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BIOACCUMULATION OF METHYLMERCURY IN A MARINE COPEPOD

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Abstract: Methylmercury (MeHg) is known to biomagnify in marine food chains, resulting in higher concentrations in upper trophic level animals than their prey. To better understand how marine copepods, an important intermediate between phytoplankton and forage fish at the bottom of the food chain, assimilate and release MeHg, the authors performed a series of laboratory experiments using the gamma-emitting radiotracer $^{203}Hg^{2+}$ and $Me^{203}Hg$ with the calanoid copepod *Acartia tonsa*. Assimilation efficiencies of Hg^{2+} and MeHg ranged from 25% to 31% and 58% to 79%, respectively, depending on algal diets. Assimilation efficiencies were positively related to the fraction of Hg in the cytoplasm of the algal cells that comprised their diet. Efflux rates of Hg^{2+} (0.29/d) and MeHg (0.21/d) following aqueous uptake were similar, but efflux rates following dietary uptake were significantly lower for MeHg (0.11–0.22/d) than Hg^{2+} (0.47–0.66/d). The calculated trophic transfer factors in copepods were >1 for MeHg and consistently low (≤0.2) for Hg^{2+}. The authors used the parameters measured to quantitatively model the relative importance of MeHg sources (water or diet) for copepods and to predict the overall MeHg concentrations in copepods in different marine environments. In general, MeHg uptake from the diet accounted for most of the body burden in copepods (>50%). For an algal diet with a MeHg dry weight bioconcentration factor $\geq 10^6$, >90% of a copepod's MeHg body burden can be shown to derive from the diet. The model-predicted MeHg concentrations in the copepods were comparable to independent measurements for copepods in coastal and open-ocean regions, implying that the measured parameters and model are applicable to natural waters. *Environ Toxicol Chem* 2017;36:1287–1293. © 2016 SETAC

Keywords: Bioaccumulation Zooplankton Assimilation efficiency Efflux rate Trophic transfer

INTRODUCTION

Phytoplankton concentrate many trace metals out of seawater [1] and serve as highly enriched sources of these metals for herbivorous animals, including zooplankton and bivalve mollusks [2–4]. Zooplankton are of particular interest because their sinking waste products and cast exoskeletons can vertically transport metals [5] and, in so doing, affect their oceanic residence times [4,6]. Overall, zooplankton play an important role in the bioaccumulation of metals in marine food webs because they link phytoplankton, whose bioconcentration of metals is the single largest enrichment step in food chains, with fish and other metazoans; thus, trace metals that are assimilated into zooplankton could ultimately be transferred to higher trophic level organisms [4].

Although phytoplankton are greatly enriched in many essential and nonessential metals, most of the nonessential metals are not appreciably assimilated from phytoplankton food by zooplankton, let alone further biomagnified in marine food chains [4]. Indeed, the concentrations of most metals in organisms at higher trophic levels typically decrease with increasing trophic level; the exception is methylmercury (MeHg), which is the only trace metal species known to substantially biomagnify in food chains. This biomagnification is the result of its high assimilation efficiency in organisms at every trophic level and very slow release rates from animal tissues [7,8]. As a consequence, MeHg is efficiently transferred from 1 trophic level to the next and is ultimately enriched in fish. It is known that MeHg accumulation in heterotrophic organisms is mainly from ingesting MeHg-containing food instead of direct uptake from water. Thus, food web structure could influence the MeHg transfer to higher trophic level organisms [9,10]. Because seafood consumption is the primary route for human intake of MeHg [11] and such exposure may lead to adverse health consequences to exposed humans [12], there is interest in the food chain buildup of MeHg in marine ecosystems among public health agencies and the general public. Although there have been comprehensive studies of the trophic transfer and cycling of many trace metals by zooplankton [4], MeHg has not been similarly studied for marine zooplankton, where few studies have examined the transfer of MeHg from phytoplankton to zooplankton.

Previous work has shown that a high assimilation efficiency (>90%) and low release rate (<5%/d) of MeHg in a fresh water daphnid, *Daphnia pulex*, feeding on a chlorophyte [13] led to a significant transfer of MeHg from phytoplankton to zooplankton. For marine organisms, Mason et al. [14] reported a high assimilation efficiency (>60%) of MeHg in marine copepods feeding on a diatom. This value was 4 times greater than Hg^{2+}, and the fractions of MeHg and Hg^{2+} accumulated in algal cell cytoplasm were strongly correlated to the assimilation efficiencies in copepods. This correlation of cytoplasmic distribution in algal cells and assimilation efficiency in herbivorous copepods is consistent with findings for other elements [15]. Lawson and Mason [16] also found high assimilation efficiencies (>50%) of MeHg in marine copepods and amphipods feeding on diatoms. Mathews and Fisher [7], examining trophic transfer of MeHg in a simple estuarine food chain, reported a high assimilation efficiency of MeHg (>76%) for both zooplankton feeding on phytoplankton and small planktivorous fish feeding on zooplankton.

In the present study, we conducted a series of laboratory experiments to evaluate assimilation efficiencies of both Hg^{2+} and MeHg in marine copepods that fed on 3 marine

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phytoplankton species belonging to different algal classes and compared the results with Hg^{2+} and MeHg uptake from the aqueous phase. We estimated Hg^{2+} and MeHg efflux rates from copepods and trophic transfer factors from phytoplankton to copepods. With those parameters, we used a simple model to estimate the relative importance of dietary and aqueous sources of MeHg in zooplankton and compared modeled MeHg concentrations in marine zooplankton with field data.

MATERIALS AND METHODS

Preparation of plankton

Three marine phytoplankton species were used to feed zooplankton: the diatom *Thalassiosira pseudonana* (clone 3H), the chlorophyte *Dunaliella tertiolecta* (CCMP1320), and the cryptophyte *Rhodomonas salina* (CCMP1319). These species, representing 3 different algal classes, have significantly different MeHg bioconcentration factors (BCFs) [17] as well as different cell wall characteristics and photosynthetic storage products, which may influence the trophic transfer of MeHg to copepod grazers. All species were held in clonal, unialgal cultures maintained axenically for generations at constant temperature (18 °C) and a 14:10 light:dark cycle (200 μmol quanta/m^2/s) with cool white fluorescent lamps. Routine cultures were maintained in sterile-filtered (0.2 μm) surface seawater (35 psu) and enriched with f/2 nutrients [18].

The coastal calanoid copepod *Acartia tonsa* was collected in early summer from Stony Brook Harbor, New York, USA, with a plankton net (160-μm mesh) and transferred into 0.2 μm filtered seawater. Immediately after collection, adult *A. tonsa* individuals were visually identified using a dissecting microscope and separated from other plankton. The separated copepods were maintained in a tank with filtered seawater under shimmer light and fed the algae *R. salina* and *Isochrysis galbana* every 12 h to allow them to acclimate to laboratory conditions for 2 d. Prior to experiments, the copepods were transferred to another tank with 0.2-μm filtered seawater without food for 12 h to evacuate their guts of ingested material. To put the amount of bioaccumulated Hg in copepods into context, we measured total background Hg concentrations in *A. tonsa* collected from Stony Brook Harbor. Total Hg, determined by analyzing freeze-dried copepods using a DMA-80 direct mercury analyzer (Milestone Inc.), was found to be 30.2 ± 4.6 ng/g dry weight ($n = 6$, water content $= 86 \pm 1\%$). The reported fraction of total Hg in the form MeHg in marine zooplankton generally ranges from 12% to 45% [19].

Hg assimilation and depuration by copepods from food

The protocol of using radioisotopes to evaluate the accumulation and assimilation of elements in copepods has been well established [2,15]. In the present study, we used the gamma-emitting radioisotope ^{203}Hg as a tracer to track the transfer of Hg (Hg^{2+} and MeHg) from phytoplankton to zooplankton. Short pulse-feeding experiments were performed to help avoid extensive excretion and recycling of Hg, which would lead to ambiguous results.

Inorganic $^{203}Hg^{2+}$ solution was obtained from Eckert and Ziegler Isotope Products (specific activity 5 Ci/g). It was further converted to MeHg ($Me^{203}Hg$) following methods described elsewhere [17,20]. In brief, the $^{203}Hg^{2+}$ solution was adjusted to pH 5 with acetate buffer and mixed with methylcobalamin ($C_{63}H_{91}CoN_{13}O_{14}P$). The reaction was allowed to proceed in the dark for 18 h to 24 h, and $Me^{203}Hg$ was formed and then extracted by dichloromethane (CH_2Cl_2) and redissolved in Milli-Q® water. The conversion yield (fraction of total $^{203}Hg^{2+}$ recovered as $Me^{203}Hg$) was $95 \pm 3\%$ ($n = 6$).

Algal cells for feeding experiments were cultured in f/2 medium without adding ethylenediaminetetraacetic acid. Late–log phase cells were concentrated from water by either filtering onto polycarbonate membranes or centrifuging and resuspending into 0.2 μm sterile-filtered seawater. The algal cells were then radiolabeled with $^{203}Hg^{2+}$ or $Me^{203}Hg$ (55–78 kBq/L, corresponding to concentrations of 0.6–2.0 μg/L Hg). The concentrations of Hg^{2+} and MeHg used in these experiments were higher by about 10^3 times and 10^4 times, respectively, than those in natural waters. Because $^{203}Hg^{2+}$ was prepared in 1 M hydrochloric acid, corresponding amounts of 1 M Suprapur NaOH were added immediately afterward to maintain the seawater pH (~8.2). In order to have uniformly radiolabeled cells, cultures were grown for at least 2 d (several cell divisions) to reach equilibrium. Analysis of the cellular distribution of ^{203}Hg (for both Hg^{2+} and MeHg) followed the protocol described by Reinfelder and Fisher [15].

Radiolabeled algal cells were concentrated, resuspended, and then added into transparent Teflon feeding bottles containing 100 mL filtered seawater with a cell density of 6×10^4 cells/mL to 8×10^4 cells/mL, depending on algal species. Three replicates were used for each algal food treatment. In each bottle, 60 copepods fed on radiolabeled cells in the dark for 45 min. After feeding, copepods were collected with a 160-μm nylon mesh, rinsed twice with 10 mL of unlabeled, filtered seawater, and immediately transferred to 25 mL clean filtered seawater for radioanalysis ($t = 0$, assigned as the starting point of the depuration period). Fecal pellets egested by copepods during the feeding experiment were also collected with a 20-μm nylon filter, rinsed with filtered unlabeled seawater, and radioassayed. Copepods were transferred back to the original Teflon bottles with clean filtered seawater to depurate their ingested radiolabeled food. They were maintained under the same conditions as the feeding experiments but with nonradiolabeled food of the same species. The radioactivity of copepods and their fecal pellets was determined periodically ($t = 4$ h, 12 h, 24 h, 36 h, 48 h, 60 h, 72 h) over the 3-d depuration period. The food was added and the seawater replaced at each time point. The gentle handling throughout resulted in negligible mortality of copepods.

During the feeding experiment, Hg^{2+} and MeHg may desorb from radiolabeled algal cell surfaces into the dissolved phase. Consequently, it was possible that the copepods may have acquired Hg from both water and food sources. Thus, a control treatment was used to assess the copepod uptake of ^{203}Hg (for both Hg^{2+} and MeHg) that had desorbed from the algae during the same time period used for radioactive feeding (45 min). To accomplish this, 100 mL of filtered seawater was exposed to radiolabeled algal cells and then passed through a 0.2 μm membrane to remove all particles. Uptake of desorbed radioactive Hg by copepods from this seawater constituted the control treatment for the feeding experiments.

MeHg uptake from the dissolved phase

Two experiments were conducted to measure MeHg uptake by copepods from the dissolved phase: 1 in which uptake was evaluated over time and 1 in which MeHg was added at varying concentrations. For the former, $Me^{203}Hg$ was added to 0.2 μm filtered seawater at 0.35 nM and equilibrated for 2 h. Copepods were then exposed to this radioactive seawater (60 individuals in a beaker containing 100 mL filtered seawater; total of 8

beakers). Periodically, at 2 h, 4 h, 8 h, and 12 h, all 60 copepods were collected from each of the 2 replicates onto 20-μm nylon membranes and rinsed with filtered clean seawater in preparation for radioanalysis. To evaluate the uptake over a range of MeHg concentrations, $Me^{203}Hg$ was added to 0.2-μm filtered seawater at 5 different concentrations (20 ng/L, 50 ng/L, 100 ng/L, 250 ng/L, 500 ng/L) and equilibrated for 2 h. Each treatment had 2 replicates. Sixty copepods were exposed to each MeHg concentration for 4 h, after which they were collected onto 20-μm nylon membranes and rinsed with filtered clean seawater for radioanalysis.

^{203}Hg measurements

Radioactivity of ^{203}Hg ingested by copepods was determined noninvasively using a large-well NaI(Tl) gamma detector. Radioactivities of labeled phytoplankton and seawater were measured using an LKB Wallac 1282 Compugamma NaI(Tl) gamma detector equipped with a well detector. The activity of ^{203}Hg was assessed at 279 keV. Intercalibration between the 2 gamma counters was performed, and all samples were counted with standards and decay-corrected. Propagated counting errors were <5%.

RESULTS

Copepods ingested radiolabeled cells during all feeding experiments. Figure 1 shows the percentage of ^{203}Hg ($^{203}Hg^{2+}$ or $Me^{203}Hg$) retained in them over different periods of depuration. All values shown are net values after subtracting radioactivity from control treatments; controls typically accounted for 5% to 15% of the total radioactivity acquired during feeding. The percentage of Hg was calculated as the radioactivity in the copepods divided by the radioactivity measured in the copepods at time 0 of depuration (immediately after feeding). The ^{203}Hg in copepods decreased over time in all experiments during depuration. Depuration patterns typically displayed an initial, rapid loss of Hg from the copepods, followed by a more gradual decline after 24 h of depuration. The initial rapid loss reflects loss of unassimilated boluses of ingested algal food through defecation, whereas the slowly exchanging pool reflects loss of assimilated Hg driven by metabolic activity [2,21]. Assimilation efficiencies (AEs) were calculated from the second stage of depuration (1–3 d), using the equation described by Wang and Fisher [2]

$$\%A = A_0\, e^{-kt} \quad (1)$$

where $\%A$ is the percentage of radioactivity retained in copepods at time t, k is the depuration rate constant during 1 d to 3 d of depuration, and A_0 is the y-axis intercept of radioactivity retained in copepods, defined as the assimilation efficiency of the ingested Hg. Calculated Hg assimilation efficiencies and efflux rate constants (k_e) are shown in Table 1. Copepods ingesting MeHg-labeled food had higher assimilation efficiencies (58–79%) than those ingesting Hg^{2+} labeled food

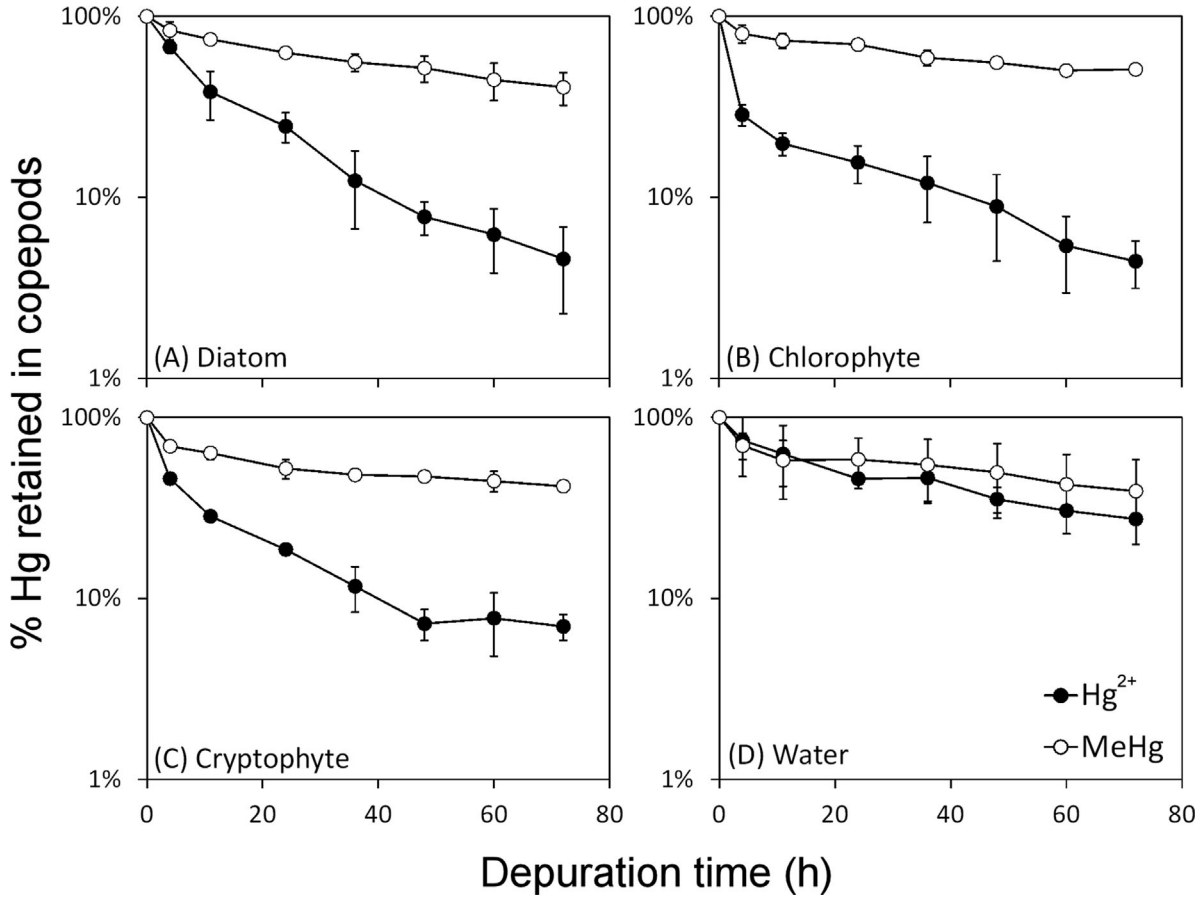

Figure 1. Depuration curves of Hg^{2+} and methylmercury (MeHg) in the copepod *Acartia tonsa* from all experiments. Mercury sources in (**A–C**) are different algal foods, and (**D**) is the dissolved phase. Time 0 represents the radioactivity measured immediately after feeding, which is assigned as 100%. In (**A–C**), radioactivity from controls (dissolved phase) was subtracted from radioactive measurements following feeding. Each point represents the average from 3 replicates, and error bars denote 1 standard deviation.

Table 1. Calculated assimilation efficiencies and efflux rate constants of Hg^{2+} and MeHg in the copepod *Acartia tonsa* from different uptake routes

	Hg^{2+}		MeHg	
Uptake route	AE (%)	k_e (1/d)	AE (%)	k_e (1/d)
Diatom	31	0.64	79	0.22
Chlorophyte	31	0.66	78	0.16
Cryptophyte	25	0.47	58	0.11
Water		0.29		0.21

MeHg = methylmercury; AE = assimilation efficiency; k_e = efflux rate constant.

(25–31%). Assimilation efficiencies for both Hg^{2+} and MeHg among different algal species did not differ significantly. Efflux rate constants following uptake from the aqueous phase (k_{ew}) were similar among the 2 Hg species, 0.29/d for Hg^{2+} and 0.21/d for MeHg. Efflux rate constants following ingestion of Hg-labeled algal food (k_{ef}) were significantly lower for MeHg (0.11–0.22/d) than for Hg^{2+} (0.47–0.66/d). In both Hg^{2+} and MeHg feeding experiments, Hg loss in the form of fecal pellets during the depuration period accounted for 10% to 40% for Hg^{2+} and 1% to 13% for MeHg of total Hg released by copepods, suggesting that excretion of Hg into the dissolved phase was the primary route of Hg loss.

There was a strong relationship between assimilation efficiencies and cytoplasmic distribution of Hg in algal cells ($r^2 = 0.95$); the slope of this regression line was about 1, and the y intercept was about 0 (Figure 2A). The clear separation of MeHg from Hg^{2+} in assimilation efficiencies in *A. tons*a reflects their different penetration into algal cytoplasm of the different algal species (Figure 2A).

Uptake of MeHg from the dissolved phase by copepods increased linearly during the 12 h of exposure (Figure 3A), suggesting that MeHg uptake did not reach equilibrium within the first 12 h of exposure. The dry weight concentration factor ($[Me^{203}Hg\ Bq/g]_{copepod}/[Me^{203}Hg\ Bq/mL]_{dissolved\ phase}$) reached 3×10^4 at 12 h. Figure 3B shows the relationship between MeHg influx rates and dissolved MeHg concentrations after 4 h of exposure. The higher aqueous MeHg concentration led to a greater influx rate, suggesting that the influx rate was directly dependent on the ambient MeHg concentration. The metal influx rate constant of 35 L/g/d, dry weight basis, was obtained from the regression slope of Figure 3B.

DISCUSSION

Hg assimilation and depuration from ingested food

Calculated assimilation efficiencies of Hg^{2+} and MeHg in copepods were comparable to those in previous studies. Fisher et al. [22] found that 21% of Hg^{2+} was assimilated by a marine copepod, *Anomalocera pattersoni*, fed a prymnesiophyte diet, *I. galbana*. Mason et al. [14] reported that 15% of Hg^{2+} and 62% of MeHg were assimilated by marine copepods (*A. tonsa*, *Temora longicornis*, *Centropages* sp.) fed a diatom diet, *Thalassiosira weissflogii*. A similar experiment conducted by Lawson and Mason [16] using marine copepods (*Eurytemora affinis*) and amphipods (*Hyalella azteca*) fed *Thalassiosira weissflogii* obtained an assimilation efficiency for MeHg in zooplankton of >50%. Williams et al. [23] reported assimilation efficiencies of Hg^{2+} and MeHg in the marine amphipod *Leptocheirus plumulosus* fed *I. galbana* to be 6% and 81%, respectively. Overall, these results suggest that the assimilation efficiencies of MeHg are much higher than those of Hg^{2+} and

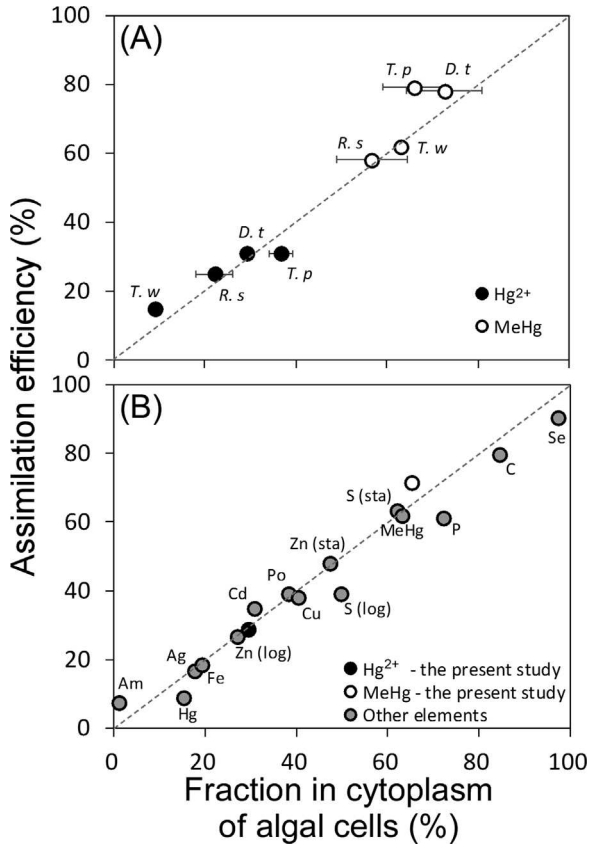

Figure 2. (**A**) Relationship between assimilation efficiencies of Hg in the copepod *Acartia tonsa* and cytoplasmic distributions of Hg in algal cells. Solid and open circles represent Hg^{2+} and methylmercury (MeHg), respectively. Dashed line is the 1:1 ratio line. The linear regression equation was $y = 1.04x + 1.01$, $r^2 = 0.95$. (**B**) Assimilation efficiency of ingested elements in copepods as a function of the cytoplasmic distributions of those elements in the ingested algal cells. Data are average values from Reinfelder and Fisher [15], Hutchins et al. [38], Mason et al. [14], Chang and Reinfelder [39], Stewart and Fisher [24], and the present study. Stationary phase (Sta) and exponential growth phase (log) of the phytoplankton cells consumed by copepods are indicated. The linear regression equation was $y = 0.91x + 2.38$, $r^2 = 0.97$. *D. t.* = *Dunaliella tertiolecta*; *R. s.* = *Rhodomonas salina*; *T. p.* = *Thalassiosira pseudonana*; *T. w.* = *Thalassiosira weissflogii* (data for *T. weissflogii* from Mason et al. [14]).

that diet composition does not have a pronounced influence on the assimilation of Hg^{2+} and MeHg in marine copepods.

As shown in Figure 2A, the pattern of assimilation efficiencies and cellular distributions of Hg in algal diets agrees with other elements reported by Reinfelder and Fisher [15]. As noted above, Hg^{2+} and MeHg showed 2 distinct groups. Methylmercury penetrated into the cytoplasm of algal cells more than Hg^{2+} did, and assimilation efficiencies in zooplankton feeding on these algae followed correspondingly. Moreover, we further compared our Hg data with other elements taken from previous copepod studies (Figure 2B). Generally, there was a strong linear relationship between assimilation efficiency and cytoplasmic distribution of the elements ($r^2 = 0.97$), with the slope close to 1 and the y intercept close to 0. Methylmercury, which is biologically nonessential, has an assimilation efficiency that exceeds some essential metals (e.g., Zn, Cu, and Fe) and is comparable to S and P (Figure 2B). Stewart and Fisher [24] reported that assimilation efficiencies of polonium in marine copepods varied significantly with different algal diets and ranged from 19% to 55%, which was also correlated to the percentage of polonium in cytoplasm of algal cells. However, we did not observe significant

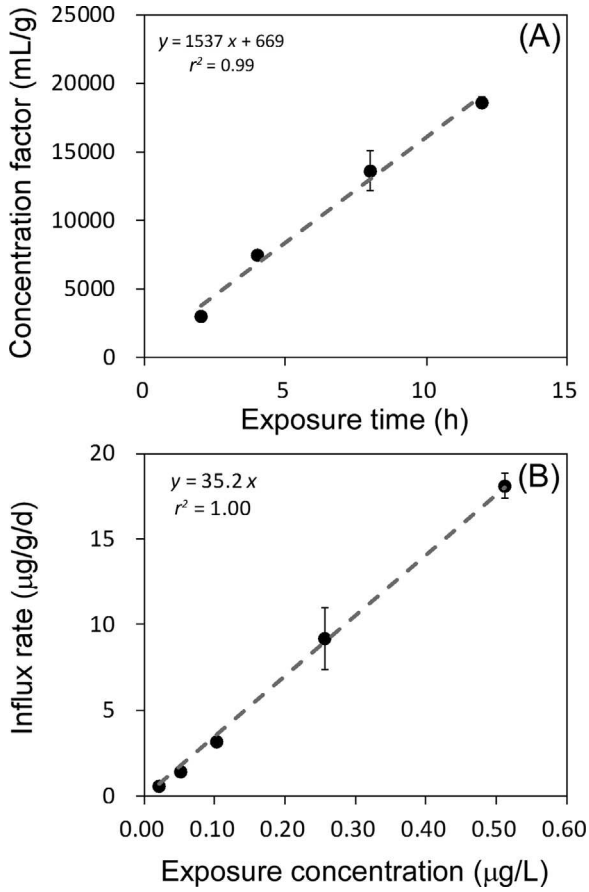

Figure 3. (**A**) Calculated concentration factors of MeHg in copepods exposed to dissolved methylmercury (MeHg) as a function of exposure time. Concentration factors represent the $[MeHg]_{copepods}$ to $[MeHg]_{water}$ ratio for each treatment. (**B**) Influx rate of MeHg in *Acartia tonsa* from the aqueous phase as a function of ambient dissolved concentrations. Each point represents the average from 2 replicates, and error bars denote 1 standard deviation.

differences in assimilation efficiencies and cytoplasmic distributions among the 3 algal diets for either Hg^{2+} or MeHg. The positive relationship of elemental distribution in algal cytoplasm with assimilation efficiencies in herbivorous animals probably reflects the degree to which the metal is released from ingested algal cells into the gut fluid of the copepod in a form that can cross the gut lining [15,24]. Thus, metals bound to compounds in algal cytoplasm that are sought by the animal (such as proteins or amino acids) may more effectively cross the gut lining (become assimilated) than metals bound to cellular components (such as cell walls) that remain undissolved in the gut.

The k_{ew} values of Hg^{2+} (0.29/d) and MeHg (0.21/d) from the copepods were 1.5-fold to 3-fold higher than those of other elements such as silver (0.17/d), cadmium (0.11/d), cobalt (0.12/d), and zinc (0.11/d) [2] and were an order of magnitude greater than that of polonium (0.01–0.03/d) [24]. For k_{ef} values, Hg^{2+} (0.47–0.66/d) was much higher than MeHg (0.11–0.22/d) and than other elements such as silver (0.29/d), cadmium (0.30/d), cobalt (0.28/d), selenium (0.16/d), zinc (0.08/d), and polonium (0.01–0.07/d) [2,24]. The present results indicate that loss rates from copepods following uptake from food were generally greater than those obtained from water, consistent with the findings of Wang and Fisher [2]. Whether this difference is attributable to different copepod tissue distributions of accumulated metal from diet versus water remains to be determined.

MeHg uptake from the dissolved phase

The calculated concentration factor and influx rate of MeHg were closer to those of silver (10.42 L/g/d) than other elements [2]. The study of the physiology of trace element uptake in crustaceans by Rainbow [25] noted that metals could enter these animals through either passive carrier-mediated transport or active transport through ionic or protein channels. It is known that Hg (including MeHg), like silver and copper, has a very strong affinity for sulfur; and this may account for MeHg's very high concentration factors and influx rates from the dissolved phase.

Trophic transfer factors of Hg

Various models have been developed to assess and quantify bioaccumulation and biomagnification of elements and compounds in aquatic ecosystems. The combination of high assimilation efficiencies and low efflux rate constants of MeHg in copepods would result in potential biomagnification in food chains. We used a simple equation to evaluate the trophic transfer factor for Hg^{2+} and MeHg [8]

$$\text{trophic transfer factor} = (\text{AE} \times \text{IR})/(k_{ef} + g) \qquad (2)$$

where AE is assimilation efficiency (%), IR is weight-specific ingestion rate (g/g/d, dry wt), k_{ef} is efflux rate constant from dietary uptake (1/d), and g is growth rate constant (1/d). For trophic transfer factor values >1, biomagnification is expected. We used assimilation efficiency and k_{ef} values obtained from the present study and literature values for growth rate (0.03/d) for *A. tonsa* [26]. A range of ingestion rates (0.33–0.43 g/g/d) of marine copepods from previous studies [26–28] was taken to calculate trophic transfer factors. Calculated trophic transfer factors are listed in Table 2. As expected, trophic transfer factors of Hg^{2+} were consistently low ($\leq$0.2), and trophic transfer factors of MeHg were all >1, suggesting that marine copepods concentrate MeHg and act as an important intermediate between phytoplankton and forage fish to transfer MeHg in marine food chains.

Modeling of Hg uptake pathways in copepods

Metal accumulation in aquatic organisms can be depicted as a balance between metal uptake and loss from aqueous and dietary routes. We used a simple first-order equation to describe the relative contribution from each route and predict the overall final concentrations in zooplankton. At steady state, when metal uptake is balanced by metal elimination and growth, the metal concentration in a given organism can be described by the following equations [8,29]

Table 2. Estimated trophic transfer factors (using Equation 2) of Hg^{2+} and methylmercury (MeHg) in the copepod *Acartia tonsa* following uptake from different food types [a]

Food type	Weight-specific ingestion rate (g/g/d)	Growth rate constant (1/d)	Trophic transfer factor	
			Hg^{2+}	MeHg
Diatom	0.33–0.43	0.03	0.15–0.20	1.04–1.35
Chlorophyte			0.15–0.19	1.37–1.78
Cryptophyte			0.16–0.21	1.40–1.83

[a]Assimilation efficiencies and efflux rate constants are given in Table 1.

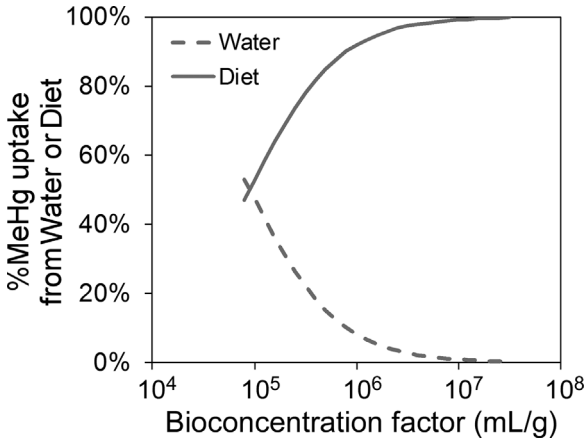

Figure 4. Model prediction of percentage of total methylmercury (MeHg) uptake in the copepod *Acartia tonsa* from dissolved or dietary sources as a function of MeHg bioconcentration factor in algae, which is proportional to the metal concentration in the prey (C_f; Equation 4). The assumed bioconcentration factor ranged from $10^{4.9}$ to $10^{7.5}$ (see [17,30]). Solid and dashed lines represent the contribution from Diet and Water, respectively. The parameters used in this calculation were the mean values from Tables 1 and 2.

$$C_{ss} = C_{ss,w} + C_{ss,f} \quad (3)$$

$$C_{ss} = [(k_u \times C_w)/(k_{ew} + g)] + [(AE \times IR \times C_f)/(k_{ef} + g)] \quad (4)$$

where C_{ss} is the metal concentration (μg/g dry wt) in an organism at steady state; $C_{ss,w}$ is the metal concentration (μg/g) obtained from the dissolved phase; $C_{ss,f}$ is the metal concentration (μg/g) acquired from the diet; k_u is the metal uptake rate constant (L/g/d) from the dissolved phase; C_w and C_f are the metal concentration in the water (μg/L) and the prey (μg/g), respectively; k_{ew} and k_{ef} are the efflux rate constants (1/d) following aqueous and dietary uptake, respectively; IR is the weight-specific ingestion rate (g/g/d, dry wt), and g is the growth rate constant (1/d). We applied Equation 4 to understand MeHg accumulation in the copepods and used average values of k_u (35 L/g/d), k_{ew} (0.21 /d), k_{ef} (0.16 /d), and assimilation efficiency (72%) obtained from our experiments. Maximum ingestion rate (0.43 g/g/d, dry wt) and average g (0.03 /d) were taken from the previous studies [26–28]. The parameter C_{ss} may vary because of variability in biologically mediated parameters such as assimilation efficiency and ingestion rate (which may vary because of food quality and/or concentration and other physiological and/or environmental conditions) as well as variability in geochemical parameters (such as C_w and C_f, which may vary regionally and temporally) in different aquatic environments. First, we used the reported bioconcentration factor (BCF, Cf to Cw ratio, dry wt basis) of MeHg between water and phytoplankton to model the relative importance of aqueous and dietary uptake to marine copepods. The volume concentration factors of MeHg in diverse marine algae ranged from $10^{4.2}$ to $10^{6.8}$ [17], which was equivalent to BCFs of $10^{4.9}$ to $10^{7.5}$ (assuming a mean cell volume to dry wt ratio of 5.0 [30]). Figure 4 shows the model predicted percentage of MeHg uptake from aqueous versus dietary sources as a function of MeHg BCFs in algal food. Overall, diet is the major source (>50%) for MeHg accumulation in copepods. As the MeHg BCF increases, the MeHg uptake contributed from the dissolved phase decreases sharply. In the open ocean, where MeHg BCFs in phytoplankton are typically $>10^6$ [31,32], our model suggests that the dietary route would be the predominant pathway (>90%) for copepods to acquire MeHg.

In addition, we incorporated field data into our model and compared the outcome with independently measured data. To date, the applicable field data we found were from 5 different marine environments (Table 3), including the northwest Atlantic [33]; the central Pacific [31,34]; Long Island Sound, USA [35]; Groswater Bay, Canada [36]; and the Gulf of Lion, France [37]. In Table 3, we present the field data and normalized them to a dry weight basis. If the water content of biota samples was not clearly noted in the original report, we assumed water content of 95% for phytoplankton and 90% for zooplankton. We used a 200 μm cutoff to differentiate between phytoplankton (microseston) and zooplankton. The size range used in each study was also noted in Table 3. Although parameters used in our model referred specifically to the copepod *A. tonsa*, previous work found no pronounced differences for other metals among different calanoid copepod species [15]. Our model-predicted values of MeHg are comparable with those directly measured in bulk zooplankton (Table 3). Zooplankton collected in the Long Island Sound study were primarily composed of calanoid copepods (*Acartia* spp.) [35], and the predicted values closely match the measured values. In the Groswater Bay study, there was only 1 sample for microseston, which may have biased the predicted value. The size range used for collecting microseston in the Gulf of Lion study was 80 μm to 200 μm, which did not comprehensively cover the size spectrum of marine nanophytoplankton and therefore may increase the uncertainty of the predicted value. Note that the k_u value for our modeling was based on experiments using elevated C_w values of Hg^{2+} and MeHg. That the model prediction of C_{ss} in zooplankton matched

Table 3. Comparison between measured methylmercury concentrations in zooplankton collected from different regions and predicted methylmercury concentrations from the model using lab-derived and field-collected parameters[a]

			Zooplankton (ng/g dry wt)		
Study area	Water (ng/L)	Microseston (ng/g dry wt)	Measured	Predicted	Ref.
Northwest Atlantic	0.0066 ± 0.0020	2.8 ± 1.2 (0.2–200 μm)	5.6 ± 5.6 (>200 μm)	5.5 ± 2.0	[33]
Central Pacific	0.019 ± 0.023	11.7 ± 10.4 (0.2–200 μm)	2–34 (>200 μm)	21.5 ± 16.9	[31,34]
Long Island Sound	0.03	4.7 ± 2.8 (0.2–200 μm)	11 ± 2 (>200 μm)	12 ± 4	[35]
Groswater Bay	0.023 ± 0.012	0.16 (5–200 μm)	7.0 (200–500 μm)	3.6 ± 1.7	[36]
Gulf of Lion	0.0045 ± 0.0026	1.4 ± 1.0 (80–200 μm)	5.2 ± 3.9 (200–500 μm)	2.9 ± 1.6	[37]

[a]For these calculations, C_w is the methylmercury concentration reported for water at each site, C_f is the methylmercury concentration in microseston at each site, and other parameters for methylmercury from Equation 4 are means from values given in Table 1. Thus, efflux rate constant following dietary uptake = 0.16 (1/d), assimilation efficiency = 72 (%), ingestion rate = 0.43 (g/g/d), growth rate constant = 0.03 (1/d), metal uptake rate constant = 35 (L/g/d), and efflux rate constant following aqueous uptake = 0.21 (1/d). Model-predicted values for copepods are compared with independently measured methylmercury concentrations in zooplankton at each site. Propagation of uncertainty (1 standard deviation) was calculated if applicable.

independent field values (Table 3) suggests that this k_u value is applicable to Hg concentrations in natural waters.

Overall, our model results suggested that it is possible to predict the concentration of MeHg in copepods given the concentration of MeHg in water and phytoplankton. Of course, it is easier to test model predictions in the field when there is a clear delineation of phytoplankton and zooplankton from other suspended particles in the same size ranges. Complications can arise when suspended abiotic particles (e.g., sediment particles) co-occur with the plankton. Recently, Lee and Fisher [17] reported that MeHg concentration factors in diverse marine phytoplankton cells are related to algal cell size. Therefore, the MeHg concentration in both phytoplankton and zooplankton can be calculated with only a few measured parameters. The parameters measured in the present study along with the established model should be useful in refining the global biogeochemical models describing Hg cycling in the marine environment.

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Data Availability—Data, associated metadata, and calculation tools are available from the corresponding author (cheng-shiuan.lee@stonybrook.edu).

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