Article

Mercury Cycling in Stream Ecosystems. 2. Benthic Methylmercury Production and Bed Sediment#Pore Water Partitioning

Mark Marvin-DiPasquale, Michelle A. Lutz, Mark E. Brigham, David P. Krabbenhoft, George R. Aiken, William H. Orem, and Britt D. Hall

Environ. Sci. Technol., **Article ASAP** • DOI: 10.1021/es802698v • Publication Date (Web): 11 March 2009

Mercury Cycling in Stream Ecosystems. 2. Benthic Methylmercury Production and Bed Sediment–Pore Water Partitioning

MARK MARVIN-DIPASQUALE,*,† MICHELLE A. LUTZ,‡ MARK E. BRIGHAM,§ DAVID P. KRABBENHOFT,‡ GEORGE R. AIKEN,|| WILLIAM H. OREM,⊥ AND BRITT D. HALL‡,#

U.S. Geological Survey, 345 Middlefield Road, Menlo Park, California 94025, U.S. Geological Survey, 8505 Research Way, Middleton, Wisconsin 53562, U.S. Geological Survey, 2280 Woodale Drive, Mounds View, Minnesota 55112, U.S. Geological Survey, 3215 Marine Street, Suite E-127, Boulder, Colorado 80303, U.S. Geological Survey, 12201 Sunrise Valley Drive, Reston, Virginia 20192

Received September 23, 2008. Revised manuscript received January 20, 2009. Accepted January 29, 2009.

Mercury speciation, controls on methylmercury (MeHg) production, and bed sediment–pore water partitioning of total Hg (THg) and MeHg were examined in bed sediment from eight geochemically diverse streams where atmospheric deposition was the predominant Hg input. Across all streams, sediment THg concentrations were best described as a combined function of sediment percent fines (%fines; particles $< 63\ \mu$m) and organic content. MeHg concentrations were best described as a combined function of organic content and the activity of the Hg(II)-methylating microbial community and were comparable to MeHg concentrations in streams with Hg inputs from industrial and mining sources. Whole sediment tin-reducible inorganic reactive Hg ($Hg(II)_R$) was used as a proxy measure for the Hg(II) pool available for microbial methylation. In conjunction with radiotracer-derived rate constants of ^{203}Hg(II) methylation, $Hg(II)_R$ was used to calculate MeHg production potential rates and to explain the spatial variability in MeHg concentration. The $\%Hg(II)_R$ (of THg) was low (2.1 $\pm$ 5.7%) and was inversely related to both microbial sulfate reduction rates and sediment total reduced sulfur concentration. While sediment THg concentrations were higher in urban streams, %MeHg and $\%Hg(II)_R$ were higher in nonurban streams. Sediment pore water distribution coefficients (log K_d's) for both THg and MeHg were inversely related to the log-transformed ratio of pore water dissolved organic carbon (DOC) to bed sediment %fines. The stream with the highest drainage basin wetland density also had the highest pore water DOC concentration and the lowest log K_d's for both THg and MeHg. No significant relationship existed between overlying water MeHg concentrations and those in bed sediment or pore water, suggesting upstream sources of MeHg production may be more important than local streambed production as a driver of water column MeHg concentration in drainage basins that receive Hg inputs primarily from atmospheric sources.

Introduction

Mercury has long been recognized as being toxic to humans and wildlife. The specific factors that control its transport, speciation, and bioaccumulation in the environment are multifaceted and interact in ways that make predicting ecological outcomes difficult. The primary pathway of Hg toxicity in the environment begins with the conversion of inorganic divalent Hg (Hg(II)) to the more bioavailable methylmercury (MeHg), a process that is largely carried out by anaerobic bacteria in aquatic bed sediment (*1*). Once formed, MeHg can enter the benthic food web directly or can migrate from the sediment to the overlying water, where it can enter the base of the pelagic food web.

Net MeHg production reflects the difference between gross MeHg production and degradation (*2, 3*), with both processes having multiple biologic and abiotic controls. Environmental factors that mediate MeHg production include those that control the presence and activity of bacteria that carry out Hg(II) methylation and those that control the pool size of the inorganic Hg(II) available for the methylation process (*4*). Both sulfate-reducing and iron-reducing bacteria have been shown to methylate Hg(II) in natural settings, although not all bacteria represented in these two groups have this capability (*5, 6*). Environmental factors that affect the presence and activity of Hg(II)-methylating bacteria include temperature, pH, and the presence of suitable electron acceptors and donors.

A number of approaches have been used to assess what fraction of the total Hg(II) pool is available to Hg(II)-methylating bacteria, including thermodynamic modeling (*7, 8*), molecular approaches (*9*), and chemical extraction (*10*). Each approach has benefits and limitations, but as a whole suggest that only a small fraction of the THg pool is available for methylation. Herein, we examine the use of whole sediment tin-reducible Hg(II) (reactive inorganic mercury ($Hg(II)_R$) as a surrogate measure of the truly available Hg(II) pool.

Compared to lakes, reservoirs, estuaries, and marine systems, streams exhibit a strong hydrologic connectivity to their catchments. Consequently, the impact of catchment characteristics on within-stream physical and chemical properties should have a significant influence on within-stream Hg cycling. Most previous studies of benthic Hg cycling in streams focus on a single ecosystem and typically involve large drainage basins (*11*), estuarine-river systems (*12*), or settings impacted by known point sources of Hg contamination (*13*). In contrast, the current study examines eight chemically and ecologically diverse streams with a wide range of basin areas and land use cover, all of which receive Hg inputs largely from atmospheric loading to the drainage basin. This study takes advantage of the large range in bed sediment geochemical gradients among the study sites, to assess the relative influence of key geochemical parameters on stream bed sediment Hg cycling. The controls on MeHg production and the physical partitioning of THg and MeHg between streambed sediment and pore water are examined.

* Corresponding author phone: 650-329-4442; e-mail: mmarvin@usgs.gov.
† USGS, Menlo Park, California.
‡ USGS, Middleton, Wisconsin.
§ USGS, Mounds View, Minnesota.
|| USGS, Boulder, Colorado.
⊥ USGS, Reston, Virginia.
Current address: Department of Biology, University of Regina, 3737 Wascana Parkway, Regina, Saskatchewan, Canada S4S 0A2.

10.1021/es802698v

Companion publications focus on the water column (*14*) and food web (*15*) portions of this study. All stream and parameter codes and chemical abbreviations used in this report are summarized in Table S1 (Supporting Information).

Experimental Design

Study Sites. The eight streams are located in three states (OR, WI, and FL). Drainage basin sizes range from 62.4 to 2640 km^2, and the extent of wetlands within the individual drainage basins ranges from 0 to 36% (*16*). Three stream basins are located in urban/developed settings, while five are largely nonurban. Stream locations and basin characteristics are described in detail in ref *16*.

Geochemical Sampling. Samples were collected five times at multiple sampling areas within each stream from February 2003 to September 2004. Sampling areas represent a range of substrates from fine-grained, organic-rich deposits to sandy, low-organic deposits. Initial sampling involved collecting bed sediment at two to four discrete areas per stream to assess spatial variability. A single sampling area was approximately 2−10 m^2 (depending on the spatial heterogeneity of the benthic substrate), from which multiple bed sediment and pore water samples were composited (*17*). On the basis of initial results of microbial MeHg production potential (MPP) rates, the area with the highest MPP per stream was selected for sampling on subsequent field trips. Spatial variability was again revisited on the fifth and final field event when three to seven areas were sampled per stream as part of a larger stream reach characterization of Hg speciation, organic content (as percent loss on ignition (%LOI)), and percent fines (%fines; particles < 63 μm) (*17*). A total of 9−13 composite samples were collected from each stream during the entire study.

Bed sediment was collected from the surface 0−2 cm depth interval, and pore water was collected from a nominal depth of 2 cm below the sediment water interface following published methods (*17, 18*). Measurements fell into two categories: (a) those conducted on samples preserved in the field and (b) those performed on sieved (1 mm) sediment, which was transferred into mason jars until completely filled and held on ice (or refrigerated) until further processing in the laboratory for microbial rate assays and ancillary sediment and pore water constituents. Sediment redox decreased an average of 120 mV (±20 mV; std. error; $n = 75$) between the time of field collection and the time of further subsampling in the laboratory (2−10 days later).

Chemical Analyses. Laboratory methods, sample preservation, and quality-control procedures are detailed in ref *18*. Analyses performed on samples preserved in the field include bed sediment THg, MeHg, $Hg(II)_R$, %LOI, and %fines and pore water THg, MeHg, sulfide, ammonium, phosphate, and DOC. Sieved sediment subsampled in the laboratory for microbial rate assays and other constituents (immediately measured or preserved) reflects conditions at the time microbial assay incubations were conducted. Rate constants for microbial Hg(II) methylation (k_{meth}) and sulfate reduction (k_{SR}) were determined in parallel sets of subsamples, using standard radiotracer techniques ($^{203}HgCl_2$ and $Na_2{}^{35}SO_4$ amendments, respectively; ~24 h incubations under anoxic conditions at room temperature (20−22 °C)). MPP rates were calculated from k_{meth} and sediment $Hg(II)_R$ concentrations, as a first-order concentration-dependent process (*18*). Microbial sulfate reduction rates (SRR) were similarly calculated from k_{SR} and the concentration of pore water $SO_4{}^{2-}$. The total amount of Hg(II) added to MPP samples as part of the radiotracer amendment was 500 ng g^{-1} of wet sediment, which is significantly higher than the in situ sediment THg concentrations (approximately 1−45 ng g^{-1} wet weight). In contrast, the amount of carrier-free $^{35}SO_4{}^{2-}$ added to SRR samples was a small fraction of the in situ pore water $SO_4{}^{2-}$ concentration in all cases. Additional measurements associated with the composite samples included sediment acid volatile sulfur (AVS) and total reduced sulfur (TRS), acid-extractable ferrous iron ($Fe(II)_{AE}$), amorphous ferric iron ($Fe(III)_a$), pH and redox via electrode, as well as pore water sulfate and chloride. Bed sediment−pore water distribution coefficients (K_d, L kg^{-1}) for both THg and MeHg were calculated as the ratio of the dry weight sediment concentration (ng kg^{-1}) of that Hg species to its pore water concentration (ng L^{-1}).

Data Analyses. Statistical and graphical data analyses were performed using either the S-Plus software (version 6.1, Insightful Corp., Seattle, WA) or SAS software (release 9.1.3, SAS Institute Inc., Cary, NC). Significance probability was set at $P < 0.05$ for all statistical tests, except where noted. Data did not meet assumptions of either the normal or log-normal distribution for all benthic measurements (all data, all streams); therefore, a nonparametric test for survival curve differences using flipped data (*19*) was used to determine whether there were differences between the urban and nonurban streams. The survival curve test is based on differences between estimates of the cumulative distribution curves for each group and is akin to the Wilcoxon rank-sum test for uncensored data. Linear regression analysis was used to examine relationships between Hg metrics and biogeochemical factors. Regression analysis of log-transformed data sets with no censoring (all values greater than the method detection limit (MDL)) was performed using PROC REG in SAS. For data sets containing censored values (<MDL), maximum likelihood regression analysis was performed using SAS PROC LIFEREG, where R^2 values are "likelihood R^2" (*19*). Site-specific linear regression relationships were developed between dependent Hg variables (bed sediment THg and MeHg concentration, MPP rates) and the independent variable log[%LOI + 1]. Spatially integrated sediment THg and MeHg concentrations and MPP rates were determined for each stream reach using these relationships and the detailed sediment %LOI transect data collected during the last sampling event. Calculations are detailed in ref *17*.

Results and Discussion

Mercury Speciation and Cycling. Spatial variation (within and among streams) ranged from 100-fold to 10,000-fold for individual benthic constituents (Table S2 and Figure S1, Supporting Information). Spatially integrated THg and MeHg concentrations and MPP rates in the surface 0−2 cm bed sediment interval ranged 90-fold (4.5−387 μg m^{-2}), 180-fold (0.06−11.1 μg m^{-2}), and 710-fold (0.05−35.5 ng m^{-2} d^{-1}), respectively, across all eight streams (Table S3, Supporting Information).

The range of streambed sediment THg concentrations from the current study (0.7−211 ng g^{-1}; Table S2) was on the lower end of the range (1.9−4517 ng g^{-1}) reported in a survey of 106 streams throughout the United States (*20*), which did not include the current eight study sites but did include some sites affected by mercury and gold mining. Sediment grain size and organic content exert a dominant influence on sediment THg distribution (see below). This may partially account for the difference in THg concentration ranges between the two studies, as the current study targeted a range of substrate types (including fine-grained, organic-rich, and sandy streambed sediment), while the reconnaissance survey targeted fine-grain, organic-rich sediment only. In contrast, the range of MeHg concentrations from the current study (<0.1−17.8 ng g^{-1}; Table S2, Supporting Information) was comparable to that from the larger reconnaissance survey (0.01−10.9 ng g^{-1}). This discrepancy reflects that fact that MeHg and THg concentrations are often correlated at low levels of THg, as in this study (see below), but little additional MeHg is produced at very high levels of THg (*20*). These data

TABLE 1. Nonparametric Test of Survival Curve Differences between Urban and Nonurban Sites[a]

variable	units	*P*	median	
			nonurban	urban
sed THg	ng g^{-1} (dry)	0.020	8.90	23.1
sed %MeHg	% of THg	0.028	2.82	2.00
sed $Hg(II)_R$	ng g^{-1} (dry)	0.0003	0.210	0.049
sed $\%Hg(II)_R$	% of THg	<0.0001	1.82	0.31
sed SRR	nmol g^{-1} d^{-1} (dry)	0.0003	1.24	8.83
sed TRS	μmol g^{-1} (dry)	0.084	2.17	5.62
sed pH[b]	standard units	0.0094	6.73	6.83
pw THg	ng L^{-1}	0.035	2.14	1.32
pw MeHg	ng L^{-1}	0.051	0.34	0.12
pw SO_4^{2-}	mg L^{-1}	<0.0001	0.72	9.71
pw Cl^-	mg L^{-1}	<0.0001	7.60	31.80
pw NH_4^+	μg L^{-1}	0.0034	49.3	89.3
pw PO_4^{3-}	μg L^{-1}	0.0021	265	732
log [THg K_d]	L kg^{-1}	0.011	3.92	4.29

[a] All streambed sediment (sed) and pore water (pw) constituents listed had significantly different survival curves when tested as described in ref *19*. The statistical probability (*P*) level of significance is indicated for each comparison, and median values are provided for the urban and nonurban groupings. [b] pH of sediment associated with microbial assays at the time of incubation.

further suggest that streams receiving Hg inputs largely from atmospheric sources can vary widely in terms of THg and MeHg concentrations and exhibit MeHg levels comparable to those from a more diverse suite of streams, including those with Hg inputs from industrial and mining sources.

Urban versus Nonurban Streams. Significant differences in key Hg and non-Hg constituents were found in urban compared to nonurban streams (Table 1). While sediment THg was higher in urban streams, sediment %MeHg and pore water MeHg concentrations were higher in nonurban streams. Even though MPP rates were not significantly different between the two groupings, the %MeHg metric is often used as a proxy for net MeHg production (*21, 22*). Thus, there is a suggestion that benthic MeHg production was higher in the nonurban sites. The higher sediment $Hg(II)_R$ concentrations and $\%Hg(II)_R$, higher pore water THg concentrations, and lower THg K_d values in the nonurban streams suggest a pool of Hg(II) that is more readily available for Hg(II) methylation in the nonurban streams. The lower sediment $Hg(II)_R$ concentrations in the urban streams may be partially driven by the higher median SRRs and the resulting higher TRS concentrations, which may bind Hg(II) (*23*). The higher SRRs are largely driven by the significantly higher pore water SO_4^{-2} concentrations in the urban streams. The elevated SO_4^{-2} levels parallel the significantly higher concentrations of several other pore water constituents in the urban grouping, including Cl^-, NH_4^+, and PO_4^{3-}, which are consistent with urban runoff (*24*). Due to the small number of streams in the current study, it is possible that the underlying differences in the geologic settings of the urban streams selected, and not solely urban effects on geochemistry, may contribute to some of the observed differences. However, no significant differences in sediment %LOI or %fines were noted for urban versus nonurban streams. Further, the indication that there may be less net benthic MeHg production in the urban stream environment parallels other recent findings that suggest lower fish Hg levels in lakes located in more urbanized settings (*25*).

Controls on Mercury Speciation. Linear regression analyses of log-transformed data (Table 2) indicate that bed sediment THg concentration can be expressed as a positive function of bed sediment %fines, %LOI, TRS, or AVS, each used as a single independent variable. These findings are similar to those of other studies that have related either decreasing grain size (*26*), increasing organic content (*27, 28*), or increasing total sulfur concentrations (*29*) individually to increasing sediment THg concentrations. The strong significant relationships across all sites presented in Table 2, compared to these same relationships tested for individual streams, where some or most proved nonsignificant (Table S4, Supporting Information), indicate that these relationships explain variability over a much larger geochemical range than typically found in single stream reach.

TABLE 2. Least Squares Linear Regression Results of Logarithm (log, base 10) Transformed Stream Data[a]

Y variable	*X* variable	slope	*Y* intercept	R^2	*N*
log[sed THg]	log[%fines + 1]	1.04	0.16	0.86	85
log[sed THg]	log[%LOI]	0.79	0.72	0.83	94
log[sed THg]	log[TRS]	0.48	0.90	0.44	90
log[sed THg]	log[AVS]	0.51	1.10	0.26	90
log[sed MeHg]	log[sed THg]	1.27	−2.13	0.77	95
log[sed MeHg]	log[MPP]	0.42	−0.13	0.75	90
log[sed MeHg]	log[k_{meth}]	0.48	0.87	0.67	90
log[sed MeHg]	log[$\%Hg(II)_R$]	0.53	−0.14	0.12	95
log[sed MeHg]	log[%LOI]	1.32	−1.44	0.75	94
log[MPP]	log[%LOI]	2.00	−2.22	0.60	90
log[MPP]	log[SRR]	0.69	−1.28	0.26	90
log[k_{meth}]	log[SRR]	0.75	−3.22	0.43	90
log[k_{meth}]	log[%LOI]	1.53	−3.97	0.45	90
log[sed $Hg(II)_R$]	log[$Fe(III)_a$]	0.21	−0.67	0.19	90
log[$\%Hg(II)_R$]	log[SRR]	−0.32	−0.05	0.36	88
log[$\%Hg(II)_R$]	log[TRS]	−0.50	0.18	0.37	90
log[$\%Hg(II)_R$]	log[THg K_d]	−0.58	2.31	0.27	68
log[THg K_d]	log[%fines + 1]	0.96	3.08	0.69	61
log[THg K_d]	log[%LOI]	0.81	3.50	0.58	69
log[THg K_d]	log[$Fe(II)_{AE}$]	0.62	4.09	0.56	69
log[MeHg K_d]	log[%fines + 1]	1.33	1.76	0.66	51
log[MeHg K_d]	log[%LOI]	1.11	2.39	0.58	58

[a] Regressions were significant at probabilities (*P*) < 0.0001. Maximum likelihood regression (MLR) statistics were used if censored (< MDL) values were present.

Using stepwise linear regression and beginning with the significant single explanatory variables in Table 2, best fit multiple regression models were constructed for THg and MeHg. Bed sediment THg concentration was best described as a function of %LOI, %fines, and an interaction term, which accounted for 92% of the variability across all sites (Figure S2, Supporting Information). While %LOI and %fines were moderately and positively related (log-transformed data, linear $R^2 = 0.55$), and thus not fully independent, this simple model could be a useful screening tool for predicting sediment THg concentrations in other streams that receive

their Hg loads primarily from atmospheric sources. Bed sediment MeHg concentration was best described as a function of k_{meth} and %LOI, which accounted for 86% of the variability across all sites (Figure S2, Supporting Information). While the individual influences of both MPP rates (*30*) and organic content (*27, 31*) on benthic MeHg concentrations have been described for other systems, the current modeling results suggest that activity of the Hg(II)-methylating community and its interaction with sediment organic content most strongly impacted MeHg concentrations across the suite of streams studied, with the %LOI term potentially influencing both overall microbial rates and Hg(II) availability.

Sediment $Hg(II)_R$ concentration was a weak positive function ($R^2 = 0.19$) of sediment $Fe(III)_a$ concentration (Table 2), supporting the hypothesis that $Hg(II)_R$, as assayed, increases with oxic conditions and represents Hg(II) that is not part of a crystalline mineral matrix (*32, 33*). Further, the negative relationship between $\%Hg(II)_R$ and both microbial SRR and sediment TRS concentration (Table 2) suggests that Hg(II) associated with solid-phase reduced sulfur minerals is largely not measured in the $Hg(II)_R$ fraction. This conclusion is consistent with studies that demonstrate the tendency of Hg to be incorporated into chemically recalcitrant solid-phase sulfur minerals such as pyrite (*23*).

Controls on Methylmercury Production. Net MeHg production is controlled by the activity of the resident Hg(II)-methylating bacteria, the availability of inorganic Hg(II) for methylation, and microbial and abiotic MeHg degradation. While an examination of MeHg degradation is beyond the scope of this paper, MPP rates calculated here as a first-order function of k_{meth} and $Hg(II)_R$ (*18*) appear to help explain the relative importance of microbial activity versus Hg(II) availability. There are two important caveats. First, the ^{203}Hg(II) radiotracer amendment used to measure k_{meth} partitions between more and less bioavailable Hg(II) pools during the incubation period, although previous studies suggest that tracer Hg(II) added to sediment may be more available for methylation than in situ Hg(II) (*3, 22*). Second, the radiotracer amendment THg concentration (500 ng/g wet sediment) used throughout this study (*18*) was roughly 10−1000 times greater than in situ THg levels, depending on the site. The extent to which radiotracer partitioning occurred is not known and will vary depending on the geochemical conditions of the sample, the size of the amendment relative to the in situ THg concentration, and the incubation time. The comparatively high amendment concentrations used in the current study may partially offset the extent to which the ^{203}Hg(II) partitions into less available pools during the short-term incubation period, and in this case, k_{meth} may be proportionally more reflective of microbial activity. Thus, while k_{meth} measured via radiotracer addition provides valuable comparative information with respect to microbiological processes, it is an imperfect measure of the actual Hg(II)−microbial community activity exclusively.

Streambed MPP rates were a function of sediment THg, %LOI, and SRR as individual independent variables (Table 2). Since both k_{meth} and $Hg(II)_R$ were used to calculate MPP, these were not included as independent variables. While sediment THg and MeHg concentrations are often poorly correlated (*31*), THg concentration, combined with the site-specific geochemical conditions, sets the upper boundary on bioavailable Hg(II) concentration, and thus on MPP rates. Organic content influences both Hg(II)-methylating bacteria activity (k_{meth}; Table 2) and sediment TRS ($R^2 = 0.34$, not shown), the latter being negatively related to $\%Hg(II)_R$ (Table 2). The weak positive MPP−SRR relationship ($R^2 = 0.26$) and somewhat stronger positive k_{meth}−SRR relationship ($R^2 = 0.43$) both reflect the well-established role of sulfate-reducing bacteria in the Hg(II)-methylation process. The strength of these relationships may reflect the role other bacterial groups (e.g., Fe(III) reducers) play in the methylation process (*6*). Alternatively, they reflect processes limiting Hg(II) availability, as it impacts either k_{meth} (as discussed above) or $Hg(II)_R$ (discussed below) or both. Stepwise multiple linear regression of MPP as a function of THg, %LOI, and SRR indicated that MPP was best described as a function of %LOI alone, although the model fit was modest ($R^2 = 0.33$, not shown).

Increasing SRRs are generally associated with increasing pools of reduced sulfur end products in both the solid phase and pore water (*34*). Thus, the negative relationship between $\%Hg(II)_R$ and both microbial SRR and sediment TRS concentration (Table 2) may partially explain why MPP rates and MeHg concentration are often poorly (*2, 30*) or inversely (*35*) correlated with microbial SRR. To the extent that reduced-S compounds decrease the pool of Hg(II) available for methylation, this represents a negative feedback on the process of MeHg production by sulfate-reducing bacteria. The corollary to this, the increase in $Hg(II)_R$ under oxic conditions, has been experimentally demonstrated (*32*) and likely reflects the release of Hg(II) adsorbed to the surface of reduced sulfur minerals upon their oxidative dissolution.

An alternative approach used to define the bioavailable Hg(II) pool for methylation is to subtract sediment MeHg concentrations from THg concentrations (*27, 36*). However, this calculation assumes that all of the sedimentary inorganic Hg(II) is equally available for methylation, which is likely not the case. Since sediment MeHg is typically a small percentage (<5%) of THg (*30*), this leads to a high calculated percentage of Hg(II) (>95% of THg), although results from sequential extraction experiments indicate that chemical "reactivity" of the THg pool varies widely (*10, 37*), suggesting that all inorganic Hg(II) is not equally available for methylation.

Previous studies indicate that the whole sediment tin-reducible $Hg(II)_R$ fraction, as assayed in the current study (*18*), includes (a) pore-water-dissolved Hg(II) that is not strongly complexed with DOC and particle-associated Hg(II) that is weakly surface-bound (e.g., $HgCl_2$, $HgSO_4$, etc.) and (b) that which is correlated with net MeHg production in controlled laboratory experiments (*33, 38*) (M. Marvin-DiPasquale, unpublished data). Thus, $Hg(II)_R$ may be a reasonable proxy for the fraction of THg that is available for Hg(II) methylation. Similar approaches to define "reactive" Hg(II) using tin reduction have been applied to overlying water (*11, 39*) and pore water (*39, 40*), but typically not to whole sediment or with the expressed intent of its use as a proxy for bioavailable Hg(II).

As a percentage of THg, sediment $Hg(II)_R$ varies widely among the current study sites (<0.01−54%, Table S2, Supporting Information) but is typically a small fraction of THg (mean ± SD, 2.1 ± 5.7%; $N = 95$). This implies that using $Hg(II)_R$, in conjunction with a given k_{meth} measurement, will result in a much lower calculated MPP rate, than if either THg or Hg(II) calculated from [THg − MeHg] were used, but one which may more accurately reflect site-specific differences in native Hg(II) availability. Other studies have relied on the tracer-derived values of k_{meth} alone as a measure of relative Hg(II) methylation (*8, 28*). To the extent that the added tracer mimics the in situ Hg(II) pool by partitioning into available and nonavailable fractions, the k_{meth} calculated is reflective of both the Hg(II)-methylating community activity and Hg(II) availability. However, the relative influence of these two factors on the measured k_{meth} value is generally unknown, and as incubation time increases, the degree to which the ^{203}Hg(II) spike is available for microbial methylation decreases. In the current study, MPP rates calculated using k_{meth} and $Hg(II)_R$ explained only slightly more (75%) of the variability in sediment MeHg concentration than did k_{meth} alone (67%; Table 2). These results suggest that either microbial activity plays a larger role than Hg(II) bioavailability in dictating MeHg production or that k_{meth} already accounted

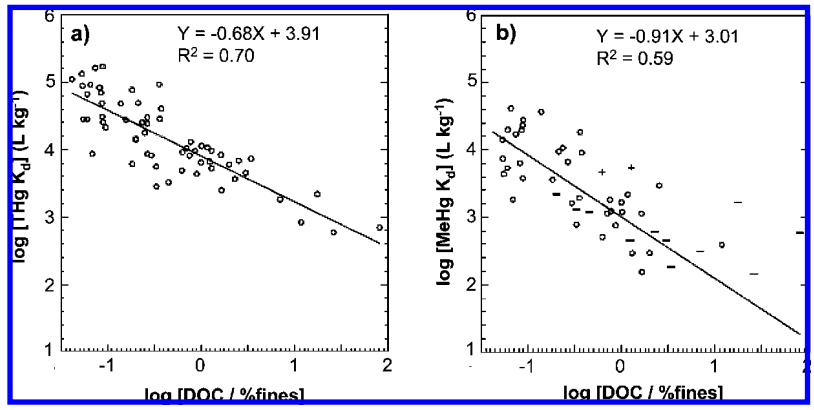

FIGURE 1. Log-transformed (bed sediment and pore water) distribution coefficients (K_d) of (a) THg and (b) MeHg versus the log-transformed ratio of pore-water dissolved organic carbon (DOC) to the percentage of fines (%fines; <63 μm fraction) in streambed sediment. Symbols denote K_d values calculated from noncensored sediment-bound and filtered Hg data (○), left-censored K_d (−) due to censored sediment-bound Hg data, and right-censored K_d (+) due to censored filtered Hg data. Where both filtered and sediment-bound forms of Hg were censored, K_d was not calculated. For MeHg (B), maximum likelihood regression statistics were used.

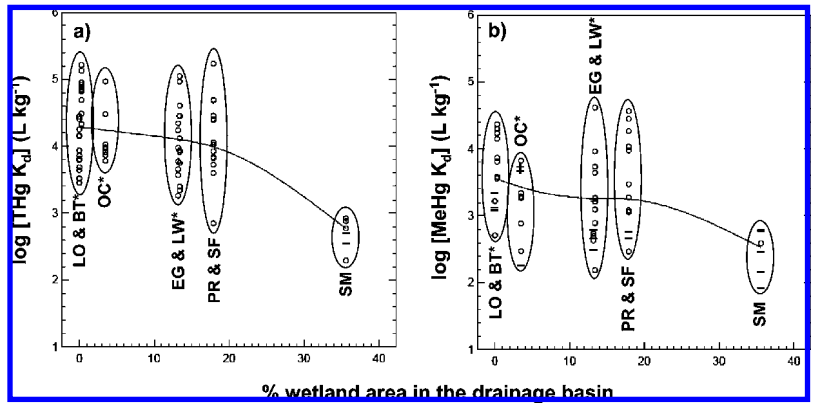

FIGURE 2. Bed sediment/pore water distribution coefficients (K_d, log transformed) for (a) total mercury (THg) and (b) methylmercury (MeHg) as a function of the percentage (%) of wetland in each stream drainage basin. Symbols, based upon the level of K_d data censoring, are defined as in Figure 1. Stream codes are defined in Table S1 (Supporting Information) and are aligned with data groupings based upon % wetland. The curve fit to the data represents a spline function. The urban sites are denoted with an asterisk (*).

for differences in Hg(II) bioavailability to a significant degree. While the value of using $Hg(II)_R$ to calculate MPP rates was modest in the current study, and questions remain as to how accurately $Hg(II)_R$ reflects truly bioavailable Hg(II), the $Hg(II)_R$ measurement provides a useful tool for examining relative differences in Hg(II) availability that are imparted by variations in sediment biogeochemistry.

Controls on Bed Sediment−Pore Water Partitioning. The distribution of Hg(II) and MeHg between streambed sediment and pore water affects the availability of these species for methylation and degradation, respectively. Partitioning of Hg species from pore water onto sediment (increasing K_d) may increase with decreasing sediment grain size (*36*), increasing organic content (*21, 27, 36*) and increasing reduced sulfur concentrations (*28*). Conversely, DOC contains strong Hg binding ligands, enhancing Hg dissolution into pore water (*41*), particularly in systems with elevated levels of pore water sulfide, where the formation of DOC−Hg−SH complexes has been proposed (*7*).

In this study, median values of log K_d ranged from <2.74 to 4.87 (130-fold variation in K_d) for THg, and from <2.53 to 4.15 (40-fold variation in K_d) for MeHg (Table S2, Supporting Information). Across all streams, log K_d values for both THg and MeHg exhibited strong and positive individual relationships to bed sediment %fines, %LOI, and $Fe(II)_{AE}$ (Table 2), the latter being indicative of solid-phase FeS or particle-adsorbed Fe(II) that forms under reducing conditions. Regressions of log K_d with pore water DOC or solid-phase sulfur species (AVS and TRS) were significant but had low R^2 values (<0.2). The negative relationship between log[$\%Hg(II)_R$] and log[THg K_d] (Table 2) suggests that an increase in partitioning of Hg(II) to the pore water phase reflects an increase in bioavailable Hg(II) by the $Hg(II)_R$ assay.

Across all sites, K_d values for both THg and MeHg were best described as an inverse function of log[DOC/%fines] (Figure 1), which indicates that, as the relative amount of pore water DOC increases or the bed sediment grain size increases (%fines decreases), more THg and MeHg partitions into pore water. Increasing sediment %fines represents an increase in the surface area to volume ratio of particles, and thus more solid phase binding sites for Hg species. This inverse function reflects competition between the solid-phase binding of Hg species and the capacity for DOC to pull Hg species into solution and is similar to the relationship described by Hammerschmidt et al. (*42*) in coastal marine sediment, where K_d values for both THg and MeHg were positively related to the K_d for organic carbon.

Importance of Drainage Basin Characteristics. Drainage basin characteristics can have a significant effect on the quantity and quality of particles and dissolved organic matter that is transferred into streams. The streams in this study exhibited large ranges in median values of sediment %fines, %LOI, and pore water DOC (Table S2, Supporting Information). There was a moderate positive relationship between

%fines and %LOI ($R^2 = 0.55$), a weak positive relationship between sediment %LOI and pore water DOC ($R^2 = 0.18$), and no relationship between sediment %fines and pore water DOC (all sites, regressions using log-transformed variables). For these eight streams, the wetland density in the drainage basin (*16*) was positively related to pore water DOC ($R^2 = 0.63$), but not to bed sediment %fines or %LOI (log-transformed median values from Table S2, Supporting Information). While log K_d values varied widely for both THg and MeHg within and among streams, there was a moderate inverse trend between percent wetland in the drainage basin and K_d values, with a decrease of approximately 1.5 log units (30-fold) in the case of THg K_d and 1.0 log unit (10-fold) in the case of MeHg K_d as streams increase in wetland density (Figure 2). These findings imply that, to the extent higher wetlands densities within a drainage basin lead to elevated pore water DOC concentrations, proportionally more THg and MeHg partitions into the pore water phase (Figure 1). To the extent that nonurban sites generally had equivalent or higher wetland densities in their drainage basins, compared to urban sites (with the exception of Lookout Cr., OR), this may partially explain our findings that nonurban sites had significantly lower log[THg K_d] values (Table 1). As lower THg K_d values reflect more $Hg(II)_R$ in the pore water phase (and higher $\%Hg(II)_R$ overall), this in turn may drive the observed higher propensity for MeHg production in the nonurban streams, as indicated by the sediment %MeHg metric. The conclusion that basin characteristics, particularly land use and wetland density, have a significant influence on the partitioning of THg, Hg(II), and MeHg in streambed sediment parallels the conclusions of the other two components of the study, where wetland density and DOC were primary factors controlling Hg concentration in overlying water (*14*) and biota (*15*).

While bed sediment and pore water MeHg concentrations were significantly correlated with each other (Pearson's $r = 0.77$; $P < 0.02$), there was no significant correlation between filtered overlying water MeHg concentration and either bed sediment or pore water MeHg concentration (not shown). This indicates that, across the range of streams studied, overlying water MeHg concentrations may be more reflective of MeHg production and transport occurring upstream, either in-channel or in hydrologically connected wetlands or soils.

Acknowledgments

This study was supported by the National Water-Quality Assessment and Toxic Substances Hydrology Programs of the USGS. We thank the following USGS staff: J. L. Agee, H. A. Harms, L. H. Keiu, J. R. Flanders, N. Ladizinski, and A.R. Stewart in Menlo Park, CA; J. F. DeWild, M. L. Olson, S. Olund, A. H. Bell, and B. C. Scudder in Middleton, WI; K. Butler in Boulder, CO; M. D. Corum in Reston, VA.; L. C. Chasar in Tallahassee, FL; and D. A. Wentz in Portland, OR.

Supporting Information Available

Table S1 summarizes definitions for stream codes, parameter codes, and chemical abbreviations used throughout this report. Table S2 details stream-specific summary statistics (medians, number of observations, and data ranges) for streambed sediment and pore water constituents discussed in this report. Table S3 includes spatially integrated data for THg and MeHg concentrations, MeHg production potential rates in streambed surface (0−2 cm) sediment, and information regarding how these values were calculated for each heterogeneous stream reach. Table S4 expands the regression analyses presented in Table 2 by including regression statistics for individual streams. Figure S1 shows the stream-specific data distribution (box and whisker plots) for bed sediment THg, MeHg, and $Hg(II)_R$ concentrations and MPP rates, and for pore water, THg and MeHg concentrations. Figure S2 shows measured versus predicted bed sediment THg and MeHg concentrations, where predicted values were derived from best-fit multiple regression models. This information is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Morel, F. M. M.; Kraepiel, A. M. L.; Amyot, M. The chemical cycle and bioaccumulation of mercury. *Annu. Rev. Ecol. Syst.* **1998**, *29*, 543–566.

(2) Marvin-DiPasquale, M.; Agee, J. L. Microbial mercury cycling in sediments of the San Francisco Bay-Delta. *Estuaries* **2003**, *26*, 1517–1528.

(3) Hintelmann, H.; Keppel-Jones, K.; Evans, R. D. Constants of mercury methylation and demethylaiton rates in sediments and comparison of tracer and ambient mercury availability. *Environ. Toxicol. Chem.* **2000**, *19*, 2204–2211.

(4) Ullrich, S. M.; Tanton, T. W.; Abdrashitova, S. A. Mercury in the aquatic environment: A review of factors affecting methylation. *Crit. Rev. Environ. Sci. Technol.* **2001**, *31*, 241–293.

(5) King, J. K.; Kostka, J. E.; Frischer, M. E.; Saunders, F. M.; Jahnke, R. A. A quantitative relationship that demonstrates mercury methylation rates in marine sediments are based on the community composition and activity of sulfate-reducing bacteria. *Environ. Sci. Technol.* **2001**, *35*, 2491–2496.

(6) Kerin, E. J.; Gilmour, C. C.; Roden, E.; Suzuki, M. T.; Coates, J. D.; Mason, R. P. Mercury methylation by dissimilatory iron-reducing bacteria. *Appl. Environ. Microbiol.* **2006**, *72*, 7919–7921.

(7) Miller, C. L.; Mason, R. P.; Gilmour, C. C.; Heyes, A. Influence of dissolved organic matter on the complexation of mercury under sulfidic conditions. *Environ. Toxicol. Chem.* **2007**, *26*, 624–633.

(8) Drott, A.; Lambertsson, L.; Bjorn, E.; Skyllberg, U. Importance of dissolved neutral mercury sulfides for methyl mercury production in contaminated sediments. *Environ. Sci. Technol.* **2007**, *41*, 2270–2276.

(9) Kelly, C. A.; Rudd, J. W. M.; Holoka, M. H. Effect of pH on mercury uptake by an aquatic bacterium: Implications for Hg cycling. *Environ. Sci. Technol.* **2003**, *37*, 2941–2946.

(10) Bloom, N. S.; Preus, E.; Katon, J.; Hiltner, M. Selective extractions to assess the biogeochemically relevant fractionation of inorganic mercury in sediments and soils. *Anal. Chim. Acta* **2003**, *479*, 233–248.

(11) Domagalski, J. Mercury and methylmercury in water and sediment of the Sacramento River Basin, California. *Appl. Geochem.* **2001**, *16*, 1677–1691.

(12) Heyes, A.; Miller, C.; Mason, R. P. Mercury and methylmercury in Hudson River sediment: impact of tidal resuspension on partitioning and methylation. *Mar. Chem.* **2004**, *90*, 75–89.

(13) Hines, M. E.; Horvat, M.; Faganeli, J.; Bonzongo, J. C. J.; Barkay, T.; Major, E. B.; Scott, K. J.; Bailey, E. A.; Warwick, J. J.; Lyons, W. B. Mercury biogeochemistry in the Idrija River, Slovenia, from above the mine into the Gulf of Trieste. *Environ. Res.* **2000**, *83*, 129–139.

(14) Brigham, M. E.; Wentz, D. A.; Aiken, G. R.; Krabbenhoft, D. P. Mercury cycling in stream ecosystems. 1. Water column chemistry and transport. *Environ. Sci. Technol.* **2009**, .

(15) Chasar, L. C.; Scudder, B. C.; Stewart, A. R.; Bell, A. H.; Aiken, G. R. Mercury cycling in stream ecosystems. 3. Trophic dynamics and methylmercury bioaccumulation. *Environ. Sci. Technol.* **2009**, .

(16) Bell, A. H.; Lutz, M. A. *Environmental settings of selected streams sampled for mercury in Oregon, Wisconsin, and Florida, 2002−06*; Open-File Report 2008-1277, U.S. Geological Survey: Reston, VA, 2008. Available at http://pubs.usgs.gov/of/2008/1277/ (accessed Feb 2009).

(17) Lutz, M. A.; Brigham, M. E.; Marvin-DiPasquale, M. *Procedures for collecting and processing streambed sediment and pore water for analysis of mercury as part of the National Water-Quality Assessment Program*; Open-File Report 2008-1279, U.S. Geological Survey: Reston, VA, 2008. Available at http://pubs.usgs.gov/of/2008/1279/ (accessed Feb 2009).

(18) Marvin-DiPasquale, M. C.; Lutz, M. A.; Krabbenhoft, D. P.; Aiken, G. R.; Orem, W. H.; Hall, B. D.; DeWild, J. F.; Brigham, M. E. *Total Mercury, Methylmercury, Methylmercury Production Potential, and Ancillary Streambed-Sediment and Pore-Water Data for Selected Streams in Oregon, Wisconsin, and Florida, 2003−04;* Data Series 375; U.S. Geological Survey: Reston, VA, 2008. Available at http://pubs.usgs.gov/ds/375/ (accessed Feb 2009).

(19) Helsel, D. R. *Nondetects and Data Analysis: Statistics For Censored Environmental Data*; John Wiley & Sons, Inc.: New York, 2005.
(20) Krabbenhoft, D. P.; Wiener, J G.; Brumbaugh, W. G.; Olson, M. L.; DeWild, J. F.; Sabin, T. J. *A National Pilot Study of Mercury Contamination of Aquatic Ecosystems along Multiple Gradients Water-Resources Investigation Report 99-4018B*; U.S. Geological Survey: Reston, VA, 1999. Available at http://toxics.usgs.gov/pubs/wri99-4018/Volume2/sectionB/2301_Krabbenhoft/index.html (accessed Feb 2009).
(21) Sunderland, E. M.; Gobas, F. A. P. C.; Branfireun, B. A.; Heyes, A. Environmental controls on the speciation and distribution of mercury in coastal sediments. *Mar. Chem.* **2006**, *102*, 111–123.
(22) Benoit, J. M.; Gilmour, C. C.; Heyes, A.; Mason, R. P.; Miller, C. L. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In *Biogeochemisty of Environmentally Important Trace Elements*; Cai, Y., Braids, O. C., Eds.; American Chemical Society: Washington, DC, 2003.
(23) Huerta-Diaz, M. A.; Morse, J. W. Pyritization of trace metals in anoxic marine sediments. *Geochim. Cosmochim. Acta* **1992**, *56*, 2681–2702.
(24) Carpenter, S. R.; Caraco, N. F.; Correll, D. L.; Howarth, R. W.; Sharpley, A. N.; Smith, V. H. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **1998**, *8*, 559–568.
(25) Chen, C. Y.; Stemberger, R. S.; Kamman, N. C.; Mayes, B. M.; Folt, C. L. Patterns of Hg bioaccumulation and transfer in aquatic food webs across multi-lake studies in the Northeast US. *Ecotoxicology* **2005**, *14*, 135–147.
(26) Hunerlach, M. P.; Alpers, C. N.; Marvin-DiPasquale, M.; Taylor, H. E.; De Wild, J. F. *Geochemistry of Mercury and other trace elements in fluvial tailings upstream of Daguerre Point Dam, Yuba River, California, August 2001; Scientific Investigations Report 2004−5165*; U.S. Geological Survey: Reston, VA, 2004. Available at http://pubs.usgs.gov/sir/2004/5165/ (accessed Feb 2009).
(27) Hammerschmidt, C. R.; Fitzgerald, W. F. Methylmercury cycling in sediments on the continental shelf of southern New England. *Geochim. Cosmochim. Acta* **2006**, *70*, 918–930.
(28) Han, S.; Obraztsova, A.; Pretto, P.; Choe, K.-Y.; Gieskes, J.; Deheyn, D. D.; Tebo, B. M. Biogeochemical factors affecting mercury methylation in sediments of the Venice Lagoon, Italy. *Environ. Toxicol. Chem.* **2007**, *26*, 655–663.
(29) Marins, R. V.; Lacerda, L. D.; Paraquetti, H. H. M.; dePaiva, E. C.; Boas, R. C. V. Geochemistry of mercury in sediments of a subtropical coastal lagoon, Sepetiba Bay, southeastern Brazil. *Bull. Environ. Contam. Toxicol.* **1998**, *61*, 57–64.
(30) Heyes, A.; Mason, R. P.; Kim, E.-H.; Sunderland, E. Mercury methylation in estuaries: Insights from using measuring rates using stable mercury isotopes. *Mar. Chem.* **2006**, *102*, 134–147.
(31) Lambertsson, L.; Nilsson, M. Organic material: The primary control on mercury methylation and ambient methyl mercury concentrations in estuarine sediments. *Environ. Sci. Technol.* **2006**, *40*, 1822–1829.
(32) Marvin-DiPasquale, M.; Cox, M. H. *Legacy Mercury in Alviso Slough, South San Francisco Bay, California: Concentration, Speciation and Mobility Open-File Report number 2007-1240*; U.S. Geological Survey: Reston, VA, 2007. Available at http://pubs.usgs.gov/of/2007/1240/ (accessed Feb 2009).
(33) Marvin-DiPasquale, M.; Hall, B. D.; Flanders, J. R.; Ladizinski, N.; Agee, J. L.; Kieu, L. H.; Windham-Myer, L. *Ecosystem Investigations of Benthic Methylmercury Production: A Tin-Reduction Approach for Assessing the Inorganic Mercury Pool Available for Methylation*, Eighth International Conference on Mercury as a Global Pollutant, Madison, WI, August 6−11, 2006.
(34) Marvin-DiPasquale, M. C.; Capone, D. G. Benthic sulfate reduction along the Chesapeake Bay central channel. I. Spatial trends and controls. *Mar. Ecol.: Prog. Ser.* **1998**, *168*, 213–228.
(35) Gilmour, C. C.; Riedel, G. S.; Ederington, M. C.; Bell, J. T.; Benoit, J. M.; Gill, G. A.; Stordal, M. C. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochem.* **1998**, *40*, 327–345.
(36) Bloom, N. S.; Gill, G. A.; Cappellino, S.; Dobbs, C.; McShea, L.; Driscoll, C.; Mason, R.; Rudd, J. Speciation and cycling of mercury in Lavaca Bay, Texas, sediments. *Environ. Sci. Technol.* **1999**, *33*, 7–13.
(37) Hall, G. E. M.; Pelchat, P.; Percival, J. B. The design and application of sequential extractions for mercury, Part 1. Optimization of HNO_3 extraction for all non-sulphide forms of Hg. *Geochem. Explor. Environ. Anal.* **2005**, *5*, 107–113.
(38) Bloom, N.; Bollen, A.; Briscoe, M.; Hall, G.; Horvat, M.; Kim, C.; Lasorsa, B.; Marvin-Dipasquale, M.; Parker, J. *International Solid Phase Mercury Speciation Exercise (ISPMSE): Introduction and Preliminary Results*, Eighth International Conference on Mercury as a Global Pollutant, Madison, Wisconsin, August 6−11, 2006.
(39) Mason, R. P.; Fitzgerald, W. F.; Hurley, J.; A.K; Hanson, J.; Donaghay, P. L.; Sieburth, J. M. Mercury biogeochemical cycling in a stratified estuary. *Limnol. Oceanogr.* **1993**, *38*, 1227–1241.
(40) Covelli, S.; Faganeli, J.; Horvat, M.; Brambati, A. Porewater distribution and benthic flux measurements of mercury and methylmercury in the Gulf of Trieste (Northern Adriatic Sea). *Estuar. Coast. Shelf Sci.* **1999**, *48*, 415–428.
(41) Haitzer, M.; Aiken, G. R.; Ryan, J. N. Binding of mercury(II) to aquatic humic substances: Influence of pH and source of humic substances. *Environ. Sci. Technol.* **2003**, *37*, 2436–2441.
(42) Hammerschmidt, C. R.; Fitzgerald, W. F.; Lamborg, C. H.; Balcom, P. H.; Visscher, P. T. Biogeochemistry of methylmercury in sediments of Long Island Sound. *Mar. Chem.* **2004**, *90*, 31–52.

ES802698V