are prepared from high-purity water (Milli-Q^{TM}, Millipore) in borosilicate glass bottles cleaned with hot 6 *N* HCl (Fisher Certified A.C.S. *Plus*). MeHg calibration standards were made daily in cleaned eluant from a stock solution of 1 µg-Hg mL^{-1} MeHgOH (Brooks Rand) in glass volumetric flasks that had been cleaned overnight in ~10

% Trace Metal Grade HCl (Fisher). The standards typically contained 0, 1, 5, 10, 25 and 50 pg-Hg mL^{-1}.

The procedure for preparing thiol-functionalized resin was developed in the P.I.'s lab by Shade (unpublished). Briefly, brominated polydivinylbenzene (pDVB, 30-70 μm mesh size) base gel (Jordi Associates) is placed in 0.08 M Na_2S solution and refluxed gently under oxygen-free N_2 gas for ~24 h. The functionalized resin is then soxhlet-extracted with MeOH for ~24 h to remove organic impurities. The resin can be stored at 4 °C in 0.15 M ascorbate solution for up to 6 months without noticeable loss of function before use in on-line thiol traps, which are typically used for two consecutive days. The resin in the off-line SPE columns are typically used for multiple samples processed on one day only.

Results

Adsorption and Elution: In order to evaluate these processes under low-pressure conditions, 40 mL volumes of *p*H 3.5 solution with [TU] = 12, 23, and 42 mM – were spiked with 16 or 18 pg of MeHg and pre-concentrated onto thiol columns. Upon elution, the MeHg was fully recovered (98.9±1.5 %, N=14). Since the concentration of $MeHgTU^+$ exceeds that of all other MeHg species by a factor of $10^{6.9}$ under these conditions and the residence time of the solution in the thiol column is only ~2 s, $MeHgTU^+$ must undergo a rapid ligand exchange reaction with the resin thiol groups:

$$MeHgTU^+ + R_{RESIN}SH \overset{fast}{\rightleftharpoons} R_{RESIN}S - MeHg + TU + H^+ \qquad (2)$$

Elution of MeHg from the thiol column with system eluant – at pH = 0 and [TU] = 200 mM – yields complete desorption in the first 2 mL, suggesting that the desorption reaction can be rapid under these conditions (data not shown). To be certain of consistent recoveries during routine sample preparation, eluant volumes of 5 mL were routinely collected. For the 10- to 40-mL sample volumes typically used here, this corresponds to a pre-concentration factor of 2-8.

Direct Adsorption from Solutions containing DOM: In contrast to the efficient ligand exchange of TU-bound $MeHg^{+}$ with the resin thiols, MeHg adsorption efficiencies from samples containing natural DOM were highly variable despite the favorable equilibria. In solutions containing Suwanee River Humic Acid (SRHA), only 10% of the dissolved MeHg was recovered at [SHRA] of 25 mg L^{-1} versus 83 % at 1 mg L^{-1} and 89 % at 0 mg L^{-1}. Such a high interference from DOM is unacceptable for an environmental analytical method since DOM ranges from 1 to >100 mg L^{-1} in freshwater systems.

Method Detection Limit (MDL). The MDL could not be determined from replicate analyses of blank samples because they contained levels of MeHg that were too low to quantify. The undetectable system blank results from being continuously cleaned with eluant, while the zero pre-concentration blank results from consistently cleaning the apparatus between samples. Note that Emteborg et al. [15] also reported an undetectable MeHg SPE-preconcentration blank when pre-cleaning with acidified TU.

In the absence of a quantifiable blank, the MDL was determined from the variability in MeHg measurements for replicate, low level standards. Seven 40-mL standards containing 1 pg of MeHg at a [TU] of 13 mM were pre-concentrated and analyzed with a mean recovery of 97 % and a standard deviation of 0.097 pg (RSD = 10%), resulting in an absolute MDL of 0.29 pg. As

expected, the absolute MDL with pre-concentration is slightly higher than the absolute LOD of our system (0.18 pg). The concentration-based MDL is ~0.007 ng L^{-1} for 40-mL samples. The MDL reported here is lower than the MDL of any other SPE method that we are aware of and is essentially equivalent to the 0.006 ng L^{-1} MDL reported for ethylation/GC with 50 mL sample volumes.

Artifacts: Extant MeHg pre-concentration techniques, including distillation/ethylation (15) and solid phase extraction (*16*), are known to produce artifactual MeHg in aqueous samples. This artifact is typically less problematic for water than for sediments since the MeHg: HgT ratio in water (5-20%) is generally much greater than in solid phases (<1%). Nevertheless, it remains necessary to investigate potential artifacts when developing a new method.

MeHg formed during pre-concentration was quantified by spiking 10^{-1} - 10^{5} ng of $Hg(NO_3)_2$ into 25-mL aliquots of a Grand Calumet wetland surface water (Hg_T = 10.45 ng L^{-1}, DOC = 16.1 mg L^{-1}). The formation of artifactual MeHg was not detectable until the total Hg (Hg_T) concentration in the sample exceeded 4000 ng L^{-1}. This 100 ng Hg^{II} spike yielded only 5 pg of artifactual MeHg, i.e., the percentage of Hg^{II} methylated (or methylation potential) was 0.005%. At the highest Hg^{II} addition, the artifactual methylation reached 0.007%. During ethylation/GC, typically 0.005 to 0.1 % of the Hg^{II} spike is methylated (*17*), an amount considered inconsequential in uncontaminated sites. For the wetland sample analyzed here, the percent artifact falls in the range of 0.66 to 0.88 %, which compares well to the typical values of 0.2 – 17.5 % found for distillation of natural aqueous samples, and is considered inconsequential.

The low tendency of this method to form artifactual MeHg is expected because i) a strong ligand (TU) known to inhibit alkylation of Hg^{II} (13) is added to the sample, ii) the sample is not heated as in distillation, and iii) no artificial alkylating agents that can themselves spuriously

generate MeHg are added (15). Note that Celo et al. (*16*) observed very high methylation – up to 40% of Hg^{II} – when directly pre-concentrating samples onto sulfhydryl-functionalized cotton fibers. However, in contrast to the present method, they did not add strong Hg^{II}-chelators to the sample nor did they treat their sorbent to remove potential alkylating agents.

Although we did not test a wide range of conditions, the very low artifacts observed herein occurred at the low end of the range of [TU] tested; even less artifact is expected at higher [TU]. At low DOC, artifact formation should be low since TU should more rapidly complex the MeHg and less alkylating agent – DOM – should be present. Although high DOM conditions could be more conducive to artifact formation, our results at ~16 mg DOC L^{-1}, which is richer in DOM than most natural surface waters, suggest that further tests, although needed, are unlikely to find high artifactual MeHg formation.

Results for Environmental Samples: The present method was applied to a variety of wetland water samples collected in July and September of 2006 as a part of ongoing field studies of Grand Calumet wetlands and an agricultural watershed in Illinois. Based on the recoveries for high DOC marshwater, a 2-h leaching step with TU concentrations of 12 mM for low DOC samples and >23 mM for high DOC (> 25 mg L-1) samples was adopted. The results of QA/QC analyses – duplicates and spike recoveries – performed for the fieldwork are reported here (Table 4). With one exception, full recovery (> 90 %) was attained on every sample. The exceptional sample was collected from a unique wetland that was highly contaminated with wastes from nearby petrochemical facilities. For this sample, the spike recovery was 61 % for 2-h and >90% for 8-h leaching steps. The precision of sample duplicates ranged from 2.5 – 11.7 % RSD (average 6%), which is typical of values reported for other methods of MeHg analysis.

Table 2. Quality Control Data for TU-SPE conducted using samples from Grand Calumet watershed and Piasa Creek.

Sample Type	[DOC] (mg L^{-1})	[HS^-] (uM)	[TU] (mM)	[MeHg] (ng L^{-1})	RSD[c]	% Recovery[d]
River Surface Water[a]	1.9		18	0.07		99.8
	2.9		18	0.036	11.7	99.2
Wetland Surface Water	7.4		12	0.132	3.8	93.1
	11.4		12	0.067	4.9	101.9
	14.8		12	0.086	5.4	99.9
	19.3		12	0.053		95.7
	23.5		12	0.172	9.6	91.6
	36.4		42	0.431	2.5	90.7
	42.7		23	0.150	3.7	94.6
	55		42	0.950		100.5
	55.4[b]		23	0.449		90.7
Landfill Leachate	68.7		12	0.478		100.0
Wetland Porewater		3.8	33	0.109		99.4
		3.15	59	0.233	4.1	90.6

A. Total Dissolved Methylmercury in Freshwaters: An Inter-comparison of Water Vapor Distillation and Thiourea-Catalyzed Solid Phase Extraction

Robert J.M. Hudson, Brian R. Vermillion, Joy Zhu, and Holger Hintelmann

Submitted to *Environmental Science and Technology* in September 2008.

Herein, we report the first inter-comparison of the most sophisticated variant of the standard procedure used for analyzing MeHg in water samples – species specific isotopic analysis using distillation/ethylation-GC coupled with ICP-MS detection (D/E-ID) – and a radically different method of extracting dissolved MeHg from water samples – thiourea-catalyzed solid phase extraction (*4*) or TU-SPE (Figure 7). Because the two methods have similar MDL and other performance metrics and were applied to samples from freshwater ecosystems in the Great Lakes basin, the comparison has direct environmental relevance. Investigations of the causes of the

observed inter-method differences were conducted as well. In particular, both methods were used to analyze MeHg in distillation residues, the unanalyzed remnants of distilled samples, for evidence of an undistillable fraction of MeHg.

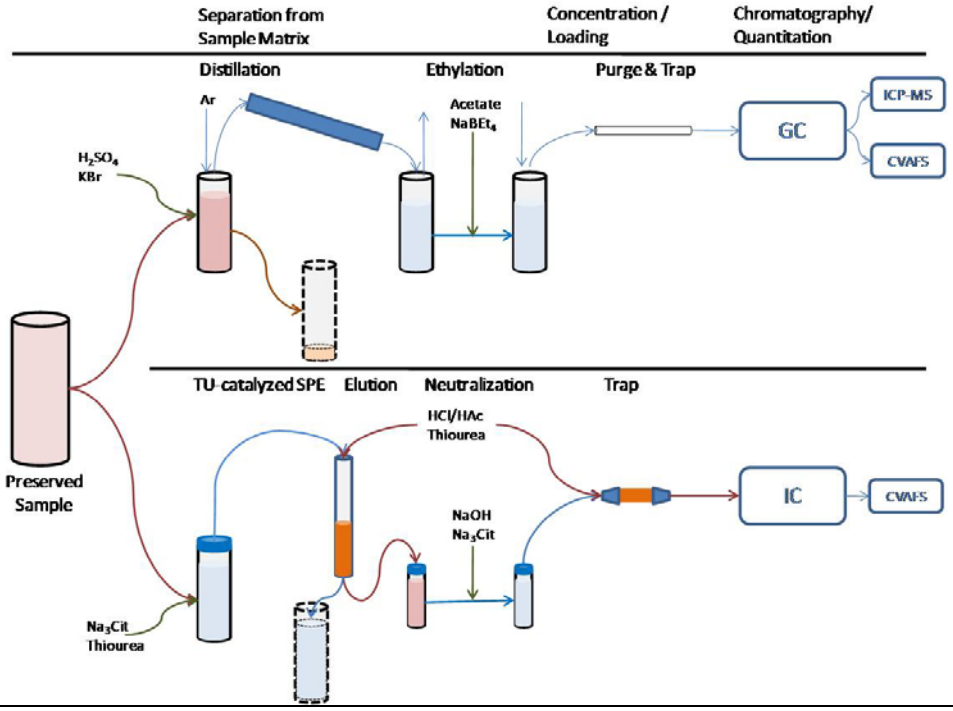


Figure 7. Schematic diagrams of sample preparation by distillation/ethylation (top) and by TU-SPE (bottom).

Methods

Water Sampling: Surface water samples were obtained using ultra-clean techniques. Samples from Lake 658 in the Experimental Lakes Area of Ontario were collected by pumping lake water through acid-cleaned Teflon tubing into acid-cleaned Teflon bottles (*13*). Surface grab samples were also collected during i) a 2-year study of wetlands located in the Calumet Region of northwestern Indiana, encompassing the Grand Calumet River basin and Indiana Dunes National Lakeshore (CR sites), and ii) occasional sampling trips to wetlands in the vicinity of Peterborough, Ontario (PW sites). Field sample processing procedures are described in the

section on the field study except that new PETE bottles were used when sampling Peterborough wetlands (PW). PW samples were filtered using an acid-cleaned glass filtration apparatus with quartz fiber filters (QFF). Lake 658 samples were QFF-filtered using an in-line Teflon filtration apparatus. All samples were preserved with 0.4% HCl (Fisher Trace Metal grade), and stored in Teflon bottles (L658), glass (CR), or PETE (PW) at 4 °C. In many cases, after analysis at one lab, samples were shipped to the other in the original bottle. Other samples were split shortly after collection with a portion sent to each lab. In all cases, samples were stored in bottles that had either been carefully cleaned (HCl/BrCl for glassware or HCl for Teflon) or were new (PETE).

MeHg Analysis by Distillation/Ethylation: At Trent University, dissolved MeHg was isolated from 50-mL samples by atmospheric pressure water vapor distillation and analyzed using ethylation/GC with ICP-MS detection (*3*). Results presented here are for MeHg (as Hg) in all isotopes, minus internal standard. Overall process (distillation+ethylation+GC steps) blanks, which were determined 2-3 times per run, averaged 1.0±0.3 pg (range 0.6-1.7 pg) in the 10 runs used here, resulting in an MDL of 0.02 ng/L. The ethylation step contributed 30-70 percent – 0.4±0.2 pg (range 0.2-1.0) – of the total process blank.

MeHg Analysis by Thiourea-Catalyzed SPE: Dissolved MeHg in 20- to 40-mL aliquots of preserved samples was analyzed at the University of Illinois using TU-SPE with analysis by HgTU-IC/CVAFS (*4*) (Table 1). Due to the rigorous cleaning with acids and/or acidic TU solutions, MeHg process blanks were undetectable (<0.2 pg). All processed standards fell within 95-109 % of the calibration curve. Standard additions (usually 25-pg per sample) were quantitatively recovered (94-109 %).

Distillation Experiments: For a limited number of samples, distillation residues generated at Trent were recovered for analysis by TU-SPE at UIUC. Most residues were obtained by distilling samples without an added internal standard. Once 85-90% of the water from a sample was visible in the receiving vessel, the distillation vessel was removed from the heating block and allowed to cool. The 3-7 mL of "residue" it contained was transferred to a new glass vial and weighed. The vessel was then rinsed with 2 mL of Milli-Q water, which was combined with the residue for analysis. At UIUC, the residues were diluted to back to the original volumes with reagent water (~50 mL), neutralized by adding sufficient NaOH, and processed as a normal water sample (see above). Process blanks for residues were 1.0±0.6 pg.

Intercomparison Results

In order to simplify the comparison of results obtained using the two methods, we focus this discussion on the ratio ($\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}}$ expressed in percent) of the MeHg concentration measured using D/E-ID ($[MeHg]_{DEID}$) to that measured using TU-SPE ($[MeHg]_{TU}$) for each split water sample:

$$\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}} \equiv [MeHg]_{DEID} / [MeHg]_{TU} \times 100\% \quad (3)$$

Of the 31 distinct surface water samples analyzed (Fig. 8), only five have results from the two methods that can be classified as equivalent ($\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}}$ between 92-108%) while 25 samples have $\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}}$ <90%. The single outlier at 130% is probably not significant, as the absolute difference is only 0.03 ng/L. Overall, the median $\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}}$ is 61% and geometric mean is 52%. Clearly, D/E-ID systematically yields lower [MeHg] than TU-SPE in the split samples analyzed here, except in those from sulfidic systems (see below).

The samples identified as being from sulfidic systems were collected in interface zones between oxic waters and anoxic water or sediments that undoubtedly contained H_2S. Two were samples from 7- and 9-m depths of the East Basin of Lake 658. These waters were oxygen depleted and at similar depths in the West Basin, hydrogen sulfide was observed within the same month (Clarisse and Hintelmann, submitted). They also agree with the corresponding $[MeHg]_{TU}$ within the limits of analytical precision ($\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}}$ = 93-104%). In the Grand Calumet region, the samples in closest agreement both exhibited MeHg levels in excess of 2 ng/L and were collected from shallow surface pools of wetlands that were sulfidic at the time of sampling (July 2007 site CRb). Finally, a sample obtained from a location within Cavan Bog (PWc) that was sulfidic at the time had a $\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}}$ of 100%, despite having the highest DOC of the PW samples.

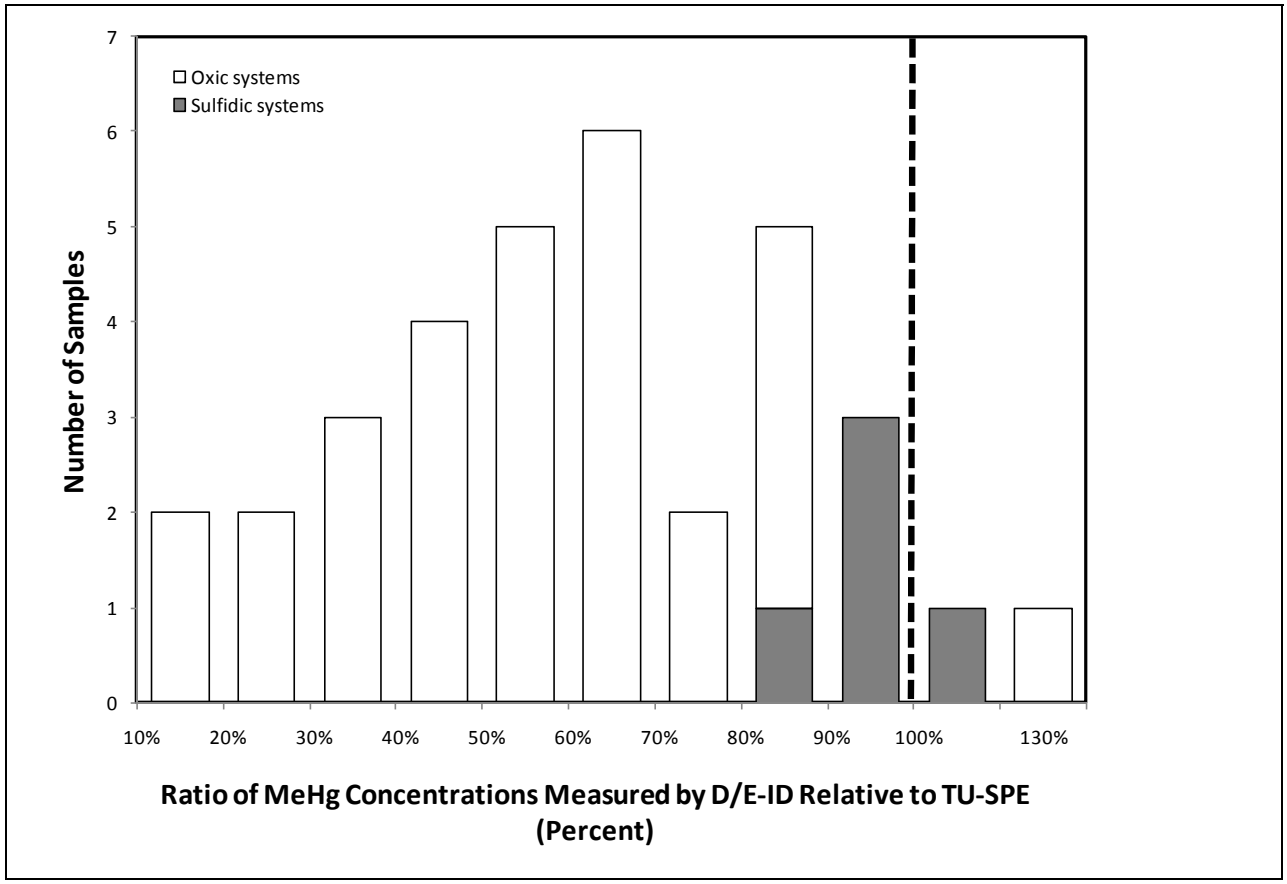


Figure 8. Frequency distribution of ratios of MeHg measured by D/E-ID/GC/ICP-MS to those measured by TU-SPE/HgTU-IC/CVAFS for all surface water samples.

A comparison across all surface water measurements (Fig. 9) shows that the $\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}}$ varies systematically with MeHg concentration, i.e., its geometric mean is 50% when [MeHg] is less than 1 ng/L, but ~92% at higher concentrations. Since high MeHg concentrations typically coincide with sulfidic zones, which have dramatically higher concentrations of strong MeHg-binding ligands in solution, these observations also suggest that there may be geochemical factors governing when the MeHg in a sample is equally available to the two methods of sample preparation.

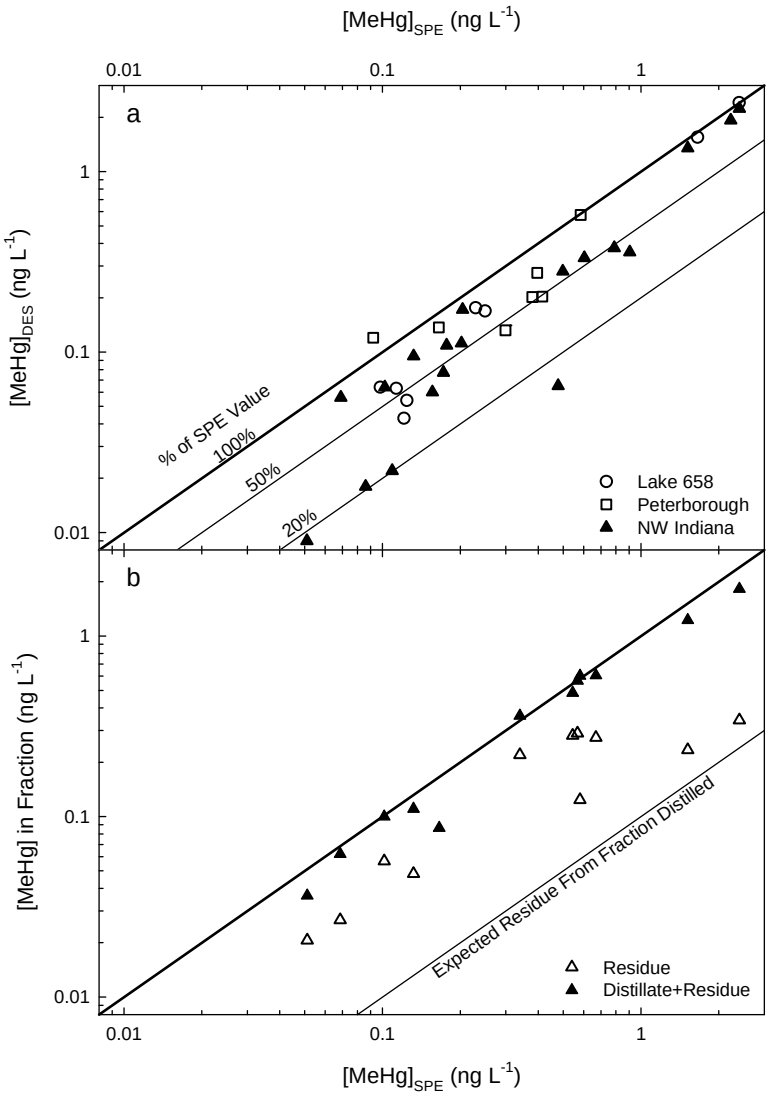


Figure 9. A) All intercomparison results: [MeHg] determined by D/E-ID/GC/ICP-MS versus TU-SPE/HgTU-IC/CVAFS, B) Residue results for 12 of the samples in panel A: residues (prepared by TU-SPE) and residues + distillates (prepared by D/E). Expected residue line is for 90% distillation (equation 4).

MeHg in Distillation Fractions.

Although distillation recovers dissolved MeHg more effectively from freshwater samples than extraction and direct ethylation, that it does not quantitatively recover MeHg has been known since the method was first published (Horvat et al.). Little is known about the fate of the

unrecovered MeHg, although it is presumed that most MeHg not volatilized during distillation is demethylated. To account for such losses, analysts routinely make standard additions. With D/E-ID, calculations based on recoveries of the isotopically-labeled internal standard added to every sample are assumed to accurately correct for incomplete recoveries of ambient MeHg. When using D/E with CVAFS detection, standard additions are most often used to check recoveries in a batch of similar samples. However, just as for any analytical method, the accuracy of conclusions drawn from standard additions, whether they are employed to quantify recoveries of ambient MeHg for routine analyses or validate a method under development, depends on the attainment of equilibrium between added and ambient MeHg. Validating the equilibrium assumption for D/E is not a simple task since it requires showing that MeHg and the end products of demethylation occur in both the distillate and residue fractions in the same proportions that they are found in the amended sample.

To examine this question further, additional aliquots of the sample with and without internal standard were distilled and the residues analyzed by TU-SPE (after dilution and buffering to *p*H 3.5). Under analysis by TU-SPE, the residues of a sample with a substantial difference between $[MeHg]_{TU}$ and $[MeHg]_{DEID}$ contained ~14 pg of MeHg, or approximately as much as the discrepancy (Fig. 10). Examination of the details of the mass balance for other samples also shows that added $Me^{201}Hg$ standard was almost completely recovered in distillates and not detectable in residues, as compared to ~50% distillation of ambient MeHg in the many $\mathbf{R}_{\mathbf{TU}}^{\mathbf{DEID}}$ samples. Unless the TU-SPE produces significant artifactual MeHg, such residue analyses are strong evidence for lack of equilibration between ambient MeHg and added tracers.

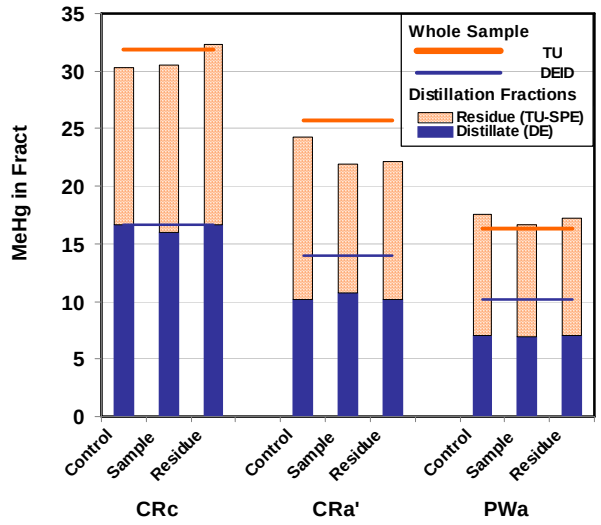


Figure 10. MeHg in fractions generated by distillation. MeHg in distillates (by D/E) and residues (by TU-SPE) fractions (No $Me^{201}Hg$ added) for samples indicated. Labels: "Control": No Hg^{II} added; "Sample" 1000-pg Hg^{II} added to whole sample prior to distillation; "Residue" Hg^{II} added to residue prior to TU-SPE analysis.

To validate the analysis of residues using TU-SPE, several QA/QC tests were conducted using inter-comparison samples. Standard additions of $MeHgCl^0$ to residues yielded >90% recoveries of MeHg (data not shown). In a test for artifact formation during residue analysis (Fig. 10), 1000 pg $HgCl_2$ amendments of both whole samples (prior to distillation) and residues (prior to TU-SPE) yielded no measurable increase in MeHg detected in residues (Fig. 10). Thus, TU-SPE analysis passes the same QA/QC tests for distillation residues as for whole samples.

Nine other fresh inter-comparison samples were also distilled without internal standards and the resultant fractions analyzed by ethylation/GC (distillates) and by TU-SPE (residues). Consistent with the trends in $\mathbf{R}_{TU}^{DEID}$, the residues of samples in the 0-0.5 ng/L range of [MeHg] contained approximately as much TU-labile MeHg as the distillates (Fig. 9) while at higher [MeHg], MeHg in residual MeHg declines as a fraction of the total. Most importantly, across all

of the samples and amended samples for which both fractions were analyzed, the sum of MeHg in the distillates and residues was highly correlated (r^2 =0.99) with the amount of MeHg in whole samples isolated by TU-SPE (Fig. 11). The linear correlation slope of 0.988 reflects a good mass balance with only a few samples exhibiting significant losses due to demethylation.

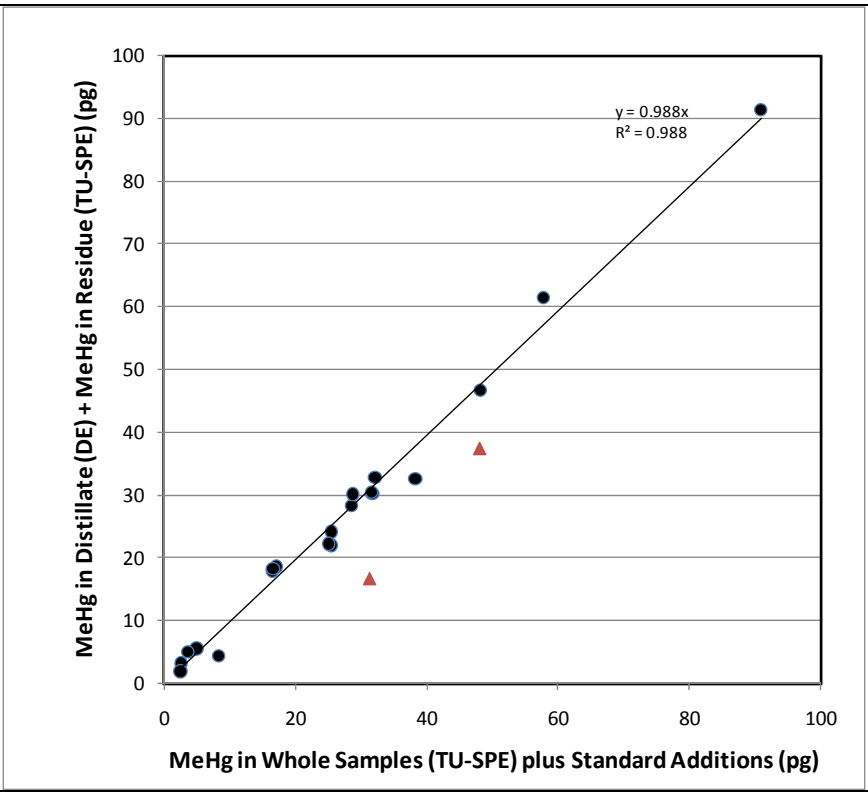


Figure 11. Comparison of MeHg measured in distillation fractions (Distillate + Residue) with MeHg in whole sample expected from TU-SPE plus added standards. Circles are surface water samples, some with added MeHg standards. Triangles are distilled standards in reagent water. X-axis values are masses of MeHg calculated to be in distilled sample from: Sample Volume×$[MeHg]_{TU}$ + MeHg in standard (when added). Y-axis values are the total masses of MeHg in distillates plus MeHg in residues. Distillate MeHg determined by ethylation/GC with $Me^{201}Hg$ internal standard in ethylation step only. MeHg in distillation residues analyzed by TU-SPE.

Synthesis

These measurements of ambient MeHg in distillation residues are the first to our knowledge and were made possible by the new TU-SPE method. Not only do they differ from estimates and

measurements of residual MeHg made using D/E, but they directly contradict the earlier report that the Hg remaining in distillation residues was largely Hg^{II} (*1*). Since isotopically-labeled MeHgCl added prior to sample preservation and distillation are recovered nearly quantitatively in studies of demethylation kinetics in lakewaters (*15*), as well as in the limited studies performed here (data not shown), it does not appear that the inter-method difference arises from DOM interferences that arise solely during the distillation process. Rather, the observations of residual Tu-labile MeHg and the significantly greater recoveries of ambient MeHg using TU-SPE than D/E-ID is most simply explained by the existence of either a particular MeHg species in natural waters that does not distill and only partially equilibrates with isotopically-labeled MeHg added during distillation.

Evidence already exists for Hg species that are inert with respect to ligand exchange reactions. Using CLE with SPE, Sedlak and coworkers (*24*) detected a fraction of Hg^{II} in some samples that failed to react with added APDC. Even more relevant is the fact that the rate of exchange of MeHg from natural ligands to TU can be slow (equilibration time of ~2 h) and depends on the concentration of TU (*4*). The presence of strong metal complexes that are inert with respect to exchange with weaker ligands – such as the Cl^-/Br^- used in D/E – but not stronger ligands – such as the thiourea used with TU-SPE – is well established in the analysis of the speciation of other metals such as Cu^{2+} (*9*). In this scenario, more rapid ligand exchange kinetics due to the strong ligand thiourea facilitating an associative pathway (*25*) or to higher *p*H and citrate diminishing MeHg occlusion by DOM could hasten the dissociation of the inert MeHg species and permit it to be detected.

Of course, without isotope dilution studies, which requires coupling the TU-SPE+HgTU/IC method to ICP-MS, it cannot be ruled out that an unknown, non-distillable compound exists in

freshwater ecosystems that methylates Hg^{II} during TU-SPE (but not during D/E). The discovery of such a methyl-donor limited artifact would in itself be quite surprising. In addition, further investigations are necessary in order to conclusively determine which, or indeed whether either, method isolates all of the MeHg in natural water samples.

Nevertheless, these results raise the distinct possibility that distillation/ethylation, the main method used in field studies of MeHg biogeochemistry since 1993 (*1*), does not isolate all of the MeHg in a significant fraction of samples from two important classes of temperate zone freshwater systems: lakes and wetland pools and outlet streams.

B. MeHg in Biological Tissues

A very convenient method for leaching MeHg from biota that is ideally suited for the HgTU-IC/CVAFS system has been developed recently (Shade, 2008). The method involves overnight leaching of a weighed sample (up to 100 mg) of tissue in HgTU-IC system eluant (TU/HCl/Acetic Acid) at 40 °C. After 0.45-µm filtration, the leachate can be buffered and directly injected into the HgTU-IC analytical system or stored frozen.

Table 3. Comparison of measurements of Hg species in DOLT-2 Certified Reference Material to measurements obtained here.

Form of Hg	Measured Hg (ppm wet)		Certified Value	
	Mean	S.D	Mean	S.D
Methyl	0.707	0.015	0.693	0.053
Inorganic	1.31	0.12	1.45	
Total	2.02	0.13	2.14	0.28

A Spatio-Temporal Study of Methylmercury Biogeochemistry in Wetlands of the Southern Lake Michigan Watershed

Brian R. Vermillion and Robert J.M. Hudson

Manuscripts in preparation.

Managing the effects of Hg pollution depends on identifying locations that may produce and/or export excessive amounts of MeHg. At the landscape scale, it is well established that wetlands are sources of MeHg to streams and lakes. In order to conduct watershed- or landscape-scale assessments of Hg pollution without requiring measurements from every site − there are hundreds of small wetlands in the Southern Lake Michigan watershed − it essential to be able to predict MeHg levels from generally available data, including water quality data and regional maps depicting distributions of historical and current Hg deposition and other biogeographic and geochemical variables.

Perhaps the most important research need for environmental management is the dependence of environmental concentrations of MeHg on the loads and/or levels of legacy contamination, since this is the basis for RAP or TMDL design as well as wetland restoration efforts. In the case of MeHg, however, the two key processes affecting methyl-Hg, methylation and demethylation, and both potentially exhibit non-linear relationships between environmental levels of Hg species and rates. By examining multiple causal factors, we should be able to determine whether differences in the ratios of MeHg to Hg^{II} in sediments with large historical accumulations of Hg^{II} contamination differ from those that do not have this contamination. This will give us a strong indication of whether immobilization in wetland sediments/soils alters in Hg^{II} bioavailability or whether other factors cause relative changes in the rates of methylation

and demethylation cause.

Study Area

The Grand Calumet River is located in Indiana at the southern tip of Lake Michigan. Its watershed contains a steep gradient in contamination, ranging from i) areas that bear the legacy of Hg pollution from the intense industrial activities in the cities of East Chicago, Hammond, and Gary to ii) parkland in the Indiana Dunes National Lakeshore. Total Hg concentrations in the water discharged by the Grand Calumet River into Lake Michigan are elevated ~10-fold relative to Lake Michigan water and are the second highest of all rivers discharging into the lake (Hurley et al., 1998). Within its watershed, fish consumption advisories due to high Hg levels have been instituted (IDEM, pers. comm.) and sediment Hg levels up to 20 ppm have been measured in the West Branch of the river (Cahill et al., 1999). MeHg levels in the river, however, are more modest. Due to the presence of these high levels of Hg and other contaminants, the Grand Calumet is currently the subject of programs to develop a Remediation Assessment Plan (RAP) and a Total Maximum Daily Load (TMDL).

Sampling Sites

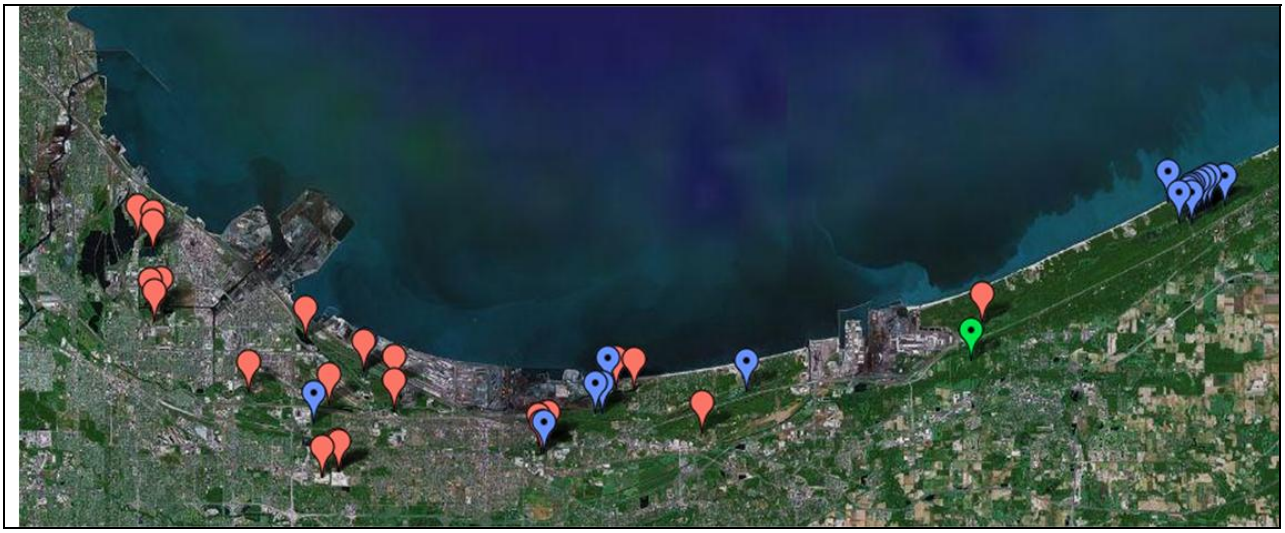


Figure 12. Aerial photograph and map of the Calumet region of Northwestern Indiana. Orange symbols are wetlands sampled in 2006 survey; Blue symbols are wetlands sampled in 2007. Green symbol is Indiana Dunes National Lakeshore field lab. Maps/photos from http://maps.google.com/. Search maps for "UIUC Calumet Study" to locate sites.

2006 Spatial Survey: A survey of lacustrine wetlands in the Grand Calumet Area of Concern was conducted in conjunction with scientists from the Indiana Department of Environmental Management (Jim Smith) and the National Fish and Wildlife Service (Thomas Simon). The sites were selected using a stratified (by surface area) random design (Fig. 12). Twenty-nine sites were sampled over a two week period in July 2006 (Table 4). At each site, 3-5 transects of 100- to 200-m length were conducted. Along each transect, three sediment cores were typically obtained. Water samples were taken near the midpoint of each transect.

2007 Temporal Survey: Nine sites from the 2006 survey were resampled in May, July and November of 2007 in order to investigate the temporal dynamics of MeHg levels in these systems. (Fig. 12).

Table 4. Geographic coordinates of sites sampled in 2006 Survey and 2007 Study. Latitudes and longitudes are in decimal degrees.

Name	Years Sampled	Longitude (West)	Latitude (North)
Wolf Lake	2006	87.508510	41.672520
George Lake-South Basin	2006	87.502230	41.666672
George Lake-North Basin	2006	87.501950	41.670610
Exxon-Mobil	2006	87.501100	41.641190
Lime Pits (Lake Mary)	2006	87.498280	41.645760
BP-Turning Basin	2006	87.487820	41.640800
Seidner Dune and Swale	2006	87.454510	41.614980
Majestic Casino Lake	2006	87.426670	41.635140
Ivanhoe	2006-07	87.420200	41.604010
Gary Landfill Constructed Wetland	2006	87.417880	41.583540
East Gary Airport	2006	87.414800	41.610540
Gary Landfill Lagoon	2006	87.410628	41.586179
Gary Landfill seep	2006	87.410020	41.585450
West Pine & Clark	2006-07	87.397830	41.622510
Bonji	2006	87.382710	41.617080
Georgia Pacific	2006	87.382610	41.608350
Wabash Railroad	2006	87.310990	41.595630
Interstate 65 West	2006-07	87.309810	41.592670
Interstate 65 East	2006	87.306320	41.596200
Miller Woods 1	2006-07	87.299140	41.603700
Miller Woods 2	2006-07	87.299140	41.603700
East Pond (INDU)	2006-07	87.276949	41.616726
Middle Marquette Lagoon (INDU)	2006	87.273030	41.617464
East Marquette Lagoon (INDU)	2006	87.264061	41.616533
North Woodbridge Lake	2006	87.230260	41.600160
Long Lake (INDU)	2006-07	87.207670	41.616982
Cowles Bog (INDU)	2006	87.091112	41.640592
Great Marsh (INDU)	2006-07	Various	

INDU denotes sites within Indiana Dunes National Lakeshore.

Sampling Methods

a) Water Samples: Clean "grab" sample bottles were filled by immersion to ~30-cm below the surface of streams and wetland pools using gloved hands. Bottles used for sampling were acid-cleaned, 1-L FDPE bottles. Water from these samples was filtered using 0.45-µm HVLP filters in a Teflon filter tower cleaned with HNO_3 and rinsed with TU cleaning solution (*4*) connected to a vacuum dessicator (*14*). Finally, samples were preserved with 0.4% HCl (Fisher Trace Metal

grade) and stored at 4 °C in borosilicate glass bottles that were stored in bottles that had been carefully cleaned (HCl/BrCl for glassware). All filtration was performed at the field lab in Indiana Dunes National Lakeshore.

b) Sediment Cores: Sediment cores were collected in 15 x 7-cm 'trace-metal clean' acrylic coring tubes from wetlands located within the Grand Calumet Area of Concern in Northern Indiana, U.S.A. Core tubes were capped while under water to prevent them from draining. After extruding the cores, any non-decomposed OM was removed and the top 5 cm of sediment thoroughly homogenized by hand or blender. This sediment was then stored frozen at –20 °C until analysis. All core processing was performed at the field lab in Indiana Dunes National Lakeshore.

c) Squeezed Porewater and Sediments: To obtain porewater, sections of sediment were loaded into into Teflon/polycarbonate squeezers (Robbins and Gustinis, 1976; Alongi, 1990; Lourey, 1999). The squeezers rely on expansion of a nitrile diaphragm, under pressure (N_2 at 100 psi), to press porewater from sediments through a cellulose filter and a Teflon flowpath into an open polypropylene syringe barrel fitted with a 0.22-μm acid-cleaned nylon syringe filter. After inserting the plunger, the porewater is pushed through the filter into I-Chem 20- or 40-mL glass vials. All squeezing and filtration was performed in a glove bag with a N_2 atmosphere. The filtered porewater and remaining sediment was refrozen and stored until analyzed. All porewater was squeezed at the field lab in Indiana Dunes National Lakeshore.

d) Fish: Fish were collected during the July 2006 survey by Thomas Simon (USFWS) using electroshocking, placed on ice in the field and then frozen at -4 °C at the end of the day.

Sample Analyses Conducted.

Table 5. Summary of Samples Collected and Analyzed from 2006-07 Grand Calumet Region Methylmercury Study.

		Spatial Study (25 sites)		**Seasonal Study (9 sites)**					
		July 2006		**May 2007**		**July 2007**		**November 2007**	
	Parameter	Samples Obtained	Samples Analyzed	Samples Obtained	Samples Analyzed	Samples Obtained	Samples Analyzed	Samples Obtained	Samples Analyzed
Surface Water	MeHg	67	67	33	33	33	33	33	33
	THg	67	67	33	33	33	33	33	33
	Cl	67	67	33	33	33	33	33	33
	Sulfate	67	67	33	33	33	33	33	33
	Nitrate	67	67	33	33	33	33	33	33
	DOC	67	67	33	33	33	33	33	33
Sediment Solids	MeHg	145	145	45		45		45	
	THg	145	145	45	45	45	45	45	45
	Organic Matter	145	145	45		45		45	
	Reducible Sulfide	145	20	45		45		45	
Sediment Porewater	MeHg	110	110	45		45		45	
	THg	110	110	45		45		45	
Biota	MeHg/THg	189	189	N/A	N/A	N/A	N/A	N/A	N/A

Results

2006 Survey: The sediment data obtained in the 2006 survey exhibit good correlations with sediment organic matter. This is a common observation in surveys of aquatic ecosystems within a region. Thus, the mercury burden of an ecosystem will depend on the organic matter content of its sediments. The ratio of total Hg (THg) to SOM reflects the level of contamination in a system. Not surprisingly, the highest levels are found in industrial sites in the Whiting area.

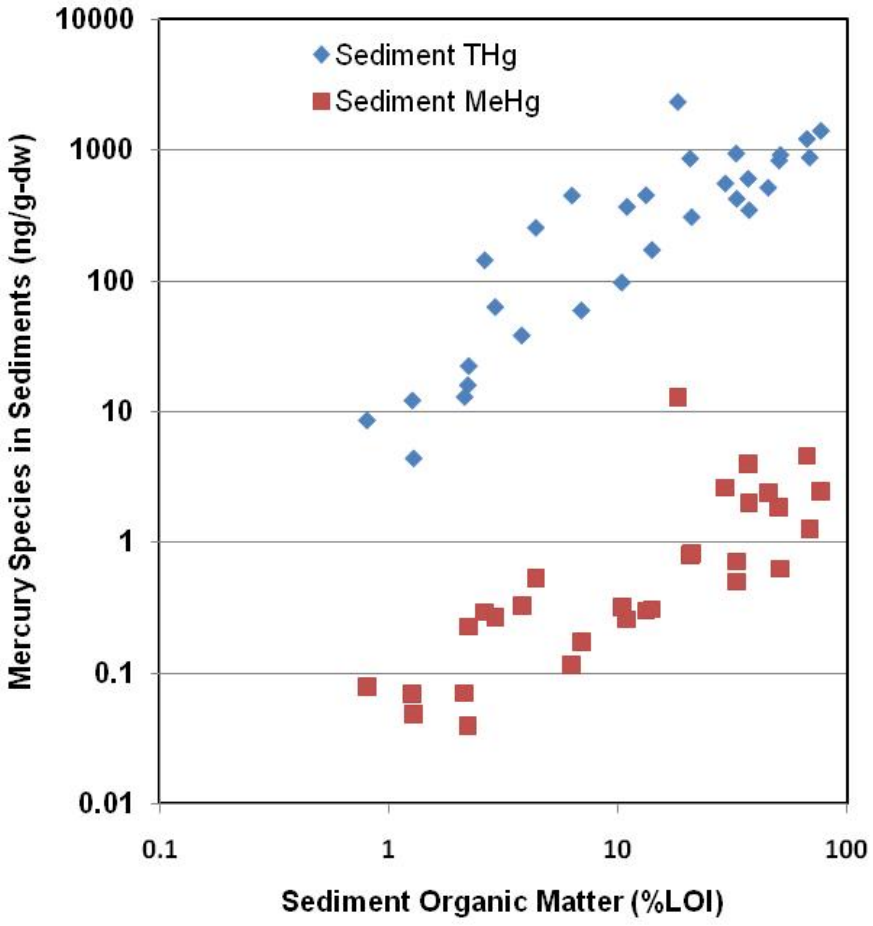


Figure 13. Dependencies of sediment total mercury and MeHg on organic matter content. Average values for each wetland from 2006 survey are plotted.

To find its way into aquatic food webs, methylmercury produced in sediments primarily diffuses out into the water column. In the data we have obtained here, both Hg species dissolved in porewater are somewhat correlated with the total MeHg levels in the sediment.

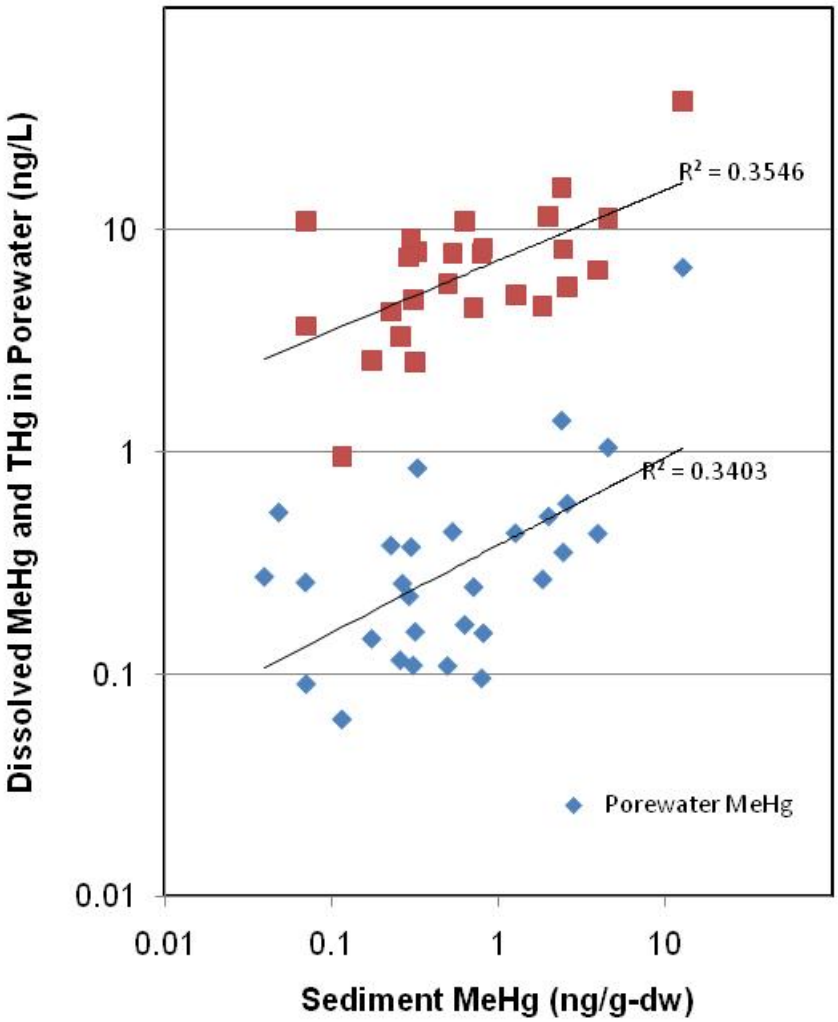


Figure 14. Relationship of porewater MeHg and THg to sediment MeHg in freshwater ecosystems.

The partitioning of MeHg and total Hg (mostly Hg^{II}) can be summarized in terms of a sediment-water partition coefficient. Interestingly, this coefficient is more systematically related to SOM for MeHg than for Hg^{II} (Data not shown).

Finally, we developed a way of partially coupling our results for Hg in fish tissues to the model structure of the USGS National Descriptive Model of Mercury in Fish. In the USGS

NDMMF modeling approach, data from a variety of combinations of species and cut (skin-on fillet, whole fish, etc.) of fish are used to derive a parameter for each sampling event (a specific site and time of fish sampling) that represents a species/cut-adjusted "mean" estimates of the potential of mercury to accumulate in the fish community at a specific site over some time-period just prior to that sampling event. To obtain this mean (β_j), one begins from the basic model equation:

$$\ln(C_{ijk} + 1) = \alpha_k \times \ln(LENGTH_{ijk} + 1) + \beta_j + \varepsilon_{ijk}$$

where i,j,k are indices for fish sample, sampling event, and fish species/cut respectively. Here, C_{ijk} is the concentration of Hg in the sample in units of µg/kg, α_k is the species/cut parameter, $LENGTH_{ijk}$ is the length of the fish in inches, and ε_{ijk} is the model error term.

The preferred method for analysis of data is to append it to the national database and re-calibrate the NDMMF, which forces the species/cut and sampling event coefficients to be re-optimized for the entire dataset. As this is not always convenient, for small data sets it does not introduce much error if one assumes that the fish species parameters (α_k) from the most-recent NDMMF calibration to the national dataset, which are available on the EMMMA website (http://emmma.usgs.gov/), remain constant and compute an approximate sampling event parameter for each sample (B_{ijk}) as follows:

$$b_{ijk} \equiv \ln(C_{ijk} + 1) - \alpha_k \times \ln(LENGTH_{ijk} + 1)$$

where β_{ijk} is assumed to be $\beta_j + \varepsilon_{ijk}$. Then the estimated B_j for each sampling event is given by:

$$B_j = \text{average of } b_{ijk} \text{ for all samples from each sampling event}$$

For the purposes of this field study, we can then compare B_j across sampling events from: different sites (that were sampled over a relatively short time period) to assess spatial variation in

fish-mercury concentrations; or different times (at the same site) to assess temporal variability. Then, one can back calculate the Hg expected in a standard fish, a 10" perch in this case, on the basis of the site and species parameter.

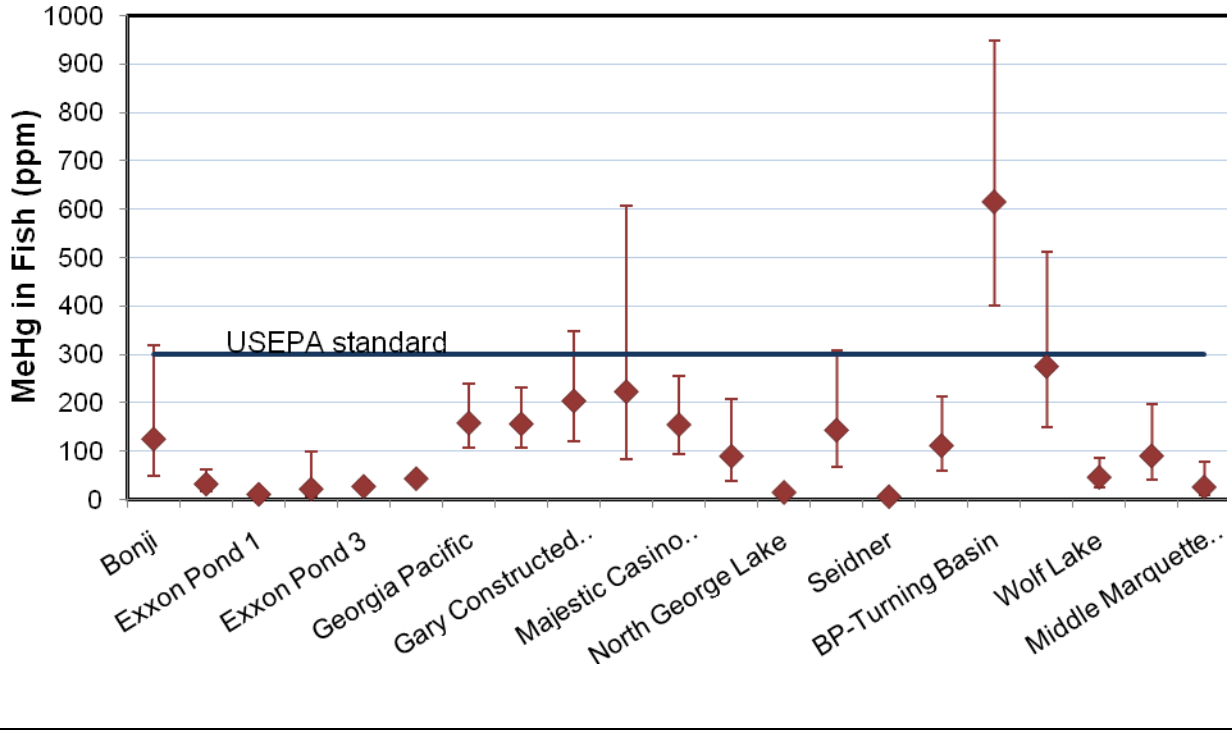


Figure 15. Normalized MeHg levels in 10" perch as computed from observations at each site using the National Descriptive Model of Mercury in Fish (http://emmma.usgs.gov/.

Our goal is to then analyze these normalized data in order to identify spatial variables that explain the variations in B_j.

Seasonal Variations

The temporal survey revealed clear differences in dissolved MeHg between seasons at that different sites, although they are not consistent and therefore somewhat hard to explain at this time.

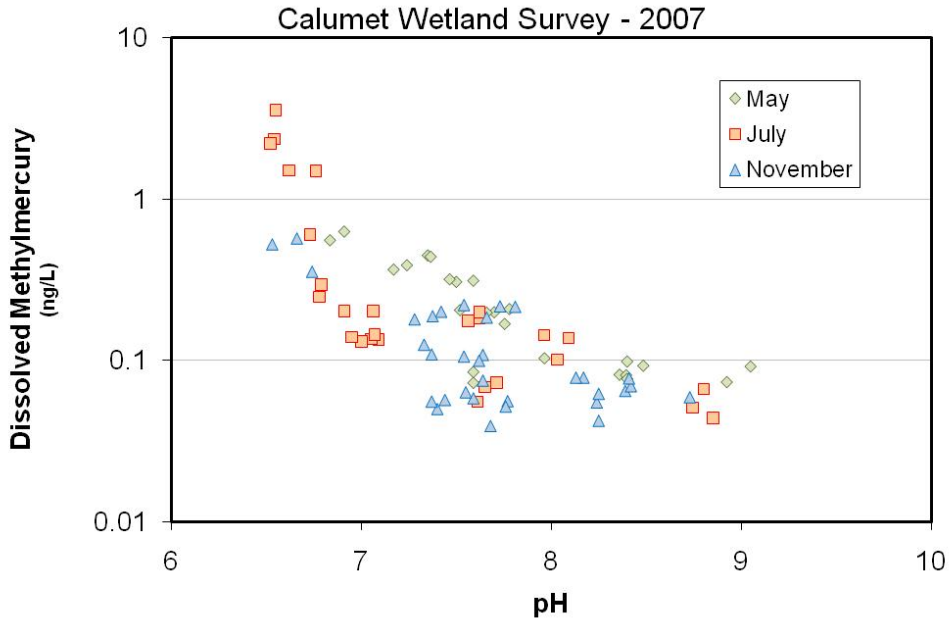


Figure 16. Dependence of dissolved MeHg on pH and season at the 9 sites repeatedly sampled during 2007.

Summary

Mercury chemists employ a variety of approaches to extract MeHg from sediments for analysis. The coupling of these methods to our new HgTU-IC system is conceptually straightforward, but had not previously been explored in great detail. In order to have a method in which we could have confidence, we compared results of coupling the HgTU/IC system with a variety of known extraction methods. Although we have some concerns about artifactual

formation of MeHg with all methods, the best results were obtained using the sulfuric acid/potassium bromide digestion method. Since this is one of the most widely used digestions, it is encouraging that we obtain good results using it and that our results for certified reference materials fall within the range of values reported in the literature.

Perhaps the most widely relevant aspect of our research is the inter-comparison between our new method (developed in 2006) and the standard method for MeHg analysis in water samples, commonly known as distillation/ethylation-GC or D/E. Although we did not expect it, we found that in about two-thirds of all samples compared, the new method yields more MeHg than D/E. In these samples, we found that D/E recovers only about 50% of the MeHg recovered by our new method; our new method never recovers significantly less MeHg. To date, we have not found any way to couple our new solid phase extraction method to the conventional method for MeHg analysis, ethylation-GC. However, when our work is published, we expect there to be a considerable effort made to "fix" the existing method.

Although further tests are needed to investigate how widespread these differences are and conclusively rule out low probability problems with the method, on the basis of this study it appears that our new analytical system will prove to be the superior method for measuring MeHg in surface water samples. The difference in results using the two methods is certainly large enough that researchers wanting to understand the transport of MeHg and its bioaccumulation will want to use the new method. Since dissolved MeHg is the main parameter to be measured to assess the impact of Hg contamination in studies of Hg biogeochemistry, as the method becomes accepted it should become the choice of environmental managers as well.

References

1) Bloom, N. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. *Canadian Journal of Fisheries and Aquatic Sciences* **1989**, *46*, 1131-1140.
2) Hintelmann, H.; Ogrinc, N. In *Biogeochemistry of environmentally important trace elements*; ACS Symp Ser: Washington, DC, **2003**; *835*, 321-338.
3) Horvat, M.; Liang, L.; Bloom, N. S. Comparison of distillation with other current isolation methods for the determination of methyl mercury-compounds in low-level environmental-samples .2. Water. *Analytica Chimica Acta* **1993**, *282*, 153-168.
4) Shade, C. W.; Hudson, R. J. M. Determination of MeHg in environmental sample matrices using Hg-thiourea complex ion chromatography with on-line cold vapor generation and atomic fluorescence spectrometric detection (HgTU/IC-FI-CVAFS). *Environmental Science &Technology* **2005**, *39*, 4974–4982.
5) Shade, C. W.; Automated Simultaneous Analysis of Monomethyl and Mercuric Hg in Biotic Samples by Hg-Thiourea Complex Liquid Chromatography Following Acidic Thiourea Leaching, *Environmental Science &Technology* **2008**, *42* , 6604–6610.
6) Vermillion, B.R., Shade, C.W., and Hudson, R.J.M.; Solid-Phase Methylmercury Determination By Hg-Thiourea Complex Ion Chromatography with On-line Cold Vapor Atomic Fluorescence Spectrometry, *Analytica Chimica Acta* (submitted).
7) Vermillion, B. R.; Hudson, R. J. M. Thiourea catalysis of MeHg ligand exchange between natural dissolved organic matter and a thiol-functionalized resin: a novel method of matrix removal and MeHg preconcentration for ultratrace Hg speciation analysis in freshwaters. *Analytical and Bioanalytical Chemistry* **2007**, *388*, 341–352.
8) Hintelmann, H.; *Chemosphere* **1999**, 39, 1093.
9) Qian, J., Skyllberg, U., Tu, Q., Bleam, W.F. , and Frech, W. *Fresen. J. Anal. Chem.* **2000**, *367* 467.
10) USEPA "Draft Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis DivisionWashington, DC, **1998**.
11) Law, S.; *Science* **1971,** *174*, 285-287.
12) Lee Y, Mowrer J.; *Analytica Chimica Acta* **1989**, *221*, 259-68
13) Clarisse, O., Hintelmann, H.; *J Environ Monit* **2007**, *8*, 1242 – 1247.
14) Emteborg H, Baxter D, Frech W.; *Analyst* **1993**, *118*,1007-1013.
15) Huang J-H, Analytica Chimica Acta **2005**, *532*, 113-120
16) Celo, V, Ananth R, Scott S, Lean D *Analytica Chim Acta* **2004**, *516*,171-177.
17) Bloom N, Colman J, Barber L Fresenius J Anal Chem **1997**, *358*,371-377.

Potential Applications or Benefits

The new method for isolating methylmercury from natural water samples developed during this project will permit those that adopt it to obtain high-quality MeHg data from water samples. These measurements will provide scientists with much more accurate and cost-effective tools for investigating the distribution and sources of methylmercury in the environment than are available at present.

In time, we expect that the mercury research community will accept that the new method of measuring Methylmercury in water samples developed under this project yields superior values as compared to the current standard method. There is a potential for far-ranging impacts on our understanding of how methylmercury is transported in the environment and how it bioaccumulates in aquatic food webs.

The results from our field study will be useful in assessing the impacts of Hg contamination in the Grand Calumet area. Although the data analysis is not complete, preliminary indications are that high levels of Hg are uncommon in the biota we studied.

The new method for analyzing MeHg in water samples is an important step for two reasons. First, it provides the first viable alternative to the standard method for analyzing MeHg in water. Simply having a method that permits comparisons to be made is important as there is no certified standard for MeHg in natural waters. Secondly, this should lead to lower costs for MeHg analysis as major parts of our IC system can be automated.

Keywords

Methylmercury, mercury, wetlands, contamination

Lay Summary

Mercury pollution in aquatic ecosystems results from a combination of atmospheric deposition and legacy contamination. In addition, many other differences between aquatic ecosystems can cause them to have very different levels of methylmercury (MeHg) in the water and ultimately in fish. Since MeHg is the only form of Hg that biomagnifies in food webs, measuring dissolved MeHg is one of the most important ways of assessing the potential of an aquatic ecosystem to have excessive levels of MeHg in fish. Thus, we devoted considerable effort in this study to developing an improved method of measuring dissolved MeHg in streams and lakes. In an extensive test conducted for this study, the new method proved to be superior to the existing method at extracting MeHg from natural water samples. The new method gives us better capabilities for investigating sources of MeHg in aquatic ecosystems.

Surveys of methyl mercury in water and sediments of wetlands and lakes in the Grand Calumet region were conducted as well. A survey of 25 sites was conducted in July 2006 and nine of these sites were revisited in a seasonal study conducted during 2007. The survey results indicate that the *p*H and concentration of dissolved organic carbon are the main water quality variables that control dissolved MeHg levels. Thus, it should be possible to predict ecosystems most likely to have high levels of MeHg on the basis of relatively simple and inexpensive measurements. Although we found a wide range of MeHg levels in fish, the samples that we analyzed did not exceed EPA standards. However, analysis of our data in combination with the

National Descriptive Model of Mercury in Fish suggests that two sites – one industrial and one in a natural area in Gary – may have levels where fish are likely to exceed the EPA standards.

International Implications

Methylmercury is a toxicant of interest around the world. The technology developed here to measure its levels in water more accurately should generate interest globally.

Media Coverage

None.

Partnerships with other institutions/individuals initiated or continued by your project.

We were only able to complete this project with extensive cooperation from the Indiana Dunes National Lakeshore (National Park Service), in particular their research coordinator Joy Marburger. We obtained the cooperation of the Indiana Department of Environmental Protection to gain access to field sites.The intercomparison of our new method with the standard method of analyzing methylmercury in water samples was conducted at Trent University during Dr.

Hudson's sabbatical. The cooperation of Prof. Holger Hintelmann was much instrumental to completing this task.

Publications

Vermillion, Brian R. and Robert J.M. Hudson. 2007. Thiourea catalysis of MeHg ligand exchange between natural dissolved organic matter and a thiol-functionalized resin: a novel method of matrix removal and MeHg preconcentration for ultratrace Hg speciation analysis in freshwaters. *Analytical and Bioanalytical Chemistry* **388**:341–352.

Vermillion, B.R., Shade, C.W., and Hudson, R.J.M.; Solid-Phase Methylmercury Determination By Hg-Thiourea Complex Ion Chromatography with On-line Cold Vapor Atomic Fluorescence Spectrometry, *Analytica Chimica Acta* (submitted).

Hudson, R.J.M., Vermillion, B.R., Zhu, J., and Hintelmann, H. Total Dissolved Methylmercury in Freshwaters: An Inter-comparison of Water Vapor Distillation and Thiourea-Catalyzed Solid Phase Extraction. *Environmental Science and Technology* (Submitted September 2008).

Graduate Student Support

This project has been the primary support for the doctoral dissertation work of Brian Vermillion. He will be completing his dissertation during fall 2008.

Related Projects

This project led to two small projects ($2700-$3000 each) being funded by the National Park Service at Indiana Dunes National Lakeshore during 2007 and 2008.

Patents/Licenses

Although there were no new patents issued during this work, the results of this study are closely linked to the patents developed under a previous Sea Grant-funded project.