

INFLUENCE OF DISSOLVED ORGANIC MATTER ON THE COMPLEXATION OF MERCURY UNDER SULFIDIC CONDITIONS

CARRIE L. MILLER,*† ROBERT P. MASON,† CYNTHIA C. GILMOUR,‡ and ANDREW HEYES†

†University of Maryland, Center of Environmental Science, Chesapeake Biological Laboratory, 1 Williams Street, Solomons, Maryland 20688, USA

‡Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, Maryland 21037, USA

(Received 17 July 2006; Accepted 27 October 2006)

Abstract—The complexation of Hg under sulfidic conditions influences its bioavailability for microbial methylation. Neutral dissolved Hg-sulfide complexes are readily available to Hg-methylating bacteria in culture, and thermodynamic models predict that inorganic Hg-sulfide complexes dominate dissolved Hg speciation under natural sulfidic conditions. However, these models have not been validated in the field. To examine the complexation of Hg in natural sulfidic waters, octanol/water partitioning methods were modified for use under environmentally relevant conditions, and a centrifuge ultrafiltration technique was developed. These techniques demonstrated much lower concentrations of dissolved Hg-sulfide complexes than predicted. Furthermore, the study revealed an interaction between Hg, dissolved organic matter (DOM), and sulfide that is not captured by current thermodynamic models. Whereas Hg forms strong complexes with DOM under oxic conditions, these complexes had not been expected to form in the presence of sulfide because of the stronger affinity of Hg for sulfide relative to its affinity for DOM. The observed interaction between Hg and DOM in the presence of sulfide likely involves the formation of a DOM-Hg-sulfide complex or results from the hydrophobic partitioning of neutral Hg-sulfide complexes into the higher-molecular-weight DOM. An understanding of the mechanism of this interaction and determination of complexation coefficients for the Hg-sulfide-DOM complex are needed to adequately assess how our new finding affects Hg bioavailability, sorption, and flux.

Keywords—Mercury Sulfide Dissolved organic matter Complexation

INTRODUCTION

The dissolved-phase complexation of inorganic Hg has been demonstrated in laboratory and field studies as a controlling factor in the bacterial production of MeHg, the form of Hg that bioaccumulates in aquatic organisms [1–4]. Both laboratory and field studies have shown that Hg complexed with dissolved organic matter (DOM) dominates the speciation of Hg under oxygenated conditions [5,6], but the complexation of Hg under natural anaerobic conditions has not been thoroughly investigated. Because Hg methylation occurs predominantly under anaerobic conditions and is partially controlled by the complexation of Hg in these environments [7], the complexation of Hg under these conditions is extremely important. Under sulfidic conditions, thermodynamic models predict that Hg-sulfide complexes dominate the Hg speciation as a result of the stronger affinity of Hg for sulfide relative to its affinity for DOM [5,8]. The importance of sulfide species in determining Hg bioavailability to methylating bacteria has been shown in culture [1], but the complexation of Hg under natural sulfidic conditions has not been reported.

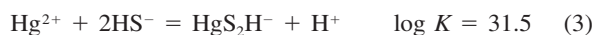
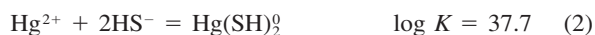
Currently, the binding of Hg with DOM is believed to occur mainly through reduced sulfur functional groups on the DOM [9,10]. Mercury also will form complexes with other functional groups, such as carboxylic acid groups [11], but these interactions are not strong enough to outcompete the reduced sulfur groups on the DOM for Hg complexation at environmentally relevant Hg concentrations [10,12]. Whereas the interaction of

Hg with reduced sulfide groups on DOM has never been directly observed, as it has been with solid organic matter [11], it has been suggested in several studies [5,6,9,12]. Published stability constants for the formation of Hg-DOM complexes (Eqn. 1) range from $10^{4.7}$ to greater than 10^{30} ; however, the lowest values probably are underestimates resulting from studies done at high Hg concentrations in which the strong-binding sites on the DOM were saturated. Therefore, the stability constants thought to be the most accurate are between 10^{22} to 10^{28} [9]. In Equation 1, the organic matter is represented as RS^- and is expressed as the concentration of the strong-binding sites on the organic matter. These constants span several orders of magnitude, most likely as a result of the heterogeneous nature of DOM and the different methods used to determine these constants [9]:



As a result of the strong affinity of Hg to reduced sulfide, Hg also forms strong complexes with dissolved inorganic sulfide that are thermodynamically more favorable than Hg-DOM complexes (see Eqns. 2–6 below). Thermodynamic models indicate that under most natural conditions, the dominant Hg-sulfide complexes are HgS^0 , $\text{Hg}(\text{SH})_2^0$, HgHS_2^- , and HgS_2^{2-} [7], but the possibility of Hg complexation with polysulfides also exists under some conditions [13]. It should be noted that although commonly written as HgS^0 , quantum mechanical calculations indicate that this complex most likely is present as HOHgSH^0 , a hydrated complex [14]. The current understanding of Hg complexation in the presence of sulfide in natural waters relies on laboratory studies and thermodynamic models that have not been validated under natural conditions:

* To whom correspondence may be addressed (millerc@uncw.edu). The current address of C.L. Miller is Department of Chemistry and Biochemistry, University of North Carolina at Wilmington, 601 South College Road, Wilmington, NC 28403, USA.



The complexation of Hg with sulfide is important in understanding the production of MeHg in the environment, because it has been demonstrated in both laboratory and field studies that the sulfide concentration affects Hg methylation rates through its impact on the bioavailability of Hg for uptake by Hg-methylating microorganisms [3,15]. Biotic production of MeHg dominates over abiotic production in aquatic systems [16], and sulfate-reducing bacteria have been shown to dominate the production of MeHg. Mercury uptake and methylation by pure cultures of sulfate-reducing bacteria is strongly dependent on sulfide concentration. Thermodynamic models suggest that uptake is diffusive and a function of the concentration of neutral Hg-sulfide complexes (HgS^0 , $\text{Hg}(\text{SH})_2^0$) [1,2,17]. To our knowledge, however, the concentration of these complexes in natural sediment interstitial water, where sulfate-reducing bacteria mediate Hg methylation, has not been measured directly. Given the importance of Hg-sulfide complexation in the production of MeHg, understanding the complexation of Hg in natural water samples is extremely important.

Because the sulfide speciation of Hg has never been measured under natural sulfidic conditions, thermodynamic speciation models used to predict the complexation of Hg in the presence of sulfide have not been validated. Using competitive ligand exchange and C-18 solid-phase extraction, Hsu-Kim and Sedlak [6] showed that in the presence of glutathione, Hg-sulfide complexes were retained on C-18 columns but that Hg-humic acid complexes were eluted from the column. In a solution containing both humic acid and sulfide, Hg was only partially retained on the C-18 column when glutathione was added, suggesting a potential interaction between Hg and humic acids in the presence of sulfide. That study could not conclude whether the observed interaction resulted from a change in the properties of the C-18 column in the presence of humic acids or from an interaction between Hg and humic acids, but it suggested that the complexation of Hg under sulfidic conditions may not be as simple as predicted by currently known thermodynamic interactions. This subject therefore warrants a more detailed investigation as a result of the importance of Hg-sulfide complexation in the production of MeHg.

In the present study, octanol/water partitioning extractions and centrifuge ultrafiltration were used to examine the complexation of Hg under natural sulfidic conditions and in laboratory solutions. Thermodynamic models indicate that inorganic Hg-sulfide complexes should dominate the species of Hg under these conditions, but contrary to current thermodynamic model predictions, these were not the most abundant species measured in natural samples and found in the laboratory studies described below. This is the result of a previously unknown interaction of Hg with DOM in the presence of sulfide.

MATERIALS AND METHODS

Octanol/water partitioning and ultrafiltration theory

Octanol/water partitioning. Octanol/water partitioning extractions involve the addition of octanol to a water sample to

Table 1. Octanol/water partitioning coefficients (K_{OW}) for several Hg complexes

Species	K_{OW}
$\text{Hg}(\text{OH})_2^0$	1.2 ^a
HgCl_2^0	3.3 ^a
HgOHCl	0.05 ^a
HgCl^+	<0.01 ^b
HgSH^+ , HgS_2^{2-}	<0.01 ^b
HgS^0 , $\text{Hg}(\text{SH})_2^0$	72 ^c
Hg-DOM ^d	0.1 ^e , 1.7–3.3 ^e

^a Mason et al. [19].

^b Charged complexes are assumed not to significantly partition into octanol (Benoit et al. [3]).

^c Present study.

^d DOM = dissolved organic matter.

^e Benoit et al. [5].

determine the fraction of Hg in the sample that will partition into octanol. Octanol/water partitioning coefficients (K_{OW}) are widely used to estimate the hydrophobicity of organic contaminants, and K_{OW} is considered to be a surrogate measure for the potential of a compound to partition into biological tissues. For Hg, neutral Hg species, such as HgCl_2^0 or HgS^0 , are more hydrophobic relative to charged Hg complexes and, therefore, partition to a greater extent into octanol [17]. The partitioning coefficient of a pure Hg species (K_{OW}) is defined using Equation 7, where HgL_{Oct} and HgL_{aq} are the concentrations of Hg bound to a specific ligand in the octanol phase and in the aqueous phase, respectively. The octanol/water partitioning of several Hg complexes have been reported previously (Table 1).

When the octanol/water partitioning of Hg in a water sample is measured, an overall partitioning coefficient (D_{OW}) is determined, because several Hg complexes, with different octanol solubilities, could be present. The D_{OW} is the ratio of the total Hg concentration in the octanol phase, Hg_{Oct} , to the total Hg concentration in the aqueous phase, Hg_{aq} , at the end of the extraction (Eqn. 8). The K_{OW} values of individual Hg complexes ($(K_{\text{OW}})_i$) are related to the D_{OW} of a sample by the fraction of the individual complexes (α_i) present in the sample (Eqn. 9) [18]:

$$K_{\text{OW}} = \text{HgL}_{\text{Oct}}/\text{HgL}_{\text{aq}} \quad (7)$$

$$D_{\text{OW}} = \text{Hg}_{\text{Oct}}/\text{Hg}_{\text{aq}} \quad (8)$$

$$D_{\text{OW}} = \sum \alpha_i (K_{\text{OW}})_i \quad (9)$$

In previous studies using octanol/water partitioning to examine Hg complexation, it has been assumed that as Hg is extracted into the octanol, equilibrium speciation is rapidly reestablished in the aqueous phase until steady-state concentrations of Hg in the aqueous and octanol phases are reached [5,13,17–19]. This is a necessary condition for the application of Equation 9. The Hg rate constant for water exchange, a parameter that has been linked to the ligand-exchange rate of metals, is one of the highest reported for trace metals (1×10^{-9} /s), which supports the rapid reestablishment of equilibrium in the aqueous phase during the octanol/water extraction [20]. In some studies examining the octanol/water partitioning of other trace metals, it has been assumed that the kinetics are too slow for equilibrium to establish once some of the metal complexes have been extracted into octanol [21–23]. In these cases, differences in the magnitude of the K_{OW} values and the octanol to water volume ratio need to be considered to calculate

the K_{OW} for different metal species [23–25]. In other words, Equation 9 cannot be used to determine the K_{OW} for individual metal species if equilibrium does not reestablish rapidly during extraction, resulting in the need for a more complex equation. Data are presented below supporting the rapid reestablishment of equilibrium speciation of Hg during the extraction procedure.

Ultrafiltration theory. Ultrafiltration of oxic water samples has been used in several studies to examine the complexation of Hg to different size classes of ligands. Previous studies have employed tangential flow ultrafiltration, which requires several liters of water, and have used membranes with a molecular-weight cutoff of 1,000 Da [26] or 10,000 Da [27]. During ultrafiltration, molecules less than the molecular-weight cutoff of the membrane will pass through the filter, whereas molecules larger than the molecular-weight cutoff will be retained by the filter. Because of the low Hg concentrations under natural conditions, filtration of large volumes of water is required. In laboratory-prepared solutions, however, the complexation of Hg can be examined using more concentrated solutions that require smaller sample volumes. This enables the use of small-volume (15-ml) centrifuge ultrafilters. The small sample volumes and relatively short duration required for centrifuge ultrafiltration (30 min) enable the examination of anoxic, sulfidic water samples, which would be difficult to do using other techniques. In the present study, Amicon Ultra-15 centrifugal filters (Millipore Corporation, Billerica, MA, USA) with a nominal weight limit of 5,000 Da were used to separate small, inorganic Hg complexes and larger complexes formed between Hg and DOM.

D_{OW} method

To use D_{OW} extractions to examine Hg complexation under natural conditions, several modifications were made to previously used D_{OW} extraction procedures to reduce Hg contamination and to eliminate analytical interference associated with residual octanol in the samples. In previous studies, high concentrations of Hg (0.5–200 $\mu\text{g/L}$) [13,17] or a radioactive Hg isotope [5] were used when D_{OW} extractions were conducted to determine Hg complexation. Therefore, issues with detection limits, which are of concern when examining Hg complexation in natural samples, were not relevant. To reduce Hg contamination, a three-step cleaning process (1 M KOH, 25% HNO₃, and 10% HCl) was performed on all labware used during the extractions. The KOH cleaning removes organic matter that is not otherwise broken down in the acid-cleaning process. Teflon® (fluorinated ethylene propylene) separatory funnels were used for all experiments.

For all extractions, 80 ml of the sample and 20 ml of octanol were added to the separatory funnels and shaken on an orbital rotation table for 2 h, unless otherwise noted. This was followed by collection of the aqueous phase in a second separatory funnel. In some samples, an emulsion formed between the octanol and the aqueous layer. When this occurred, as much of the emulsion as possible was included in the aqueous layer, but the difficulties associated with separating the layers likely resulted in some of the variability observed in the measured D_{OW} values. For experiments involving sulfide and for extraction of natural waters, extractions were conducted in an anaerobic chamber, and all Teflon-ware, including separatory funnels, were placed in the anaerobic chamber for a minimum of 5 d before use to remove oxygen.

Because octanol has a slight solubility in water (0.58

g/L) and interferes with the Hg analysis via the SnCl₂ reduction/gold-trap method, traces of octanol remaining in the aqueous phase were removed using a hexane extraction. Twenty milliliters of a 4.4 M potassium chloride solution in 5% trace metal-grade hydrochloric acid were added to each aqueous-phase sample before hexane extraction to ensure that greater than 95% of the Hg was present as charged Hg-chloride complexes (HgCl₃⁻, HgCl₄²⁻) [19] that would remain in the aqueous phase. This was followed by the addition of 15 ml of hexane (ultra resi-analyzed 95% *n*-hexane). After shaking for 1 h, the aqueous phase was collected, and the sample was purged with ultrahigh purity-grade argon in a 70 to 80°C water bath for 1 h to evaporate any remaining hexane. The octanol was not presaturated with water, because at the water to octanol ratio used in the present study, less than 1% of the water in the aqueous phase would partition into the octanol.

In some previous experiments [5,17,19], the Hg concentration in the octanol phase was determined by difference, based on the Hg concentration in the sample before extraction and the amount measured in the aqueous phase after extraction. In the present study, to ensure that no Hg was lost to Teflon-ware or evasion during the extractions, the Hg concentration in the octanol phase also was measured. A back-extraction technique was developed to remove Hg from the octanol phase into an aqueous medium, allowing standard Hg analysis. A potassium chloride solution (1.32 M in 4% hydrochloric acid) was added to the octanol phase after the aqueous phase had been removed, resulting in conversion of more than 95% of the Hg to charged Hg-chloride complexes, which ensures their partitioning into the aqueous solution. This aqueous solution was separated from the octanol, and the residual octanol in the aqueous solution was removed as described above.

Before applying D_{OW} extractions to natural water samples, several validation experiments were conducted to examine the background level of Hg associated with the extractions, the loss of Hg during the procedure, and the effect of extraction time on the results. Potential Hg contamination could arise from the octanol, hexane, and potassium chloride solutions used during the extractions. To determine both the level and the variability of Hg contamination as a result of the labware and reagents, the revised D_{OW} extraction procedure was carried out using unamended ultrapure water (18.2 M Ω /cm; Millipore). To examine whether Hg was lost during the extraction procedure, the mass recovery of a stable isotope of Hg added to ultrapure, lake, and estuarine water samples was assessed at the end of the D_{OW} procedure. The use of a stable isotope for recovery studies allowed clear distinction of any Hg contamination from the spike addition. For all experiments using an Hg stable isotope spike, stable isotope stock solutions were prepared using Hg-199-enriched solid HgO. This stable isotope, purchased from Oak Ridge National Laboratories (Oak Ridge, TN, USA), had a purity of 91.95%. The stock solution was prepared in a dilute acid solution, and the Hg-199 was in the form of Hg-199 chloride when added to the experimental solutions. For these studies, Hg-199 was added to the water samples 12 h before the extractions at a level of approximately 1.0 ng/L. The Hg-199 was measured in samples before extraction and in the aqueous and octanol phases after extraction. Water was collected from Lake Lariat, a high-DOM lake in Calvert County (MD, USA), and from the Patuxent River, a low-DOM and high-chloride estuarine system in Solomons (MD, USA).

Previously used Hg D_{OW} extraction methods have employed

Table 2. Characteristics of dissolved organic matter (DOM) isolates from the Suwannee River (USA) and the Florida Everglades (USA) used in the laboratory-prepared solutions

DOM	Isolation method	Average MW ^a	%C	%S	Reduced S (% of total S)	Carboxyl mol/[kg-C]	Aromatic C (%)
Suwannee ^b River DOM	Reverse osmosis	NR ^c	48.8	0.6	NR	9.85	NR
F1-HPoA ^d	XAD resin	1031	52.2	1.73	22.1	10.43	18.2

^a MW = Molecular weight.

^b Data for the Suwannee River natural organic matter was obtained from the International Humic Substances Society (St. Paul, MN, USA).

^c NR = Not reported.

^d F1-HPoA is a hydrophilic acid isolated from within Water Conservation Area 2A of the Florida Everglades and was provided by George Aiken (U.S. Geological Survey, Boulder, CO).

extraction times ranging from 20 min to 2 h [13,17,28]. To determine the amount of time required for steady-state concentrations to establish in the aqueous and octanol phases, two extraction time series were conducted. For both experiments, samples were amended with Hg-199 at approximately 1.0 ng/L. In the first time-series experiment, Lake Lariat water was used, and the D_{OW} was measured at 1, 2, and 4 h. In the second experiment, a 3.2×10^{-5} M, laboratory-prepared solution of sodium sulfide was used, and the D_{OW} was measured at 1, 2, 4, and 8 h. All laboratory solutions were prepared in 0.01 M phosphate buffer (pH 6), unless otherwise noted, to maintain an environmentally relevant pH. Use of a phosphate buffer also enabled comparison with previous Hg octanol/water partitioning studies in which phosphate buffers were used to prepare laboratory solutions [5,17].

To compare D_{OW} values measured using the modified extraction procedure presented here with those of previously conducted extractions, the D_{OW} values of Hg-chloride and Hg-DOM solutions were examined. Chloride solution extractions were conducted on a 4.3 ng/L Hg-199 solution in 0.014 M sodium chloride at pH 6.8. Extractions also were performed on Hg-DOM solutions using Suwannee River natural organic matter and a hydrophilic acid DOM isolate from the Florida Everglades (USA). Suwannee River natural organic matter was purchased from the International Humics Substance Society (St. Paul, MN, USA), and a hydrophilic isolate from the Florida Everglades (2BS-HPiA) was obtained from G. Aiken (U.S. Geological Society, Boulder, CO). The Suwannee River DOM is a freeze-dried isolate collected using reverse osmosis, resulting in a sample containing hydrophobic humic and fulvic acids, along with other soluble organics. The Florida Everglades DOM isolate was collected in the northern Everglades within the Water Conservation Area 2B; details of the location and characteristics of this isolate have been presented elsewhere [29]. The characteristics of the two DOM isolates are shown in Table 2. The Suwannee River DOM solution contained 30 mg/L of DOM and 1.4 ng/L of Hg-199, whereas the Florida Everglades solution contained 10 mg/L of DOM and 0.6 ng/L of Hg-199. Both solutions were prepared in 0.04 M phosphate buffer (pH 6), and as a result of no other strong complexing ligands being in solution, the Hg was completely complexed to the DOM. The modified extraction technique also was applied to two sulfidic, oxygen-free, ultrapure water solutions to test the method under anoxic conditions and to compare results with previous measurements of the K_{OW} of HgS^0 and $Hg(SH)_2^+$ [17].

Measurement of D_{OW} on natural and laboratory-prepared solutions

Extractions were performed on surface water collected from Lake Lariat, interstitial water separated from sediments col-

lected at Lake 658 at the Experimental Lakes Area (ON, Canada), and interstitial water collected from the Patuxent River in the community of California (MD, USA). The surface water from Lake Lariat and the interstitial water from Lake 658 were both oxic, whereas the interstitial water from the Patuxent River contained measurable sulfide. The characteristics of Lake Lariat are described above. Lake 658, at the Experimental Lakes Area in northwestern Ontario, has been amended with a Hg stable isotope, Hg-202, since 2001. Sediment was collected from Lake 658 in July 2004 and held under anaerobic conditions for 48 h before the interstitial water was isolated using 0.2- μ M disposable filter units (Nalgene, Rochester, NY, USA) in an anaerobic chamber. Lake Lariat surface water was filtered through quartz-fiber filters before extraction in an anaerobic chamber. Samples also were collected for sulfide and pH analysis.

To examine the species of Hg in a more controlled system while still preserving the important interactions occurring under natural conditions, a sediment slurry was prepared in the laboratory using 200 g of surficial (depth, 0–10 cm) estuarine sediment collected from Mackall Cove, a small inlet off the Patuxent River in St. Leonard (MD, USA), and 2 L of filtered and deoxygenated Patuxent River water. Enriched Hg-199 was added to the sediment slurry. The slurry was shaken in an anaerobic chamber for 48 h to allow time for bacterial sulfide production and equilibration of the isotope spike. Before the D_{OW} extraction, the water in the slurry was separated from the solid phase using quartz-fiber filters. Octanol/water extractions were performed in quadruplicate on the isolated water. The sulfide concentration and pH of the slurry water also were determined.

To examine the influence of DOM on the species of Hg in the presence of sulfide, octanol/water extractions were performed on laboratory solutions of Hg with two DOM isolates, each with and without sulfide. The DOM isolates were dissolved in a 0.04 M, deoxygenated phosphate buffer (pH 6) in an anaerobic chamber to a final concentration of 15 mg C/L. Sulfide was added to half of each DOM solution to a final concentration of approximately 20 to 30 μ M. Enriched Hg-199 also was added to reach an approximate concentration of 1.25 ng/L, and the solutions were allowed to equilibrate overnight before the extraction. Extractions were performed as described above for the slurry experiment.

Validation of centrifugal ultrafiltration

To our knowledge, the present study is the first to use centrifuge ultrafiltration to examine Hg complexation; therefore, to demonstrate the applicability of the centrifuge ultrafilters for this purpose, several solutions containing known complexes of Hg were examined. Amicon ultrafilters with a mo-

Table 3. Ultrafiltration validation experimental results

Solution	Speciation	Recovery	% Above filter	% In filtrate	% On filter
No ligands	Hg(OH) ₂ , 100%	90%	11%	21%	68%
0.01 M Chloride	HgCl ₂ , 91.3% HgCl ₃ ⁻ , 7.3% HgClOH 1.1%	93%	11%	21%	68%
0.01 M acetate	Hg(acetate) ₄ ²⁻ , 98.2% Hg(acetate) ₃ ⁻ , 1.7 %	96%	8%	16%	75%
1.9 × 10 ⁻⁵ M sulfide	HgS ⁰ , 53.1% HgS ₂ H ⁻ , 37.3 %	99%	16%	14%	70%
12.1 mg C/L of DOM ^a	Hg-DOM	102%	86%	2%	12%

^a DOM = dissolved organic matter.

lecular-weight cutoff of 5,000 Da were used for all ultrafiltration experiments. Solutions were prepared in 0.04 M phosphate buffer (pH 6) for all extractions. Five solutions were used, each containing only one Hg-complexing ligand. One solution was prepared without the addition of any strong Hg-complexing ligands, resulting in Hg(OH)₂ as the dominant Hg complex. Solutions also were prepared using 0.01 M NaCl, 0.01 M acetate, 1.9 × 10⁻⁵ M sulfide, and DOM (12.1 mg C/L); the dominant species in each of these solutions, as determined through thermodynamic calculation, are shown in Table 3. The sulfide solution was prepared in an anaerobic chamber using deoxygenated phosphate buffer (pH 6). Suwannee River DOM was used to prepare the DOM solution. An initial solution containing 35 mg of DOM (17 mg of C) was dissolved in 150 ml of phosphate buffer. This solution was ultrafiltered, and the DOM greater than 5000 Da was diluted in phosphate buffer and filtered through a glass-fiber filter. This solution was further diluted with phosphate buffer to obtain a DOM solution (12.1 mg C/L) containing only DOM greater than 5,000 Da. For all solutions, enriched Hg-199 was added to an approximate concentration of 24 ng/L. Ultrafiltration was performed on the solutions after they had equilibrated overnight. Inorganic complexes of Hg (Hg-chloride, Hg-hydroxide, and Hg-sulfide) and Hg complexed to acetate should all pass through the filter, because these complexes are less than 5,000 Da. The Hg-DOM complexes, however, should remain above the filter.

Three 15-ml aliquots of each solution were ultrafiltered. To prevent the sorption of Hg in the filtrate to the walls of the centrifuge tubes, 300 μl of 50% HCl were added to the bottom of the centrifuge tubes so that the filtrate was acidified to 1% HCl during filtration. After filtration, the solution above the filter was collected by rinsing the filters with three 5-ml aliquots of phosphate buffer and pouring off the sample into a separate centrifuge tube. This solution was acidified to 1% HCl to examine the amount of Hg sorbing to the filters, 15 ml of a bromine monochloride solution (0.13 M KBr and 0.06 M KBrO₃) were added to the filters after the solution remaining above the filter was collected. The bromine monochloride solution was held in the filter for a minimum of 24 h, and the amount of Hg extracted in this solution was determined. All of these fractions—the Hg in the unfiltered solution, the Hg in solution above the filter, the filtrate, and any Hg sorbed to the filter—were measured to calculate mass balance and spike recovery.

Examination of Hg complexation using ultrafiltration of DOM and DOM/sulfide solution

The ultrafiltration technique was applied to Hg, DOM, and sulfide mixtures to investigate the influence of DOM on the

complexation of Hg under sulfidic conditions. Anaerobic DOM solutions were prepared at 19.2, 12.1, and 5.9 mg C/L in deoxygenated phosphate buffer, as described above, using Suwannee River DOM greater than 5,000 Da. Sulfide was added to an aliquot of each DOM solution to obtain an approximate concentration of 10 μM. Enriched Hg-199 was added to each solution (~24 ng/L) at the same time the sulfide was added, and the solutions were equilibrated overnight. Each solution was ultrafiltered in triplicate. The amount of Hg in the unfiltered solution, the filtrate, the solution remaining above the filter, and that sorbed to the filter was determined as above. The pH and sulfide concentration in the solutions at the time of filtration also were determined.

Analytical methods

Total Hg analysis was done using standard Hg analytical methods [30] with inductively coupled plasma-mass spectrometry (ICP-MS) [31]. Stable isotope analysis of total Hg using the ICP-MS has been used in several studies in our laboratory [32,33], and the detection limit for this method generally is approximately 10 pg/L of Hg in aqueous samples. To oxidize any organic matter, bromine monochloride was added to the samples for a minimum of 24 h before analysis. Before analysis, hydroxylamine hydrochloride was added to the samples to reduce any excess bromine monochloride. The Hg in the samples was reduced using stannous chloride immediately before purging and amalgamation onto traps containing gold beads. The traps were heated, and the pulse of Hg released was detected using a Hewlett Packard 4500 ICP-MS (Agilent Technologies, Santa Clara, CA, USA) [31]. Background Hg concentrations were quantified using the Hg-202 peak. The calculations for determining the amount of enriched Hg-199 spike have been presented elsewhere [31]. Quantification was performed using external standards with appropriate spikes, blanks, and replicates.

A solid-state, ion-selective electrode and a reference electrode (Thermo Electron, Waltham, MA, USA) were used to analyze for sulfide [34]. Sample and standards were preserved in sulfide antioxidant buffer and analyzed within 4 h. Samples of DOM were analyzed using a Total Organic Carbon 5000 analyzer (Shimadzu, Kyoto, Japan) by the Nutrient Analytical Services Laboratory at the Chesapeake Biological Laboratory (Solomons, MD, USA).

Thermodynamic modeling

Speciation calculations were performed using the program MINEQL+ (Environmental Research Software, Hallowell, ME, USA). The formation constants for Hg-sulfide complexes [17] and a complexation constant for Hg to DOM based on

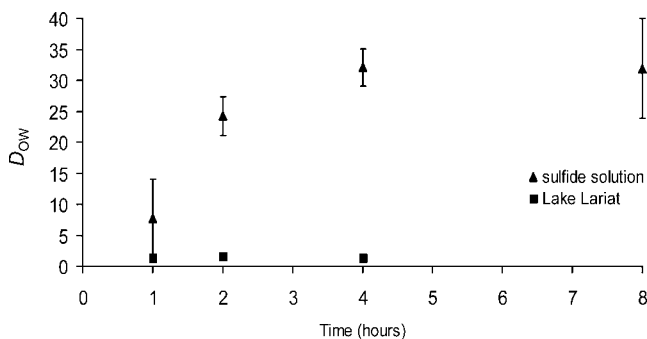


Fig. 1. Measured overall partitioning coefficient (D_{OW}) values over time in a sulfide solution and lake water spiked with Hg-199. Error bars represent the standard deviation of triplicate samples and fall within the symbols for the Lake Lariat (Calvert County, MD, USA) samples.

the molar concentration of DOM, as presented in Benoit et al. [5], were added into the MINEQL+ database. All other complexation constants were used directly from the MINEQL+ database.

RESULTS

Octanol/water partitioning experiments

D_{OW} method validation. The experiments designed to examine the detection limit and extraction recovery both validate the use of D_{OW} extraction for low-level natural samples. The average Hg blank associated with the extraction of 80 ml of ultrapure water was 34 ± 10 pg, based on three trials, each consisting of three blank measurements. The relative standard deviations were lower within each individual trial. In all subsequent extractions of natural waters, blanks were measured and subtracted from the sample concentrations. The detection limit, calculated as threefold the standard deviation of the blanks, for this method was 0.4 ng/L, making this method applicable to examine Hg in unamended samples. As a comparison, the concentration of Hg used in previous octanol/water studies ranged from 0.5 to 200 $\mu\text{g/L}$ [13,17,19].

The average recovery as determined using ultrapure, lake, and river water spiked with Hg-199 was $94\% \pm 4\%$, $95\% \pm 6\%$, and $96\% \pm 1\%$, respectively. These recoveries include Hg in the aqueous and octanol phases, which also could include Hg adsorbed to the separatory funnel wall during the initial extraction step. Because the recovery was high and consistent across different water matrices, subsequent extractions only examined the amount of Hg in the unextracted water and the aqueous phase. The amount of Hg associated with the octanol phase was determined by the difference between the whole-water and aqueous-phase Hg.

Previous Hg D_{OW} extraction methods have employed a range of extraction times. Two extraction time series using Lake Lariat water and a laboratory-prepared solution of sulfide showed no differences in the measured D_{OW} after 2 h (Fig. 1), suggesting that equilibrium was rapidly reestablished once Hg complexes were extracted into octanol. Thermodynamic calculations indicated that Hg-DOM complexes were the dominant Hg complexes in Lake Lariat water, and no difference in the measured D_{OW} was found over time. In the sulfide solution, the calculated Hg speciation was 8.4% HgS^0 , 33.6% Hg(HS)_2^0 , and 57.5% HgS_2H^- . The measured D_{OW} at 2, 4, and 8 h were not statistically different ($p = 0.01$), supporting the idea that equilibrium is rapidly reestablished once Hg is ex-

tracted into the octanol phase. For all subsequent extractions, an extraction time of 2 h was employed.

To compare our present D_{OW} study, which was done at environmentally relevant Hg concentrations, with previous studies, extractions were performed using chloride, sulfide, and DOM solutions. In the chloride solutions, thermodynamic calculations predict that HgCl_2 (81.5%), HgCl_3^+ (11.4%), and HgClOH (6.4%) dominate the speciation. Using the reported K_{OW} values for these species [19], the fraction of the three species present in the solution, and Equation 3, the predicted D_{OW} for this solution would be 3.1. The D_{OW} , as measured using the modified procedure at low levels of Hg, was 3.1 ± 2.3 , indicating that the modified D_{OW} extraction procedure is comparable to previously used extraction techniques.

The modified extraction technique also was applied to two sulfidic, oxygen-free, ultrapure water solutions to test the method under anoxic conditions and to compare results with previous measurements of the K_{OW} of HgS^0 and Hg(SH)_2^0 [17]. The measured D_{OW} values were 54 ± 9 for the first solution (1.0 ng/L of Hg-199, 20 μM sulfide, pH 6.1) and 28 ± 6 for the second solution (0.8 ng/L of Hg-199, 30 μM sulfide, pH 6.3). Using the predicted species of Hg in the two solutions, the calculated K_{OW} was 72 ± 15 . In calculating the K_{OW} values of HgS^0 and Hg(SH)_2^0 , it was assumed that charged Hg-sulfides do not partition into octanol and HgS^0 and Hg(SH)_2^0 partition into octanol to the same extent, as shown by Benoit et al. [17].

The calculated K_{OW} for both HgS^0 and Hg(SH)_2^0 were approximately a factor of three larger than the K_{OW} values for the same species determined by Benoit et al. [17] ($K_{OW} = 25$) but similar to those calculated based on Jay et al. [13]. Using D_{OW} data for Hg-sulfide solutions presented in Jay et al. [13], the calculated K_{OW} for HgS^0 and Hg(SH)_2^0 would be 66 ± 20 , a value similar to the K_{OW} calculated in this study. The lower value reported by Benoit et al. [17] could be explained by the differences in the extraction techniques. In the study by Benoit et al. [17], 96% of the Hg adsorbed to the extraction container walls or precipitated during the extraction. In the present study, both MINEQL+ calculations and filtration of the aqueous phase demonstrated that precipitation was insignificant and that adsorption to the container walls was, on average, 24.6%, with the highest adsorption to the walls occurring in samples containing DOM isolates.

Extractions also were performed on Hg-DOM solutions using Suwannee River natural organic matter (DOM) and a hydrophilic acid DOM isolate from the Florida Everglades [35]. The D_{OW} values for Hg bound to Suwannee River DOM and the Everglades DOM were 1.7 ± 1.1 and 3.3 ± 2.0 , respectively. The large variability associated with these measurements likely is a combination of the low concentration of Hg used in the present study and the difficulties associated with separation of the aqueous and octanol phases because of formation of an emulsion at the interface in some instances when DOM was in the samples. In the previous higher-concentration studies, samples were not collected at the interface of the aqueous and octanol phases, because only small subsamples of these phases were needed for analysis. As a result of the low Hg concentrations used in the present study, the entire aqueous and octanol phases were needed for analysis. Collecting the entire phase could account for the observed variability in the measurements, because the octanol/water interface was not always clearly defined.

Benoit et al. [5] reported a D_{OW} of 0.12 ± 0.03 for the Everglades DOM used in the present study, which is much

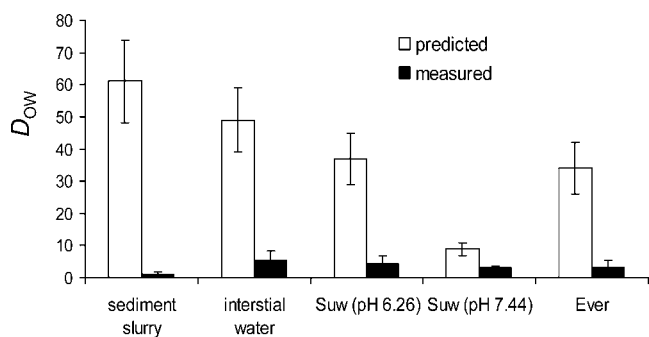


Fig. 2. Predicted (white bars) and measured (black bars) measured overall partitioning coefficient (D_{OW}) values for the sediment slurry, interstitial water, and three laboratory-prepared solutions. Two of the laboratory solutions contained Suwannee River (USA) natural organic matter (Suw) at two different pH values, and the third solution contained dissolved organic matter from the Florida Everglades (USA; Ever). Sulfide was added to each of these solutions. The predicted D_{OW} values were determined from thermodynamic speciation of Hg and known K_{OW} values for Hg-sulfide and Hg-DOM complexes. Error bars represent the standard deviation of three samples for the measured values. For the predicted values, the error bars are a result of the error associated with the K_{OW} values of the Hg complexes used to predict the D_{OW} values.

lower than our measured values. Namjesnik-Dejanovic and Cabaniss [36] used reverse-phase high-performance liquid chromatography for a suite of DOM samples to estimate D_{OW} values, which ranged from 12.9 to 37.2, suggesting that some fraction of DOM will partition into octanol. These D_{OW} values are much larger than our measurement D_{OW} values for Hg-DOM complexes, suggesting that only a small portion of the Hg would have to complex with the more hydrophobic portion of the DOM to explain our measurements. This is supported by the observation that a significant fraction of dissolved Hg is associated with large organic complexes or colloidal matter [26,37], which is more hydrophobic than Hg associated with small organic molecules. Using C-18 columns, it has been shown in several estuarine water samples that more than 50% of the Hg is present as hydrophobic organic complexes [38], also supporting the D_{OW} values measured for Hg-DOM complexes in the present study. Differences in the D_{OW} values presented here when compared to those reported by Benoit et al. [5] also could be a result of differences in the extraction techniques, as discussed above.

D_{OW} values of natural and laboratory-prepared solutions

Thermodynamic modeling and D_{OW} extractions of Lake Lariat surface water and Lake 658 interstitial water suggest

that Hg-DOM complexes were the dominant species in both of these systems. The ambient Hg concentrations for Lake Lariat and Lake 658 interstitial water were 0.8 and 1.2 ng/L, respectively. The sulfide concentrations in both water samples were less than the detection limits of our sulfide analysis ($<1 \times 10^{-7}$ M); therefore, the Hg complexation was predicted to be controlled by Hg-DOM complexes. The measured D_{OW} from Lake Lariat surface water and from Lake 658 interstitial water was 1.1 ± 0.4 and 1.2 ± 0.2 , respectively. These are lower than the D_{OW} values determined for DOM isolates, which ranged from 1.7 to 3.3. It is not unreasonable to assume, however, that the Hg in these natural water samples was bound to DOM, because it has been shown that the polarity of DOM varies between systems [36]. It is not possible with this technique to determine if inorganic Hg complexes, such as Hg-chloride complexes, are present, because the K_{OW} values for Hg-DOM and $HgCl_2$ are similar. As a result of the low chloride concentration and the high affinity of Hg for organic matter, MINEQL+ calculations show that any inorganic Hg-chloride complexes were unlikely to be present in these solutions.

Measured D_{OW} values for Hg in the sulfidic interstitial waters collected from the Patuxent River were much lower than predicted based on thermodynamic equilibrium modeling using the constants for Hg-sulfide and Hg-DOM complexes (Fig. 2). The interstitial water collected from the Patuxent River had a Hg concentration of 2.3 ng/L, a sulfide concentration of 2.2 μ M, and pH 7.25. Based on the equilibrium constants reported by Benoit et al. [17], the speciation of Hg in the interstitial waters should have been dominated by HgS^0 (68%), $HgHS_2^-$ (30%), and HgS_2^{2-} (2%) (Table 4). The predicted D_{OW} , as calculated using Equation 3 with the predicted speciation and the K_{OW} values for the dominant species, was 49.0 ± 10 . However, the measured D_{OW} was 5.4 ± 3.0 (Fig. 2). Because the affinity of Hg for sulfide is much higher than the affinity of Hg for DOM [5,9], models for Hg complexation under sulfidic conditions predict that inorganic sulfide complexes (HgS^0 , $Hg(SH)_2^0$, $HgHS_2^-$, HgS_2^{2-}) would dominate. At sulfide concentrations in the low micromolar range, the neutral complex should dominate, and D_{OW} values should be high, reflecting the K_{OW} of approximately 70 for HgS^0 . However, this did not appear to be the case for the interstitial water collected from the Patuxent River.

Similar discrepancies between the predicted and observed D_{OW} values also were observed with the water collected from the estuarine sediment slurry and from laboratory-prepared solutions of Hg, sulfide, and DOM isolates (Fig. 2). The Hg

Table 4. Characteristics of the natural and laboratory-prepared solutions used in the overall partitioning coefficient (D_{OW}) extractions^a

Experiment	pH	Sulfide (μ M)	Speciation
Patuxent River	7.25	2.2	HgS^0 , 68.1%
Interstitial water			$HgHS_2^-$, 29.7%
Sediment slurry	6.50	2.4	HgS^0 , 83.4%
			$HgHS_2^-$, 14.0%
Suwannee DOM ^b	6.29	20.8	HgS^0 , 44.1%
			$HgHS_2^-$, 47.6%
Suwannee DOM	7.44	26.0	HgS^0 , 13.1%
			$HgHS_2^-$, 79.0%
Everglades DOM	6.25	29.7	HgS^0 , 37.1%
			$HgHS_2^-$, 52.9%

^a The speciation was calculated using the MINEQL+ software program (Environmental Research Software, Hallowell, ME, USA).

^b DOM = Dissolved organic matter.

and sulfide concentrations and the pH for each of these experiments, along with the predicted Hg speciation, are presented in Table 4. In all cases, the measured D_{OW} was well below the predicted value (Fig. 2).

The low measured D_{OW} values relative to the predicted values suggests that neutral Hg-sulfide complexes either were not present or were present at much lower concentrations than predicted based on measured stability constants for HgS^0 , $Hg(HS)_2^0$, and Hg-DOM alone. It is important to note that because octanol/water extractions can only detect the presence of neutral Hg-sulfide complexes, it cannot be determined from these data if charged Hg-sulfide complexes are still present in the aqueous phase. Clearly, however, DOM influences the complexation of Hg under sulfidic conditions. Because the complexation constant for this interaction is not known, current thermodynamic models cannot accurately predict the speciation of Hg under sulfidic conditions.

Ultrafiltration studies

Ultrafiltration method validation. The results from the validation study for the ultrafiltration method (Table 3) demonstrate its applicability in examining Hg complexation. For all solutions, the recovery of the added Hg isotope was greater than 90%. In solutions containing Hg complexes of less than 5,000 Da (Hg-acetate, Hg-chloride, Hg-sulfide, and Hg-hydroxide complexes), 16% or less of the Hg remained above the filter after centrifugation. A large portion of the Hg (68–75%) sorbed onto the filter (measured as Hg desorbing from the filter with the addition of bromine monochloride), whereas the remaining Hg was in the filtrate.

Ultrafiltration of the Hg-DOM solutions further validated this method for use in examining the complexation of Hg. In the solution containing Hg and DOM, all the Hg should have been complexed to the DOM and, therefore, remained above the filter after filtration. The retention of Hg above the filter was 86%, whereas 12 and 2% was sorbed to the filter or present in the filtrate, respectively. The recovery of the added Hg was essentially 100% (Table 4). The ultrafiltration results of solutions containing large Hg-DOM complexes and smaller organic and inorganic Hg complexes support the use of this technique to examine the association of Hg with large complexes. For all experiments using this technique, it was assumed that all the Hg remaining above the filter was associated with DOM; therefore, this fraction of Hg will be referred to as DOM-associated Hg. It should be noted that as a result of the slight adsorption of Hg-DOM complexes to the filters, the abundance of Hg-DOM likely is slightly underestimated using this assumption.

Ultrafiltration of laboratory solutions. The complexation of Hg under anoxic conditions was examined at three DOM concentrations in the presence and absence of micromolar levels of sulfide. When sulfide was not present, greater than 80% of the Hg was DOM-associated, indicating that Hg-DOM complexes dominated the Hg speciation (Fig. 3), as would be predicted from thermodynamic models. In the solutions containing sulfide, where Hg-sulfide complexes were predicted to be the dominant species, 100% of the Hg should have either passed through or sorbed onto the filter. This was not observed. The amount of Hg remaining above the filter was between 38 and 63%, indicating that a mixture of small, inorganic Hg-sulfide complexes and larger-molecular-weight, Hg-DOM complexes were present in the experimental solutions. The amount of Hg associated with DOM increased with increasing

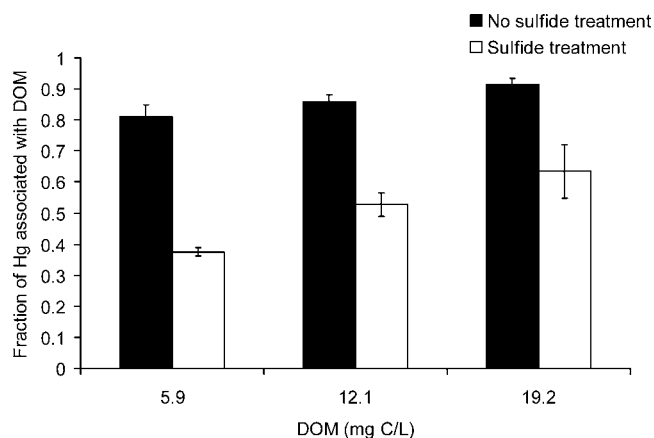


Fig. 3. Ultrafiltration results for solutions containing three concentrations of Suwannee River (USA) dissolved organic matter (DOM) in the absence (black bars) and presence (white bars) of sulfide. Dissolved organic matter concentrations are reported as mg C/L. Error bars represent the standard deviation of triplicate samples.

DOM concentration, suggesting that the interaction between Hg, sulfide, and DOM both exists and is dependent on the DOM concentration.

DISCUSSION

There appears to be an interaction between Hg, sulfide, and DOM that is not captured by the single-ligand complexation coefficients for Hg with sulfide and DOM. In both natural sulfidic interstitial waters and artificial solutions of Hg, sulfide, and DOM, the measured D_{OW} values were lower than would be predicted if the species of Hg were dominated by inorganic Hg-sulfide complexes. This interaction was further supported by the ultrafiltration experiments, which indicate that Hg is associated with DOM in the presence of sulfide. As a result of the much stronger interaction of Hg with inorganic sulfide relative to DOM, current thermodynamic equations and calculations predict that sulfide complexes dominate, but this does not accurately predict the results obtained, which demonstrate an interaction occurring between Hg, sulfide, and DOM.

The interaction of Hg with DOM in the presence of sulfide likely is not the result of a simple interaction of Hg with DOM, because this type of interaction is less thermodynamically favorable than the formation of inorganic Hg-sulfide complexes. The complex that forms likely involves Hg, DOM, and sulfide. A possible interaction between these three species involves a direct binding of DOM to a Hg-sulfide complex. As mentioned earlier, HgS^0 likely exists as a hydrated complex, $HOHgSH^0$. It is unlikely that DOM can displace the Hg-sulfide bond, but DOM could be interacting with the hydroxyl group on the $HOHgSH^0$ complex, resulting in a DOM-Hg-sulfide complex as described by Equation 10:



In this equation, HR represents an organic compound, with H being the proton on some functional group, such as a thiol or carboxylic acid.

Several studies have shown that DOM both inhibits the formation and enhances the dissolution of solid $HgS_{(s)}$ minerals [29,35,39], an interaction that could be similar to that between DOM and Hg-sulfide complexes observed in the present study. It has been proposed by those previous authors that DOM could be interacting with the surface of the solid $HgS_{(s)}$, resulting in

the dissolution of Hg from the solid as a dissolved Hg-DOM complex. Based on the stability constants of Hg-DOM and Hg-sulfide complexes, it is not likely that Hg-DOM complexes would be thermodynamically favorable in these solutions. The interaction between Hg, sulfide, and DOM shown in the present study provides a more likely explanation for the observed dissolution of HgS_(s) in the presence of DOM.

In studies examining HgS_(s) minerals, correlations were found between the extent of dissolution of HgS_(s) and the molecular weight, aromaticity, and specific ultraviolet absorbance of different DOM isolates, but it appeared that functional groups, such as thiols and carboxyl groups, on the DOM did not explain the observed dissolution of HgS_(s) [29,35,39]. This suggests that the interaction between Hg, sulfide, and DOM could be a function of the aromaticity and size of the DOM, suggesting that the interaction could involve a hydrophobic interaction between neutral Hg-sulfide complexes and hydrophobic DOM molecules.

Our determination that a significant interaction occurs between Hg-sulfide and DOM will require revision of our models for dissolved Hg species, solid partitioning, and bioavailability of Hg in anoxic environments. Because Hg-sulfide-DOM complexes exist in anoxic environments, reducing the fraction of the Hg in the inorganic Hg-sulfide complexes, our previous thermodynamic models [3,17] overestimated the abundance of dissolved inorganic Hg-sulfide complexes. To update previous thermodynamic models, a better understanding of the mechanism controlling the interaction of Hg, sulfide, and DOM and the formation constants for this interaction are required. Although the concentration of neutral dissolved Hg-sulfide in new models will be substantially lower than those we estimated when studying Hg bioavailability to *Desulfobulbus propionicus* [3], the concentration is sufficient to supply Hg for methylation by passive diffusion, because the estimated diffusion rates in previous experiments [7] were two to three orders of magnitude above the rate of MeHg production by cells. The complexation of Hg with larger organic complexes also may affect Hg uptake, because small organic Hg complexes could be taken up via active [4] or facilitated [40] transport. An understanding of the mechanism of this interaction and a determination of complexation coefficients for the Hg-sulfide-DOM complex are needed to adequately assess how our new finding affects Hg bioavailability, sorption, and flux.

Acknowledgement—The authors thank George Aiken at the U.S. Geological Survey for the use of a DOM isolated from the Florida Everglades. We also thank Tyler Bell and Georgia Riedel for help with sample collection. This work was partially supported by the National Science Foundation (NSF DEB 0451345) and formed part of the Ph.D. dissertation at the University of Maryland of C.L. Miller.

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