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Bioavailability of Inorganic and Methylmercury to a Marine Deposit-Feeding Polychaete

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We measured the assimilation efficiencies (AEs) from various types of sediments and the uptake rate constants from the dissolved phase of inorganic mercury (Hg(II)) and methylmercury ($CH_3Hg(II)$) in the marine deposit-feeding polychaete *Nereis succinea*. AEs of Hg(II) ranged between 7 and 30% and were unaffected by sediment composition, whereas AEs of $CH_3Hg(II)$ ranged between 43 and 83% and were strongly affected by sediment composition. Sediment grain size had no apparent effect on Hg(II) and $CH_3Hg(II)$ assimilation. AEs for Hg(II) associated with anoxic sediment were slightly lower than with oxic sediment, whereas $CH_3Hg(II)$ displayed comparable AEs for both oxic and anoxic sediment. Dissolved uptake rate constants of $CH_3Hg(II)$ were 2.2 times those of Hg(II). A bioenergetic-based kinetic model was used to separate the pathways (solute vs sediment) and sources [Hg(II) vs $CH_3Hg(II)$] of Hg accumulation in *N. succinea*. The model predicted that, under conditions typical of coastal sediment environments, $CH_3Hg(II)$ accumulation contributes about 5−17% of total Hg accumulation in polychaetes. Most of the Hg(II) (>70%) accumulation is predicted to derive from sediment ingestion, whereas for $CH_3Hg(II)$ the relative importance of dissolved vs sediment ingestion depends greatly on its partition coefficient for sediments. Uptake from the dissolved phase and sediment ingestion can be equally important for $CH_3Hg(II)$ accumulation in *N. succinea*.

Introduction

Sediments are major repositories of many metals, including mercury, in coastal environments. Hg associates primarily with particulate organic matter in oxidized sediments and with sulfides in anoxic sediments (*1*−*3*). Metal bioavailability to benthic invertebrates from sediments can be affected by sediment geochemistry (e.g., binding with different geochemical components) and the physiology of benthic invertebrates (e.g., assimilation of metals) (*4*). The extent to which sediments can act as a source of contaminants for aquatic organisms is currently under active investigation. Metal bioavailability and toxicity from contaminated sediments has been quantified by relating measurements of simultaneously extracted metal (SEM) to acid volatile sulfides (AVS) in sediments (*5*−*7*). Other methods for quantifying metal bioavailability include chemical extraction of sediments by 1 N HCl (*8*, *9*) or by gut juices extracted from deposit-feeding invertebrates in vivo (*10*).

Concerns regarding Hg contamination are mainly due to its methylation in sediments to organic methylmercury (CH_3Hg), which exhibits much higher mobility, bioavailability, and toxicity than Hg(II) to aquatic organisms. The biomagnification of $CH_3Hg(II)$ in aquatic food webs has been well documented (*11*, *12*), in contrast to other metals (*13*). Once $CH_3Hg(II)$ is accumulated by benthic invertebrates from contaminated sediments, they may serve to transfer the $CH_3Hg(II)$ to benthic predators, including fish and mammals. Although $CH_3Hg(II)$ comprises only a small fraction (typically <1%) of the total Hg in sediments (*14*, *15*), concentrations in many fish and mammals can increase by several orders of magnitude, and most Hg in the muscle tissue of these animals is in the methylated form (*16*, *17*). However, the extent to which sedimentary $CH_3Hg(II)$ can be accumulated by benthic invertebrates and its subsequent transfer to higher organisms remain less well studied.

An unknown factor is the pathway of Hg accumulation in benthic organisms. Benthic animals can be potentially exposed to Hg(II) and $CH_3Hg(II)$ from both the dissolved phase (including porewater and overlying water) and sediments (including benthic diatoms and detritus). Although $CH_3Hg(II)$ is present in the subsurface anoxic layer (due to sulfur reducing bacterial methylation of inorganic Hg), it can also be released to the overlying water or surface oxic porewater by bioturbation, bioirrigation, and simple diffusion. A fundamental challenge is to separate the pathways (solute vs sediments) and sources (Hg(II) vs $CH_3Hg(II)$) of Hg accumulation in benthic invertebrates, as this could influence sediment quality criteria and allow prediction of the trophic transfer and biomagnification of Hg in aquatic food webs. Empirically it is well established that Hg concentrations in biota correlate well with sediment concentrations (*9*, *18*), leading to the assumption that sediments can be an important source of Hg accumulation, although this has not been tested rigorously and other factors may also affect the availability of sediment-bound Hg (*9*).

In this study, we conducted a series of experiments to assess the range of assimilation efficiencies of inorganic and methylmercury from ingested sediment and their uptake from solution. The assimilation of Hg was measured using a pulse-chase feeding technique, and the solute uptake was measured using short-term exposure. This kinetic approach has been shown to be more appropriate than long-term exposure (e.g., equilibrium approach) in evaluating metal uptake rates in aquatic animals (*19*). These measurements were then applied in a bioenergetic-based kinetic model that was used to separate the pathways and sources of Hg accumulation in the deposit-feeding polychaete, *Nereis succinea*. *N. succinea* is a nonselective deposit feeder living in shallow burrows and consumes mainly surface oxic sediments with associated detritus, microorganisms, algae, and occasional meiofauna. Previously this worm has been used as an indicator of the temporal dynamics of Hg in a small Hawaiian estuary (*20*). The kinetic model has been used to predict metal concentrations in mussels (*21*) and to separate pathways (dissolved vs particulate) and species of Cr accumulation in mussels (*22*). The kinetic model can effectively separate pathways of metal uptake because it can incorporate varying biological and geochemical conditions that are likely encountered by animals in nature (*23*).

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Materials and Methods

Polychaete worms (*N. succinea*) of 30−50 mg dry wt were collected from the sediments (<10 cm depth) of the Flax Pond salt marsh, Long Island, NY. The animals were individually acclimated for 2−3 days (without feeding) in the laboratory in a flexible plastic U-tube (inner diameter of 3.2 mm) with both ends connecting to the bottom of plastic beakers each containing 70 mL of filtered seawater (DOC = 1.5 mg L^{-1}). Experiments were conducted at 12 °C and a salinity of 28 ppt (the salinity of Flax Pond).

Experiments used the γ-emitting radioisotope ^{203}Hg to follow the kinetics of Hg uptake and release from the worms; the radioisotope ($^{203}HgCl_2$, in 1 N HCl, specific activity 25.486 mCi mg^{-1}) was purchased from the Buffalo Materials Research Center, Buffalo, NY. $CH_3{}^{203}$Hg(II) was synthesized from ^{203}Hg(II) according to methods described elsewhere (*24*, *25*) and was dissolved in 5 mM Na_2CO_3 until use.

Hg and CH_3Hg(II) Assimilation Efficiency from Radiolabeled Sediments. The purpose of these experiments was to measure the range of assimilation efficiencies of inorganic and methylmercury from sediments with different characteristics; varying organic content, grain size, and anoxic and oxic sediments were considered. Sediments from Flax Pond (FP) and the Verrazano Bridge (VZ, in the Hudson Estuary) were used to test the effect of organic carbon content on Hg assimilation. Organic carbon contents of FP sediments and VZ sediments (<500 μm size) were 5.76% and 2.83%, respectively. For FP sediments, we also examined anoxic and oxic sediments (both <500 μm) and the effects of particle size (<63 μm vs 63−500 μm) on Hg(II) and CH_3Hg(II) assimilation, whereas for VZ sediments only oxic sediments <500 mm was used. Sediments (60 μg wet wt in 2 mL of seawater) were radiolabeled with ^{203}Hg(II) and $CH_3{}^{203}$Hg(II) for 1 d. Concentrations in sediments due to radioisotope additions were 0.1 μg g^{-1} for Hg(II), comparable to many clean coastal sediment concentrations (*26*), and 0.03 μg g^{-1} for CH_3Hg(II), about 10 times higher than uncontaminated surface sediments (*15*). Short-term radiolabeling minimized any potential methylation of ^{203}Hg(II) or demethylation of $CH_3{}^{203}$Hg(II), although conversion rates can reach 1% h^{-1} (*27*). Adsorption of Hg(II) to marine sediments occurs within hours (*28*). Anoxic sediments were radiolabeled in oxygen-free seawater (which was previously bubbled with N_2 gas), and preparation of encapsulated anoxic radiolabeled sediment was also performed in an N_2 bag. The stable mercury concentrations and geochemical properties (e.g., AVS concentration) of the sediments were not measured.

Following 1 d radiolabeling, sediments were centrifuged to remove any overlying radioactive water. This procedure was repeated three times by adding nonradioactive filtered seawater. Sediments were cut into small pieces (<0.5 mg wet wt) with a needle and coated with a thin layer of Knox Unflavored Gelatine (7 g dissolved in 100 mL of seawater at 60 °C) to make the encapsulated sediment attractive to the worms. We then immediately immersed the sediment in a thin layer of cod liver oil atop filtered seawater (maintained on ice) to make spherical sediment pills.

The encapsulated sediments were then immediately fed to the worms maintained in clean U-tube systems. About 2−3 pellets were added to the tube opening (head side of worms). The worms ingested these sediments rapidly (<1 h), and loss of Hg from these encapsulated sediment pills was presumed to be negligible. Following ingestion, the radioactivity of individual worms was counted for 1 min. There were 5−7 replicate individuals for each treatment. The worms were then replaced in the U-tubes and allowed to depurate the ingested radioactive materials for 80 h, during which the water was changed daily to minimize buildup of radioisotope desorbed from unassimilated feces. Nonradioactive sediments (<500 μm, 1 cm thickness) were added into one beaker (in the worm's head side), allowing the worms to feed on these sediments to purge their guts of undigested radioactive material. Any egested radioactive fecal materials were collected from another beaker (without sediment). Radioactivity retained in the worms was counted regularly throughout the depuration period.

We also simulated ^{203}Hg(II) or $CH_3{}^{203}$Hg(II) desorption from radiolabeled sediments. Radiolabeled sediments (<0.5 mg) were placed in 3 mL of filtered pH 8.0 seawater (control) or pH 6 seawater. Recent studies by Ahrens and Lopez (unpublished) show that the gut pH of *N. succinea* is about 6.1 and rather uniform throughout the gut. After 1 d, the sediments were filtered onto 0.2-μm polycarbonate membranes, and radioactivity of ^{203}Hg(II) or $CH_3{}^{203}$Hg(II) remaining in the sediments was counted.

Uptake of Dissolved Hg and CH_3Hg(II). Uptake rates of dissolved Hg(II) and CH_3Hg(II) were determined by exposing worms at different Hg concentrations. Concentrations were 1.49, 5.23, 20.75, and 81.42 ng L^{-1} for CH_3Hg(II) and 0.1, 0.5, 2, and 10 μg L^{-1} for Hg(II). The lowest concentration of Hg(II) used was the lowest concentration we could employ to obtain accurate measurements of radioactivity over short counting periods. For the CH_3Hg(II) experiment, only radioactive $CH_3{}^{203}$Hg(II) was used to prepare different concentrations, whereas for the Hg(II) experiment, stable Hg (1.02 mg mL^{-1} of stock Hg atomic absorption standard solution from Aldrich Chemical Co.) was used to prepare different Hg(II) concentrations, while radioisotope ^{203}Hg(II) was used as a tracer to follow the uptake of Hg(II) by worms. There were five replicate individuals for each concentration treatment. Worms were individually placed in 80 mL of 28 ppt 0.2-μm filtered surface seawater (collected 10 km off Southampton, NY, and diluted with deionized distilled water). Periodically, individual worms were rinsed with filtered seawater, counted for their radioactivity, and then returned to new batches of seawater containing the same Hg concentration. After the exposure, worms were dried overnight at 80 °C, and their dry weights were determined.

Radioactivity Measurements. Radioactivity of ^{203}Hg(II) or $CH_3{}^{203}$Hg(II) was determined using an LKB Pharmacia Wallac 1282 Compugamma counter equipped with a NaI(Tl) well detector. γ-Emission was determined at 279 keV. Counting times were adjusted to yield propagated counting errors <5%.

Kinetic Separation of the Uptake Routes and Sources of Hg Accumulation in Worms. Assuming that metals are available to worms through uptake from the dissolved phase and ingested sediment and that metal influx is directly proportional to metal concentrations in both phases, metal concentrations in polychaetes under steady-state conditions (C_{ss}, μg g^{-1}) can be described by the following first-order mathematical equation (*21*, *23*, *29*):

$$C_{ss} = \frac{([k_u C_w] + [AE \times IR \times C_f])}{(k_e + g)} \quad (1)$$

where k_u is the metal uptake rate constant from the dissolved phase (L g^{-1} d^{-1}), C_w is the metal concentration in the dissolved phase (μg L^{-1}), AE is the assimilation efficiency from ingested sediments, IR is the weight-specific ingestion rate of polychaetes (g g^{-1} d^{-1}), C_f is the metal concentration in ingested food particles (μg g^{-1}), k_e is the metal efflux rate constant (d^{-1}), and g is the growth rate constant of the polychaete (d^{-1}). Mercury speciation in the dissolved phase is not considered in this model, and solute uptake is expressed as a function of total dissolved Hg.

If C_f is not known, it can be calculated from

$$C_f = K_d C_w \quad (2)$$

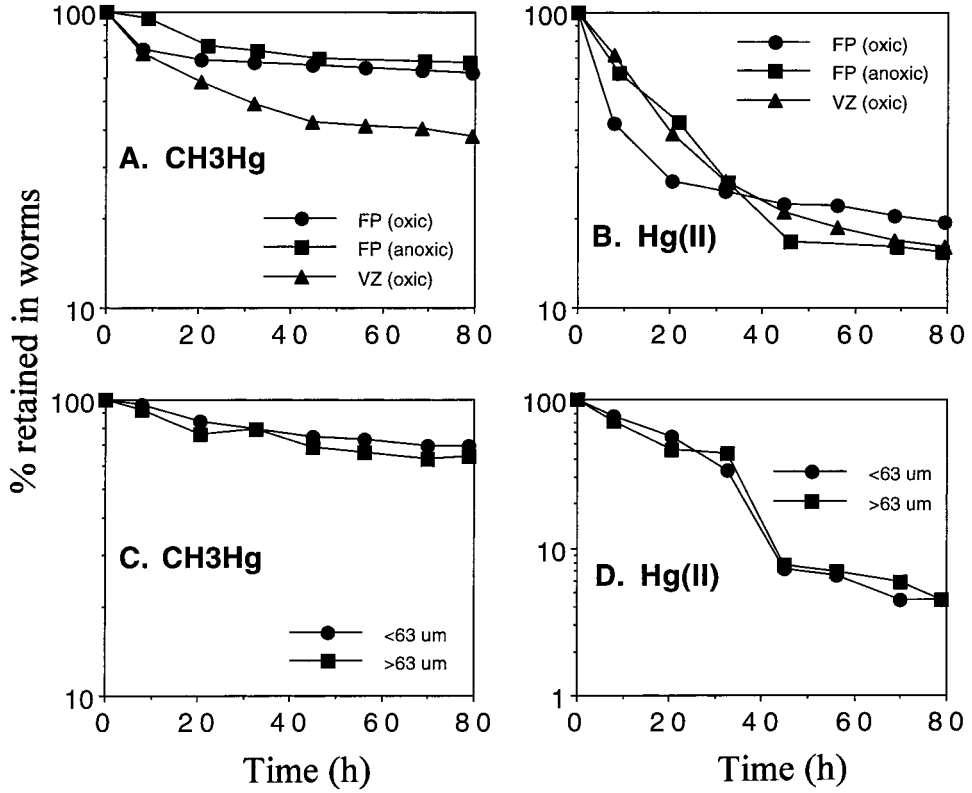

FIGURE 1. Retention of $^{203}Hg(II)$ and $CH_3{}^{203}Hg(II)$ in *Nereis succinea* following ingestion of radiolabeled sediment. Only means are given ($n = 5-7$); standard deviations of calculated assimilation efficiencies are given in Table 1. FP, Flax Pond salt marsh sediment; VZ, Verrazano Bridge sediment; <63 μm, Flax Pond sediment <63 μm size; >63 μm, Flax Pond sediment 63−500 μm.

where K_d is the partition coefficient (L g^{-1}) of the metal in the sediments. This equation assumes an equilibrium partitioning of Hg between sediment and dissolved phases (including porewater and overlying water). Thus, eq 1 can be rewritten as

$$C_{ss} = \frac{(k_u + AE \times IR \times K_d)C_w}{(k_e + g)} \quad (3)$$

However, this equation does not necessarily suggest that metal concentration in the worms can be directly predicted based on C_w as other parameters in this equation can vary greatly under different environmental conditions.

The metal bioconcentration factor (BCF) is calculated here as the ratio of metal concentration in the animals to the metal concentration in the dissolved phase:

$$BCF = C_{ss}/C_w = \frac{(k_u + AE \times IR \times K_d)}{(k_e + g)} \quad (4)$$

Thus, the relative importance of Hg vs $CH_3Hg(II)$ in the overall Hg accumulation in the worm can be calculated as

$$R_{Hg} = BCF_{Hg}/[BCF_{Hg} + (BCF_{CH_3Hg}C_{w,CH_3Hg}/C_{w,Hg})] \quad (5)$$

$$R_{CH_3Hg} = 1 - R_{Hg} \quad (6)$$

where R_{Hg} is the fraction of total Hg in the worm arising from uptake from Hg(II), and R_{CH_3Hg} is the fraction of total Hg in the worm arising from uptake from $CH_3Hg(II)$. BCF_{Hg} is the bioconcentration factor of Hg(II) in the worm, and BCF_{CH_3Hg} is the bioconcentration factor of $CH_3Hg(II)$ in the worm and can be calculated from eq 4.

The relative importance of dissolved vs food uptake for Hg(II) and $CH_3Hg(II)$ can also be calculated by

$$R_w = (k_u)/(k_u + AE \times IR \times K_d) \quad (7)$$

$$R_f = 1 - R_w \quad (8)$$

where R_w is the proportion of metal obtained from the dissolved phase and R_f is the proportion of metal from ingested food.

Results

Hg(II) and $CH_3Hg(II)$ Assimilation from Radiolabeled Sediments. Concentrations of Hg(II) and $CH_3Hg(II)$ in worms due to ingestion of radiolabeled sediments were 2 and 0.7 ng g^{-1}, respectively. Depuration patterns of Hg(II) and CH_3-Hg(II) in *N. succinea* feeding on different types of sediments are shown in Figure 1. Loss of Hg and $CH_3Hg(II)$ was fastest during the first two days. Because the gut processing time differed greatly for different individuals, the fraction of radioisotopes retained in the worms varied greatly within the first two days of depuration. Because the fecal pellets contained negligible radioactivity after 2 d, AEs were calculated by two methods. The first method assumed that the worm completed assimilation within 2 d, and thus AEs were calculated as the percentage of ingested Hg retained in the worm following 2 d depuration. The second method modeled the depuration of radioisotopes in the slower compartment (between 45 h and 79 h), with AEs calculated as the *y*-intercepts of the regression between log % radioisotopes retained in worms and time of depuration (45−79 h), according to eq 9:

$$A = A_o \exp(-kt) \quad (9)$$

where A is the percent of isotopes retained in worms during the slower compartment of loss, A_o is the assimilation efficiency, t is time (h), and k is the depuration rate constant (h^{-1}) during the second compartment of loss.

TABLE 1. Assimilation Efficiencies (%) of Hg(II) and $CH_3Hg(II)$ in the Polychaete *Nereis succinea* Feeding on Different Types of Sediments[a]

sediment (μm)	Hg(II)		$CH_3Hg(II)$	
	method 1	method 2	method 1	method 2
		Flax Pond Sediment		
oxic, <500	22.4 ± 2.3*	27.8 ± 2.6*	65.9 ± 13.8*	71.0 ± 14.6 *
anoxic, <500	16.7 ± 3.9*	19.1 ± 3.4*	69.8 ± 8.4	73.1 ± 9.7
oxic, <63	7.1 ± 3.0	15.3 ± 7.1	74.8 ± 5.5	83.8 ± 8.9
oxic, 63−500	7.6 ± 5.6	14.4 ± 8.4	69.3 ± 9.7	76.4 ± 12.5
		Verrazano Bridge Sediment		
oxic, <500	21.1 ± 5.9	30.5 ± 9.7	42.7 ± 13.9*	49.3 ± 12.9*

[a] Mean ± SD (n = 5−7). Method 1 assumes worms completed Hg assimilation at 2 d. Method 2 calculates assimilation from the y-intercept of the regression between percent retained in worms and time (45−79 h). An asterisk (*) indicates statistically significant difference between two treatments (P < 0.05, t-test).

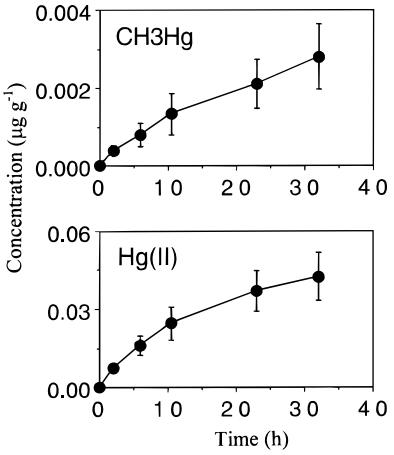

FIGURE 2. Accumulation of $CH_3Hg(II)$ and Hg(II) from the dissolved phase in *Nereis succinea* over time. For $CH_3Hg(II)$, the dissolved concentration was 0.0014 μg L^{-1}, and for Hg(II) the dissolved concentration was 0.1 μg L^{-1}. Means ± SD (n = 5).

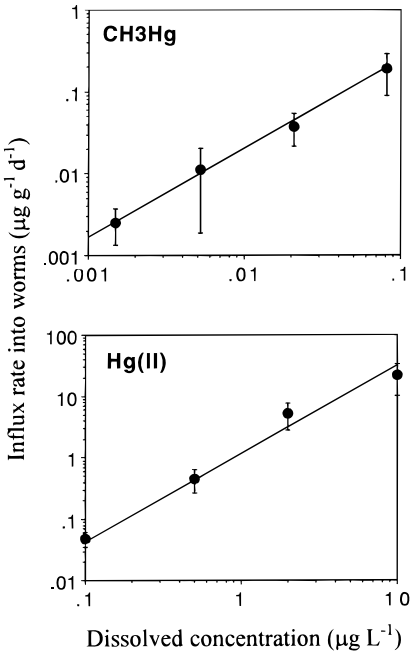

FIGURE 3. Influx rates of $CH_3Hg(II)$ and Hg(II) into *Nereis succinea* at different dissolved concentrations. Means ± SD (n = 5).

AEs for Hg(II) and $CH_3Hg(II)$ from each sediment type are shown in Table 1. AEs determined by the two methods did not differ significantly from each other. AEs determined for FP sediments were 66−84% for $CH_3Hg(II)$ and 7−28% for Hg(II), suggesting that the bioavailability of $CH_3Hg(II)$ from ingested sediments was 2.4−12 times higher than Hg(II). Sediment grain size did not affect Hg(II) and $CH_3Hg(II)$ assimilation. The AE of Hg(II) from anoxic FP sediment was significantly lower than the AE from oxic sediments (<500 μm) (P < 0.05, t-test), but $CH_3Hg(II)$ AEs were comparable between oxic and anoxic sediments. AEs of $CH_3Hg(II)$ from VZ sediment were significantly lower than from FP sediments, whereas AEs of Hg(II) were comparable between the two sediments.

Both Hg(II) and $CH_3Hg(II)$ were tightly bound to the sediments; under most conditions there was very little desorption of both Hg species (<12%) in either pH 8.0 seawater or pH 6.0 seawater. Generally, lowering the pH from 8 to 6 did not influence Hg desorption from sediment. There was no evidence that Hg(II) and $CH_3Hg(II)$ assimilation in *N. succinea* were dependent on their desorption either in pH 6 or pH 8 seawater.

Uptake of Dissolved Hg and $CH_3Hg(II)$. Uptake of dissolved Hg by worms over time is shown in Figure 2. Generally there was a linear pattern of metal uptake with time between 2 and 11 h, after which the uptake rate decreased. The higher uptake within the first 2 h might have been due to rapid surface adsorption when the worms were first exposed to Hg. After 11 h of exposure, a decrease in metal uptake rate may have been due to a decrease in animal ventilating activity, a saturation of binding sites available for Hg, or possibly efflux of accumulated Hg. We therefore calculated Hg uptake rate constants using uptake data between 2 and 11 h.

To calibrate for the initial surface adsorption of Hg to the worms, we performed a linear regression between metal concentration in the worms and time of exposure (between 2 and 11 h), and the calculated y-intercept was assumed to be the amount of metal adsorbed. This amount was then subtracted from metal accumulated between 2 and 11 h. Mean influx rates (I_u, μg g^{-1} d^{-1}) of Hg were calculated based on measurements at 2, 6, and 11 h and correlated with dissolved concentration (C_w, μg L^{-1}) of Hg and $CH_3Hg(II)$ (Figure 3). With this approach, the calculated influx rates were comparable at 2, 6, and 11 h of measurements, suggesting that duration of exposure (up to 5 h) between 2 and 11 h had no effect on Hg uptake rates. The relationship between I_u and C_w suggests that uptake of dissolved Hg(II) and $CH_3Hg(II)$ conformed with Freundlich adsorption isotherms. The coefficients (b) of this relationship for CH_3-Hg(II) are close to 1.0, so the uptake rate constant (k_u, L g^{-1} d^{-1}) can be calculated as I_u/C_w (Table 2). For Hg(II), b values for two experiments were 1.339 and 1.367, implying that uptake of dissolved Hg(II) increased disproportionately with an increase in Hg dissolved concentration.

TABLE 2. Regression of Influx Rates (I_u, μg g^{-1} d^{-1}) of Hg(II) and $CH_3Hg(II)$ into *Nereis succinea* with Different Hg Concentrations in the Dissolved Phase (C_w, mg L^{-1})[a]

Hg	expt	eq	r^2	mean k_u (L g^{-1} d^{-1})
Hg(II)	1	$I_u = 1.260\ [C_w]^{1.367\pm0.119(SE)}$	0.985	1.260
	2	$I_u = 1.287\ [C_w]^{1.339\pm0.106(SE)}$	0.988	1.287
$CH_3Hg(II)$		$I_u = 2.584\ [C_w]^{1.059\pm0.042(SE)}$	0.997	2.584

[a] The calculated uptake rate constants (k_u, L g^{-1} d^{-1}) are also shown.

Kinetic Separation of the Sources and Pathways of Hg Accumulation. Parameters required in the kinetic model include AE, k_u, k_e, C_w, and K_d of Hg(II) and $CH_3Hg(II)$, and IR and g in *N. succinea* (eq 4). Values of AE and k_u are taken from this study (Tables 1 and 2). A linear pattern of Hg uptake from the dissolved phase was observed across a range of Hg concentrations (0.1−10 μg L^{-1} for Hg(II), 1−80 ng L^{-1} for $CH_3Hg(II)$). The k_u values obtained from these ranges are assumed to be applicable to lower Hg concentrations (that is, k_u is independent of concentration whereas influx rate is directly proportional to concentration). There are very few direct field measurements for generating reliable K_d values for Hg(II) and $CH_3Hg(II)$ for marine coastal sediments. Gagnon et al. (*15*) reported concentrations of $CH_3Hg(II)$ in both porewater and sediment for organic-rich sediments (Saguenay Fjord and St. Lawrence Estuary, Canada). The calculated K_d values of $CH_3Hg(II)$ between 1.5 and 10 cm depths were 2300 ± 1600 and of Hg(II) were 1−3.7 × 10^4 (calculated from ref *3*), consistent with previous reported values (*30*). We therefore used a mean K_d value of 2 × 10^3 for $CH_3Hg(II)$ and of 2 × 10^4 for Hg(II) in our modeling. Because the K_d may vary considerably in nature, we also allowed the K_d to vary by an order of magnitude to evaluate the effects of K_d variation on Hg accumulation. For IR, we used a mean ingestion rate of 3.5 g g^{-1} d^{-1} for *N. succinea* (*31*).

The k_e values of Hg(II) and $CH_3Hg(II)$ in *N. succinea* have been recently determined in experiments in which worms were either exposed to ^{203}Hg-labeled oxic sediment or $CH_3{}^{203}Hg(II)$-labeled anoxic sediment for 1 month and then depurated in nonradiolabeled sediment for 12−18 d (Gagnon et al., unpublished). Efflux rate constants calculated after 4 d of metal depuration (to avoid interference from metal digestion) were 0.027 d^{-1} for Hg(II), comparable to earlier measurements with *N. succinea* (0.031 d^{-1}) (*20*), and 0.014 d^{-1} for $CH_3Hg(II)$. These values were used in the modeling. The growth rate constant g was ignored in the modeling calculation as it was assumed to be negligible as compared to k_e for adult worms.

To determine the relative importance of Hg(II) vs CH_3-Hg(II) as sources for the overall Hg accumulation in *N. succinea*, the proportion of total dissolved Hg (porewater and overlying water) that is methylated needs to be known. Methylmercury concentrations are very low (often below detection) in porewater recovered from surficial oxic sediment layers. Gagnon et al. (*15*) found that dissolved CH_3-Hg(II) generally represented 10−30% of the total dissolved porewater Hg from an organic-rich contaminated anoxic sediment, and in our modeling we assumed values up to 30%.

Table 3 summarizes the mean numeric values for each parameter for Hg(II) and $CH_3Hg(II)$ used in the kinetic modeling. We modeled the effect of AEs and K_d values on the relative importance of dissolved uptake vs sediment ingestion for Hg(II) and $CH_3Hg(II)$ accumulation (Figure 4). In modeling the effect of each variable, mean numeric values of other parameters were assumed (Table 3). Most Hg(II) (>70%) is calculated to derive from ingested sediment, whereas for $CH_3Hg(II)$ both dissolved sources and sediment can be equally important (Figure 4).

TABLE 3. Mean Numeric Values of Parameters Used in the Kinetic Modeling of Hg Accumulation in the Polychaete *Nereis succinea*

parameter	definition	Hg(II)	$CH_3Hg(II)$
AE	assimilation efficiency	0.20	0.70
K_d (L g^{-1})	partition coefficient	20	2
k_u (L g^{-1} d^{-1})	dissolved uptake rate constant	1.27	2.58
k_e (d^{-1})	efflux rate constant	0.027	0.014
IR (g g^{-1} d^{-1})	ingestion rate	3.5	3.5

The relative importance of dissolved uptake vs sediment ingestion was found to depend greatly on K_d values for both Hg(II) and $CH_3Hg(II)$ and was less dependent on AEs. For Hg(II), a significant effect of AEs on the uptake pathway was only evident at lower K_d values. We also used the mean numeric values of each parameter to separate the sources of Hg accumulation in worms (Figure 5). Our calculations suggest that the proportion of $CH_3Hg(II)$ accumulated in worms increased linearly with the proportion of total dissolved Hg that is methylated. Under conditions likely to occur in coastal sediment, where 10−30% of total dissolved Hg is $CH_3Hg(II)$ (*15*), about 5−17% of the total Hg in worms is predicted to be $CH_3Hg(II)$.

Discussion

The higher AEs of $CH_3Hg(II)$ than Hg(II) in *N. succinea* are consistent with many previous measurements in other marine invertebrates and fish (*32*−*35*). Our preliminary experiments suggest that the gut passage time of sediments is about 8 h in *N. succinea*, consistent with previous estimates (11−14 h at 10 °C and 4−6 h at 20 °C; *36*) but is significantly longer than the gut passage in many other deposit feeders (*37*). Our results also indicate that the time required for complete assimilation of Hg (about 2 d) is about 6 times longer than the gut passage of food in polychaetes. This has also been observed in marine mussels (*38*) and copepods (*39*). This difference between gut transit time of food and of Hg suggests that unassimilated Hg might have desorbed from the ingested sediments in the gut and was eventually egested; however, the gut behavior of Hg was not studied.

Metal bioavailability from contaminated sediments has been studied by measuring metal desorption in gut juices extracted in vivo from polychaetes by Mayer et al. (*10*), who also found a linear relationship between metal (Cu and Pb) desorption from contaminated sediments in gut juices and metal desorption in seawater. In our experiments, both Hg-(II) and $CH_3Hg(II)$ were strongly bound to sediments, and there was little desorption in seawater. No relationship between Hg assimilation and Hg desorption rate was evident, implying that desorption probably was not the sole process controlling Hg assimilation in *N. succinea*. This has also been reported for Hg assimilation in mussels (*35*). Because the ultimate form of metal available for assimilation in the gut is likely to be in the dissolved phase, a lower desorption (<10%) than metal AE (10−30% for inorganic Hg and 48−80% for methylmercury) suggests that desorption in seawater underestimates Hg desorption in the worm gut. Presumably surfactants and enzyme activity play a major role in the solubilization of sediment-bound metals in the gut (*10*, *40*).

Many field studies have shown that Hg concentrations in benthic invertebrates from organic-rich sediments are lower than from organic-poor sediments and normalizing sediment Hg concentration to organic matter content greatly tightens correlations of tissue burdens in invertebrates with sediment concentrations (*1*, *18*, *41*, *42*). In our experiments, however,

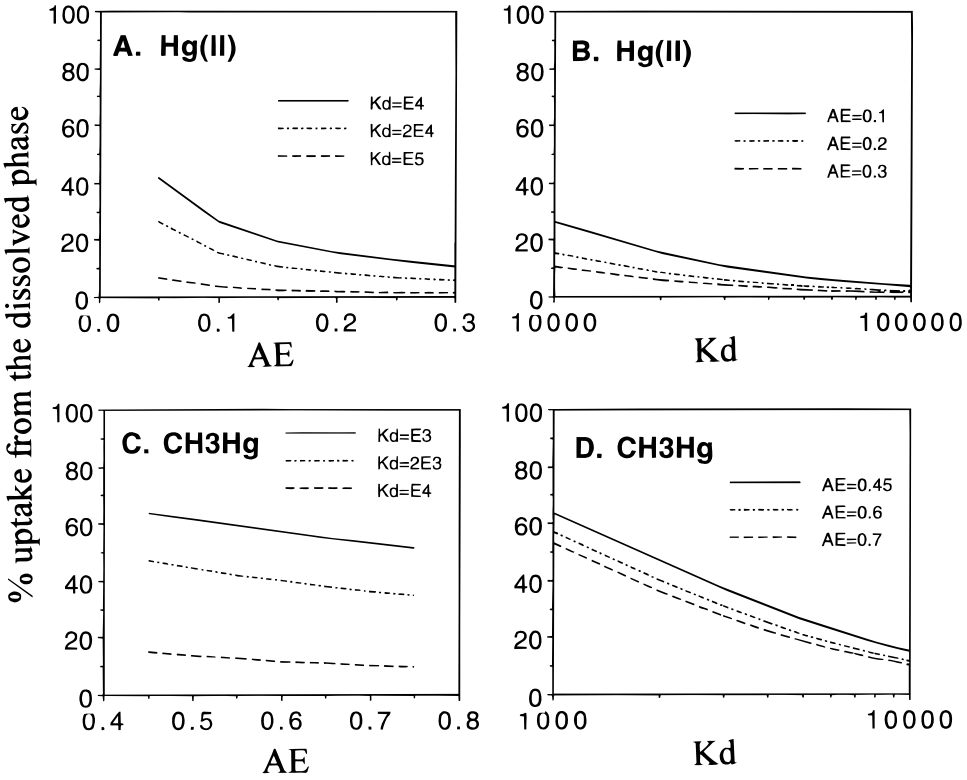

FIGURE 4. Uptake from the dissolved phase of Hg(II) and $CH_3Hg(II)$ in *Nereis succinea* calculated by the kinetic model for different Hg assimilation efficienies (AE) and partition coefficients for sediments (K_d). Values are percentages of Hg(II) or $CH_3Hg(II)$ obtained from the dissolved phase for different conditions. (A) Hg(II) as a function of AE. (B) Hg(II) as a function of K_d. (C) $CH_3Hg(II)$ as a function of AE. (D) $CH_3Hg(II)$ as a function of K_d.

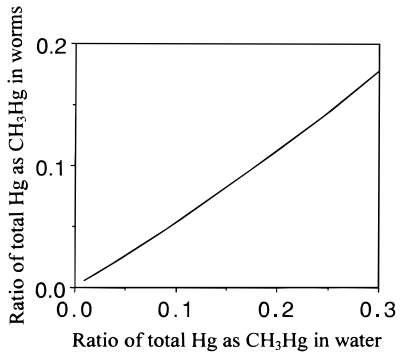

FIGURE 5. $CH_3Hg(II)$ as a percentage of total Hg in the polychaete *Nereis succinea* calculated by the kinetic model, as a function of the percent of total Hg as $CH_3Hg(II)$ in the dissolved phase.

AEs of Hg(II) and $CH_3Hg(II)$ were comparable for different sediment grain sizes, suggesting that sedimentary organic carbon content has little effect on Hg assimilation in *N. succinea*. Hg(II) AEs were comparable for FP and VZ sediments in which the organic carbon content differed 2-fold; however, AEs of $CH_3Hg(II)$ were significantly different for these two sediments. Furthermore, organic coatings of sediments have been found to increase the AEs for CH_3Hg-(II) bound to all types of particles but have no effect on AEs for Hg(II) in marine mussels (*35*). It is thus likely that other factors in addition to sedimentary organic carbon content are responsible for Hg assimilation in *N. succinea*. Presumably Hg assimilation from sediments is related to the binding strength of Hg for sediment particles; when binding sites (which may well be associated with organic matter) are greatly undersaturated with Hg, Hg assimilation may be less affected by the organic carbon of sediments.

Hg concentration in worms is also a function of the uptake rate of dissolved Hg and feeding rate (which can vary among different sediments), both of which may contribute to the negative correlation between Hg concentration in polychaetes and organic carbon content of sediments (*42*). For example, Hg in sandy environments may be more labile and diffuse more readily into porewater, which could then be taken up rapidly by organisms due to the strong affinity of Hg for protein.

Comparable AEs of $CH_3Hg(II)$ between oxic and anoxic sediments suggest that $CH_3Hg(II)$ can be very bioavailable to *N. succinea* from anoxic sediments, which typically exhibit high concentrations due to bacterial methylation (*43*). In anoxic sediment, our short-term experiments may have allowed insufficient time for Hg to bind to acid-volatile sulfides (AVS) (which presumably would be very unavailable for animals), and therefore Hg(II) was assimilable from these sediments. It is also possible that $^{203}Hg(II)$ bound to AVS or other sulfide ligands was reoxidized and released during the long gut digestive period.

Uptake rates of dissolved Hg conformed to Freundlich adsorption isotherms, indicating that uptake is a passive process, consistent with findings for Hg uptake in phytoplankton (*44*, *45*). The high k_u of $CH_3Hg(II)$ may also contribute to the overall $CH_3Hg(II)$ accumulation in benthic organisms. We have recently measured the k_u of other trace metals in *N. succinea* and found the highest values for CH_3-Hg(II), followed by Ag > Hg(II) > Zn > Cd > Co > Se (*46*), consistent with findings for other invertebrates (*21*, *39*, *47*).

The high *b* coefficients (1.33) for dissolved Hg(II) uptake as a function of the dissolved concentration remain unexplained. This may have been due to increased membrane permeability with increasing Hg concentration (i.e., Hg toxicity) or possibly to lower proportional complexation by DOC at higher dissolved Hg concentrations. In marine mussels and copepods, *b* coefficients are generally close to 1, implying that uptake is directly proportional to metal concentration in the dissolved phase (*21*, *39*). Similarly, *b*

coefficients for $CH_3Hg(II)$ uptake are close to 1.0. For *N. succinea*, *b* coefficients are close to 1.0 for Ag, Co, and Se, but only 0.66 for Zn, suggesting that Zn may be regulated by the polychaete during its uptake from the dissolved phase (*46*).

Assuming that $CH_3Hg(II)$ comprises about 10−30% of total dissolved Hg in anoxic sediment porewater (*15*), our model predicts that about 5−17% of the total Hg in *N. succinea* may be $CH_3Hg(II)$. This is comparable to recent field measurements of Muhaya et al. (*42*), who found that $CH_3Hg(II)$ accounts for about 18% (range of 3−50%) of the total Hg in the polychaete *N. diversicolor*. The relative importance of Hg(II) and $CH_3Hg(II)$ as sources for Hg accumulation in marine deposit feeders depends, of course, on the type of sediments ingested by the animals and the depth at which the animals feed. Any change in Hg geochemistry in sediments may greatly affect the separation of Hg(II) and $CH_3Hg(II)$ accumulation. Although the concentration of $CH_3Hg(II)$ is <1% of the total Hg concentration in sediments and <30% of total Hg in porewater (*15*), it can become an important source for Hg accumulation because of its higher dissolved uptake rate, higher assimilation efficiency, and lower efflux rate. Typical concentrations of Hg(II) in coastal waters are about 1 ng L^{-1} (*48*) and of $CH_3Hg(II)$ are about 0.01 ng L^{-1} (*49*) but may be as high as about 0.4 ng L^{-1} in hypoxic waters (*50*). In addition, $CH_3Hg(II)$ may also accumulate through bacterial methylation in the gut of benthic invertebrates, but this has not been examined.

Of the 83−95% of total Hg that may accumulate through Hg(II) uptake, our model calculation demonstrated that Hg(II) is primarily obtained from ingested sediment. In many field studies, particulate ingestion has been suggested to be a major source for Hg accumulation in benthic animals (*9*, *18*, *51*, *52*). Hg tissue burden in these organisms is also strongly correlated with sediment-bound Hg but not dissolved Hg (*1*, *18*, *53*). In field studies, however, accumulation of $CH_3Hg(II)$ was not measured.

We should note several limitations of the experiments and modeling in our study. First, the general applicability of our AE measurements is as yet unresolved. AE was measured following short-term radiolabeling of sediment, and it is likely that the AE we measured represents assimilation from an easily exchanging geochemical sediment fraction. We employed short-term radiolabeling to minimize changes in Hg and $CH_3Hg(II)$ species during the labeling period (e.g., Hg methylation and CH_3Hg demethylation). In the kinetic model, the sediment Hg concentration considered should be for the same geochemical pool as for the AE measurements. By applying the AE values measured here to total Hg concentrations in sediment would likely lead to overestimates of overall influx of Hg in worms from sediments. For example, our model predicts that, for sediments containing total Hg(II) at 0.3 μg g^{-1}, worms should have a Hg concentration >8 μg g^{-1}, yet *N. succinea* in such sediments have been found to contain only 0.02−0.1 μg g^{-1} (*20*). Alternatively, applying Hg K_d values of 2000−20 000 L kg^{-1} (assuming K_d represents partitioning of Hg with the exchangeable geochemical fraction in the sediment) and a dissolved concentration of 1 ng L^{-1} would produce worms containing 0.05−0.5 μg g^{-1}, within the range of Luoma's measurements. Subsequent bioaccumulation studies need to consider the fractionation of Hg in different sedimentary components and the rates at which the Hg equilibrates with these components. Furthermore, we did not specifically test the effects of sediment encapsulation on AE of Hg and $CH_3Hg(II)$ or the diffusion of $CH_3Hg(II)$ into the gel coating.

Second, although we used ^{203}Hg with the highest specific activity available, the added ^{203}Hg elevated the total Hg concentration in the sediments. It is not known whether changes in Hg concentration can affect its assimilation in worms, although in mussels the effects of metal concentration on assimilation are marginal (*54*). Third, the influence of geochemical properties of sediments, especially AVS concentrations, and how these may have changed during the radiolabeling and encapsulation on Hg assimilation was not addressed in this study; consequently, the AE values obtained here may not be applicable to sediments with very different characteristics. Fourth, we did not consider the effect of water chemistry (e.g., DOC, dissolved sulfide) and its influence on Hg speciation on mercury uptake from the dissolved phase. We recognize that changes in water chemistry can strongly affect the bioavailability of the dissolved pool. We extrapolated k_u measurements obtained from relatively high dissolved Hg concentrations to waters with lower Hg levels. Furthermore, the uptake rate constant from the dissolved phase was obtained from overlying water exposure only, and Hg uptake from porewater may display different uptake rates. For example, the porewater in Flax Pond sediment contained 2.76 mM DOC, and the mercury in this porewater may be complexed by this organic matter, resulting in lower k_u values. Finally, a field study to specifically test the validity of AE measurements and the kinetic model predictions of metal concentrations in these worms would be appropriate.

In conclusion, our study suggests that $CH_3Hg(II)$ is more bioavailable than Hg(II) to the deposit-feeding polychaete *N. succinea* from both the dissolved and particulate (sediment) phases. Assimilation of Hg is little affected by sediment grain size and organic carbon content, although exceptions are noted. $CH_3Hg(II)$ from anoxic sediments is highly assimilated by the worms, whereas Hg(II) shows slightly lower assimilation from anoxic sediments than from oxic sediments. The high bioavailability of $CH_3Hg(II)$ results in a significant contribution to total Hg accumulation in *N. succinea*, even though it accounts for <1% of the total Hg in sediment and <30% of total Hg in porewater. Of all potential sources considered, ingested sedimentary Hg(II) represents the greatest source for Hg in polychaetes.

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