Biogeochemistry **40:** 235–247, 1998.

Accumulation of mercury in estuarine food chains

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Key words: fish, invertebrates, ligands, mercury, methylmercury, phytoplankton

Abstract. To understand the accumulation of inorganic mercury and methylmercury at the base of the estuarine food chain, phytoplankton (*Thalassiosira weissflogii*) uptake and mercury speciation experiments were conducted. Complexation of methylmercury as methylmercury-bisulfide decreased the phytoplankton uptake rate while the uptake rate of the methylmercury-cysteine and -thiourea complexes increased with increasing complexation by these ligands. Furthermore, our results indicated that while different ligands influenced inorganic mercury/methylmercury uptake by phytoplankton cells, the ligand complex had no major influence on either where the mercury was sequestered within the phytoplankton cell nor the assimilation efficiency of the mercury by copepods. The assimilation efficiency of inorganic mercury/methylmercury by copepods and amphipods feeding on algal cells was compared and both organisms assimilated methylmercury much more efficiently; the relative assimilation efficiency of methylmercury to inorganic mercury was 2.0 for copepods and 2.8 for amphipods. The relative assimilation is somewhat concentration dependent as experiments showed that as exposure concentration increased, a greater percentage of methylmercury was found in the cytoplasm of phytoplankton cells, resulting in a higher concentration in the copepods feeding on these cells. Additionally, food quality influenced assimilation by invertebrates. During decay of a *T. weissflogii* culture, which served as food for the invertebrates, copepods were increasingly less able to assimilate the methylmercury from the food, while even at advanced stages of decay, amphipods were able to assimilate mercury from their food to a high degree. Finally, fish feeding on copepods assimilated methylmercury more efficiently than inorganic mercury owing to the larger fraction of methylmercury found in the soft tissues of the copepods.

Introduction

The presence and behavior of mercury in aquatic systems is of great interest and importance. For example, mercury is the only metal which bioaccumulates through all levels of the aquatic food chain (e.g. Lindqvist et al. 1991). Accordingly, mercury contamination in fish has been a widespread health concern (Clarkson 1990; Fitzgerald & Clarkson 1991). Lower trophic levels play a major role in mercury bioaccumulation as, for example, in the pelagic realm, the greatest bioconcentration occurs between the water and phytoplankton (Lindqvist et al. 1991; Mason et al. 1996).

Previous experiments (Mason et al. 1996) showed that uptake of inorganic mercury (Hg(II)) and methylmercury ($CH_3Hg(I)$) by phytoplankton in estuarine environments involved passive diffusion of neutral mercury species

across the membrane. These experiments demonstrated the impact of pH and salinity on the uptake of Hg(II) and $CH_3Hg(I)$ by phytoplankton and demonstrated that the accumulation rate was a function of the water chemistry – pH, salinity, and ligand concentration – which influenced mercury speciation, i.e. uptake was controlled by mercury complexation. To extend these studies, we investigated how different organic ligands affected Hg(II)/$CH_3Hg(I)$ uptake and sequestration within phytoplankton cells.

Previous results also demonstrated that assimilation efficiency by primary consumers was directly related to the fraction of the element within the algal cytoplasm (Reinfelder & Fisher 1991; Mason et al. 1996). Thus, if Hg(II) and $CH_3Hg(I)$ complexation influences sequestration within phytoplankton cells, it will also affect the subsequent trophic transfer of Hg(II) and $CH_3Hg(I)$ to primary consumers. We therefore examined how different ligands influenced subsequent uptake of mercury by copepods. The food chain studies were extended to compare the assimilation efficiency of copepods relative to amphipods. As amphipods typically feed at the sediment-water interface, we performed an experiment to see how degraded phytoplankton material, as might be found at the end of a spring bloom, influenced assimilation. To complete the food chain studies, we investigated assimilation of Hg(II)/$CH_3Hg(I)$ by fish feeding on copepods to ascertain whether assimilation was related to the sequestration of Hg(II)/$CH_3Hg(I)$ within the copepods.

Experimental design

All mercury concentrations in the following experiments were determined by cold vapor atomic fluorescence spectroscopy (Bloom & Fitzgerald 1988). Samples were brominated, then neutralized, and bubbled with argon in the presence of tin chloride (Bloom & Crecelius 1983). The mercury was collected on gold columns under clean conditions prior to quantification by cold vapor atomic fluorescence spectroscopy.

For short term phytoplankton uptake experiments, cultures of the marine diatom *Thalassiosira weissflogii* in 500 ml polycarbonate bottles were spiked with the appropriate ligand and Hg(II) or $CH_3Hg(I)$ was added at pM to nM concentrations. The phytoplankton cultures were stored at 20 °C under light for four to six hours to allow assimilation to occur. A sample of culture was then taken and the fluorescence determined. This allowed calculation of cell concentration from a predetermined relationship between fluorescence and cell concentration. A known volume of culture was then filtered through a 3 μm polycarbonate filter for total mercury analysis. Samples were stored frozen in Teflon vials until the analyses were performed.

The overall octanol/water partitioning for the methylmercury complexes was determined in the presence of the different ligands (sulfide, thiourea, and cysteine) in order to calculate the K_{ow}'s of the various $CH_3Hg(I)$-ligand complexes. Extractions were performed with equal volumes (25 ml) of octanol and 10 ppt seawater at different ligand concentrations. The solutions were adequately mixed then allowed to separate and equilibrate. The water layer was then removed and its total mercury concentration measured. From the calculated speciation in each experiment, determined using MINEQL, the concentration of neutral complex was determined. It was assumed that only the neutral species partition into octanol, and thus the K_{ow} of the neutral complexes could be estimated (see Mason et al. 1996 for details).

For the phytoplankton fractionation experiments, samples of *T. weissflogii* cultures, which had been incubated in the presence of $CH_3Hg(I)$ and each ligand for at least 12 hours, were removed for fractionation. The samples were placed in polycarbonate centrifuge tubes and sonicated for 10 minutes at an output of 5 to 6 and a 70 to 80% duty cycle. The sonicated samples were then centrifuged for 20 minutes at 10,000 rpm to separate the mixture into two fractions: cell membrane and cytoplasm. These fractions were individually placed in separate vials for total mercury analysis.

Subsamples of these cultures were also resuspended into fresh seawater and the copepod *Eurytemora affinis* added for the copepod feeding experiments. Feeding was allowed for four to six hours. After this period of time, copepods were removed from the algal suspension using a 165 μm mesh screen, placed in fresh seawater, and allowed 30 to 45 minutes to clear their guts. Copepods were then filtered onto polycarbonate filters. Copepod fecal pellets were separated from algal cells using a 64 μm mesh screen and both were filtered separately onto 3 μm polycarbonate filters. All fractions were stored frozen in Teflon vials and analyzed for total mercury. Assimilation efficiency was calculated as the ratio of mercury assimilated by the copepods to mercury found in both the copepods and their fecal pellets:

$$\text{Assimilation Efficiency} = \{(A - B) * C\}/\{[(A - B) * C] + D\}$$

where A = [Hg] in copepods after feeding; B = [Hg] in copepods before feeding; C = # of copepods; and D = [Hg] in fecal pellets.

Invertebrate assimilation experiments were similarly performed with the copepod *E. affinis* or the amphipod *Hyallela azteca*. Feeding was again for four to six hours. The invertebrates were then removed from the cultures and allowed to clear their guts. Copepods were collected on polycarbonate filters while the amphipods were hand-picked and placed in Teflon vials for total mercury analysis. The fecal pellets of the invertebrates and samples of algae were also collected for total mercury analysis.

To investigate how the availability of $CH_3Hg(I)$ changed during the decay of a phytoplankton culture after senescence, a large culture of *T. weissflogii* was grown and supplied with $CH_3Hg(I)$. After at least 12 hours of exposure, growing phytoplankton from the culture were fed to *E. affinis* and *H. azteca*. The culture was then placed in darkness and no longer aerated. As the culture proceeded to deteriorate and decompose due to bacterial activity over the following 33-day period, feeding experiments were conducted at regular intervals with both of the above organisms.

To determine the fractionation of Hg(II) and $CH_3Hg(I)$ within copepods, a culture of *E. affinis* was raised from nauplii and fed on mercury-laden phytoplankton for two to three weeks. A sample of adult copepods was collected and analyzed for the total mercury present in the intact organism. Other samples were collected, digested at 45 °C for 60 minutes in 0.2 N NaOH, filtered, rinsed, and the filtrate analyzed for total mercury. This procedure allowed us to measure how much of the mercury was in the exoskeleton of the copepods, and by difference the amount of mercury in the soft tissues of the copepods.

The assimilation efficiency of mercury by fish feeding on copepods was determined using pre-exposed copepods. Starting with nauplii, *E. affinis* were fed mercury-laden phytoplankton for two to three weeks. Adult copepods were strained from the tank, counted, and fed to *Cyprinodon variegatus* (sheepshead minnow) larvae. After all the copepods were eaten, which took at most 25 hours, the fish were placed in fresh seawater and allowed to clear their guts for at least 20 hours. The fish total mercury concentration was determined on a wet weight basis. Fish samples were digested in 70% sulfuric/30% nitric acid prior to analysis. Assimilation efficiency was calculated as the ratio of mercury assimilated by the fish to mercury present in the copepods eaten:

$$\text{Assimilation Efficiency} = \{(F - E) * G\}/\{(H * I)\}$$

where E = Average [Hg] in ng/g wet wt of control fish; F = [Hg] in ng/g wet wt of fish at end of experiment; G = Final fish wet wt; H = [Hg] in copepods (ng/copepod); and I = # of contaminated copepods eaten.

Results and discussion

Phytoplankton uptake and mercury speciation

Experiments were performed in the presence of varying concentrations of cysteine, thiourea, and sulfide. At the salinity of the experiments (14 ppt) essentially all the $CH_3Hg(I)$ is present as CH_3HgCl in the absence of the

organic ligands (Mason et al. 1996). Thus, the relative uptake rates, normalized to the rate of uptake in the absence of organic ligands, show the rate of $CH_3Hg(I)$ assimilation relative to that of the chloride complex. For the ligands, speciation calculations were made using MINEQL and literature values for the complexation constants. It was assumed that the sulfide and organic ligands were adequately stable throughout the short term experiements. Once a complex is formed between the ligand and mercury, the complex itself is much more stable owing to the strong binding constants of sulfide with mercury. Also, the half time for sulfide in oxygen saturated seawater is a few hours (Stumm & Morgan 1981); and thus, although there may have been a potentially significant loss at the lowest sulfide concentrations, all of the sulfide at low concentrations is bound to mercury. At higher sulfide concentrations, oxidation was likely insufficient to change speciation.

The data in Figure 1 show that for CH_3HgSH, there was a decreasing trend in relative uptake rate as the relative concentration of the neutral sulfide complex increased. For the organic ligands used in these experiments – cysteine and thiourea – the uptake rate increased at the highest ligand concentrations where a significant fraction of the $CH_3Hg(I)$ was bound as an organic complex. The concentrations of sulfide used in these experiments are comparable, though slightly higher than, concentrations found in the ocean, while the concentrations of cysteine are somewhat higher than those expected in oxic waters, but could easily be encountered in pore water. Though we are not aware of any measurements of thiourea concentrations, the concentration is likely comparable to that of other sulfur-containing ligands found in aquatic systems. From the relative uptake rates at the highest ligand exposures, and based on the experimentally determined permeability for CH_3HgCl (Mason et al. 1996), it is possible to estimate the permeabilities (P) of the $CH_3Hg(I)$-ligand complexes:

$$P_{ligand} = P_{Cl} * [(\text{relative uptake} * \chi_{Cl(L=0)}) - \chi_{Cl}]/\chi_L$$

where χ is the mole fraction of the ligand or chloride (Cl) complex; $\chi_{Cl(L=0)}$ represents the mole fraction of the chloride complex with no added ligand. The permeabilities were calulated based on relative uptake at the highest ligand concentrations. This ensured that any oxidation of the ligands which might have occured did not influence the permeability calculations.

The octanol-water partition coefficients (K_{ow}'s) were determined for the CH_3Hg-ligand neutral complexes. The more lipophilic thiourea complex (log K_{ow} = 2.8) (see Table 1) has a higher permeability than the similarly-sized cysteine complex (log K_{ow} = 1.7). The chloride complex has a relatively high permeability even though it has a low K_{ow}. These differences occur because

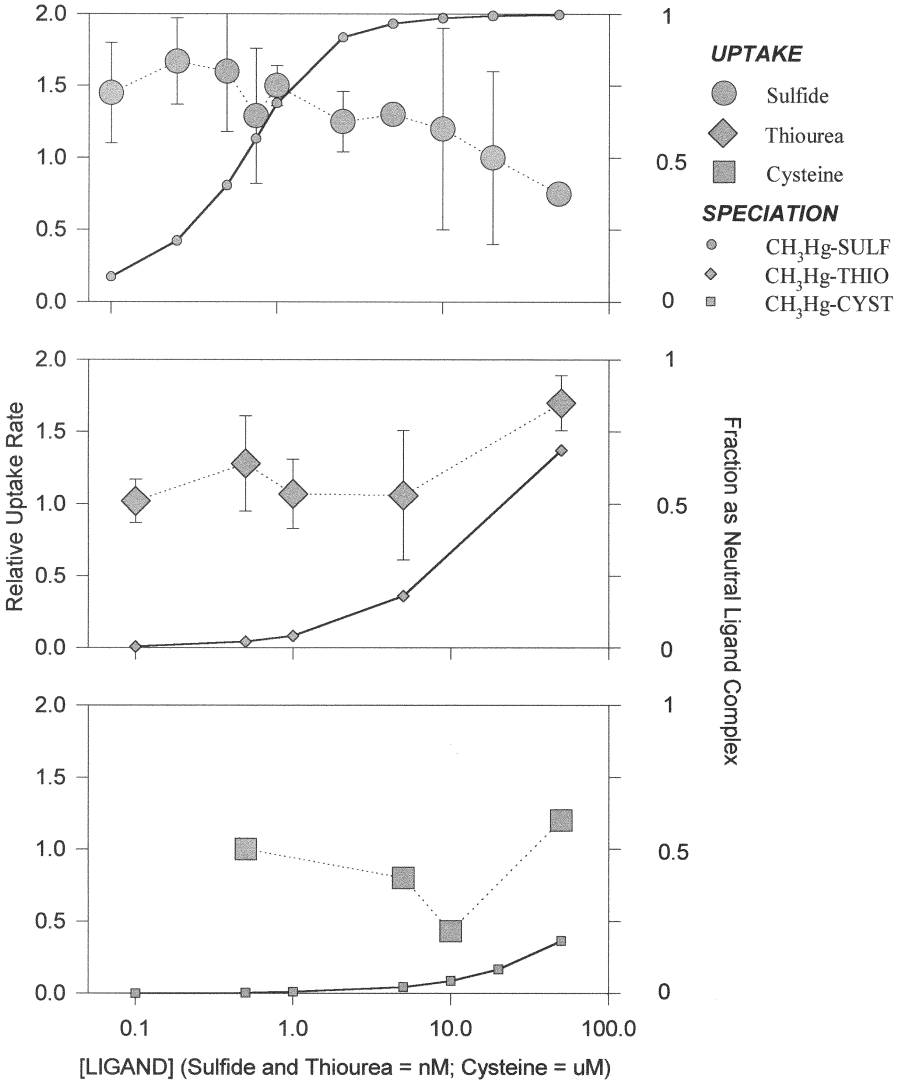

Figure 1. The effect of ligands on uptake of $CH_3Hg(I)$ by the diatom *Thalassiosira weissflogii*.

P is not directly related to K_{ow} (Mason et al. 1996) but is also dependent on the diffusion rate of the compound through the membrane ($D_{membrane}$) i.e.:

$$P \propto (K_{ow} \times D_{membrane})$$

To correct for the effect of diffusion, it is necessary to know the molecular volume of the complexes. Various empirical relationships do exist for estimating molecular volume but these are not suitable for the determination of the

Table 1. Estimated permeabilities (P) for the methylmercury-ligand complexes and corresponding octanol-water partition coefficients (K_{ow}) for the ligand complexes.

Ligand	Estimated P $\times$ 10^{-4} cm·s^{-1}	Log K_{ow}
Sulfide	4.7	1.45
Thiourea	12.6	2.8
Cysteine	3.6	1.7
Chloride	7.2	0.23

molecular volume for organo-metallic complexes. The data presented here indicate, however, that the CH_3Hg-organic complexes have permeabilities lower than expected based on their relative K_{ow}'s compared with CH_3HgCl; this is expected because of their larger size (smaller $D_{membrane}$). For CH_3HgSH and CH_3HgCl, which have similar K_{ow}'s and similar molecular volumes, the uptake rates are similar.

Phytoplankton fractionation and invertebrate assimilation efficiencies

The influence of the binding ligands on both the assimilation efficiency of $CH_3Hg(I)$ by copepods and the cellular fractionation within the diatoms is shown in Figure 2. No clear trend was evident when the fraction of mercury which is contained in the algal cell cytoplasm is plotted against the various ligands used. Thus, when phytoplankton are exposed to CH_3Hg-ligand complexes, the complexing ligand does not have a major effect upon where $CH_3Hg(I)$ is sequestered within the cell. This is expected as it is likely that once the mercury-ligand complex crosses the lipophilic membrane, it dissociates as a result of the changed chemical environment. The mercury then binds to other ligands and/or reactive sites within the algal cell that are present at relatively high concentrations. Thus, when copepods ingest phytoplankton exposed to such conditions, the ligands appear to have no effect on assimilation efficiency of mercury by the copepods. Therefore, the water chemistry influences only the rate of uptake at the base of the pelagic food chain but not the subsequent trophic transfer to consumers.

Copepods and amphipods were also fed diatoms that had been exposed to Hg(II) and $CH_3Hg(I)$, to determine their assimilation efficiencies for both compounds (Figure 3). Both copepods and amphipods were able to assimilate $CH_3Hg(I)$ much more efficiently than Hg(II). The relative assimilation of $CH_3Hg(I)$ to Hg(II) was 2.0 for copepods and 2.8 for amphipods. The relative assimilation for the copepods was similar to that found by Mason et al. (1996) for a mixed assemblage of copepods collected from Massachusetts Bay.

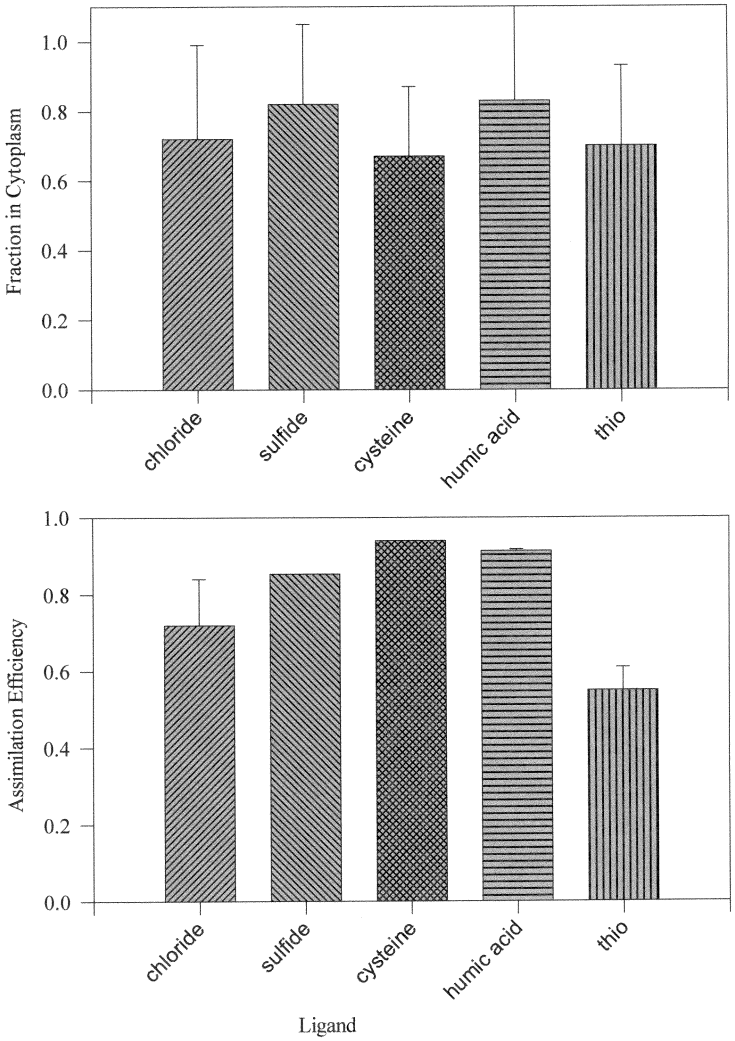

Figure 2. The effect of ligands on the percent of $CH_3Hg(I)$ which is found in the cytoplasm of *T. weissflogii* and the effect of ligands on the assimilation efficiency of $CH_3Hg(I)$ by the copepod *Eurytemora affinis* fed *T. weissflogii*.

Thus, both organisms are able to readily assimilate $CH_3Hg(I)$ from living cells. However, this was not the situation when $CH_3Hg(I)$-laden algal cultures were allowed to naturally degrade (in the presence of the natural bacterial assemblage) prior to being fed to the invertebrates. A difference in assimilation efficiency was found between copepods and amphipods, as shown in Figure 4. The quality of the food seems to have no effect on assimilation efficiency of $CH_3Hg(I)$ by amphipods, for even at the end of the experiment

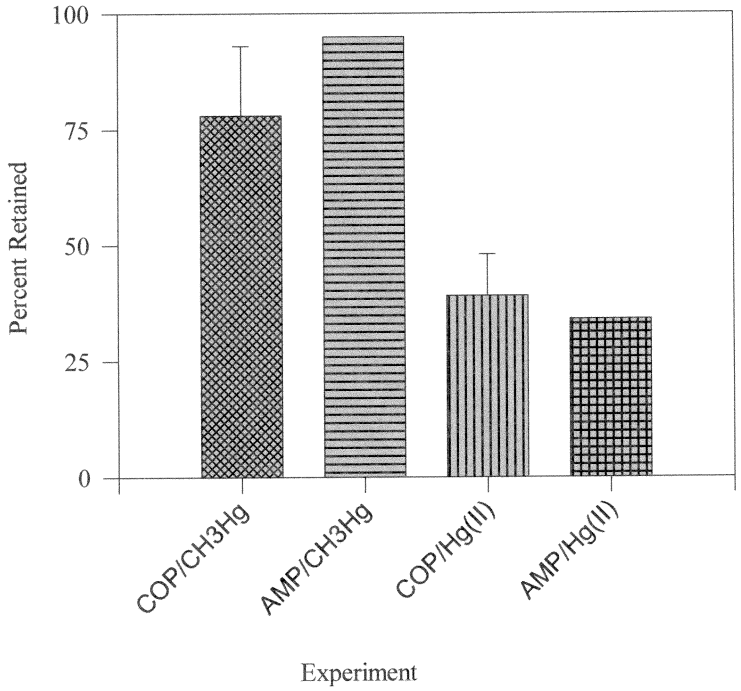

Figure 3. Percent of $CH_3Hg(I)$ and Hg(II) retained by the copepod *E. affinis* and the amphipod *Hyallela azteca* fed contaminated phytoplankton.

(33 days) when the phytoplankton biomass has reached an advanced state of deterioration (Harvey et al. 1995), the amphipods still assimilated the mercury to a high degree. However, the older and more degraded the culture, the less copepods were able to assimilate the $CH_3Hg(I)$. One obvious explanation for these findings concerns the location in the water column at which these organisms feed and their typical food source. Copepods swim and capture particles suspended within the water column, and typically only encounter and feed on live algal cells. Food passes through their digestive system rapidly and only the readily assimilated material is taken up by the copepods. Amphipods, on the other hand, are benthic organisms which feed on detritus and other decaying material which sinks out of the water column. Obviously, amphipods have therefore developed mechanisms for extracting nutrition from such material and it is likely that this feeding strategy also results in a better assimilation of $CH_3Hg(I)$ from the degraded phytoplankton.

Effect of exposure concentration

It is possible that the relative distribution of mercury within the phytoplankton is a function of the total assimilated concentration. If membrane binding sites

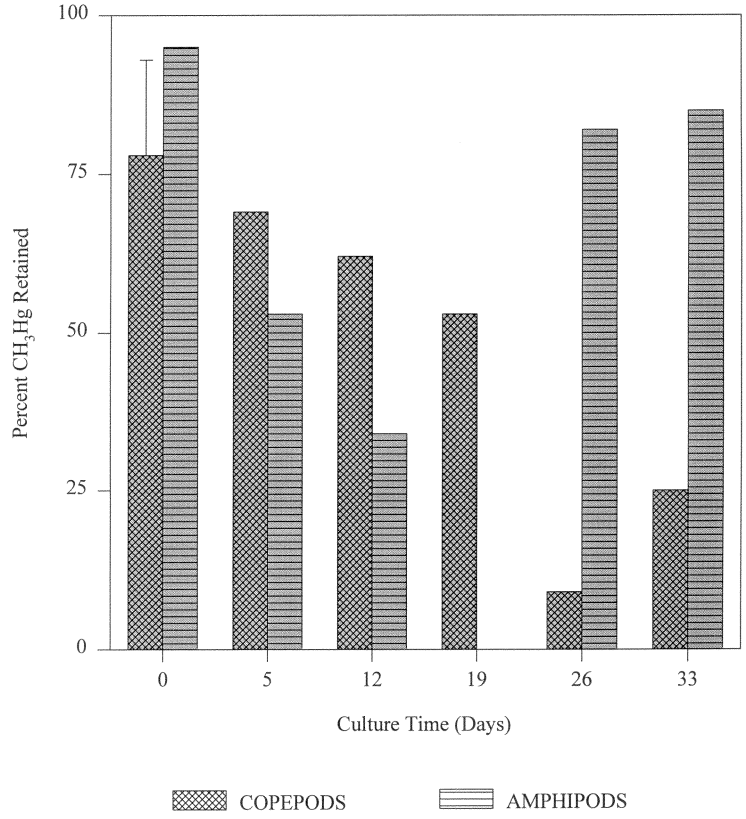

Figure 4. The effect of "food quality" on the percent of $CH_3Hg(I)$ retained by the copepod *E. affinis* and amphipod *H. azteca* when they are fed degrading phytoplankton biomass.

are limited, then the fractionation of the $CH_3Hg(I)$ in the cell cytoplasm should change with increasing cell concentration. This is indeed the case (Figure 5). At low exposures, a higher fraction of the $CH_3Hg(I)$ resides in the cell membrane. In concert, the concentration of $CH_3Hg(I)$ found in copepods feeding on these algae increases with exposure concentration with the increase in fraction of the $CH_3Hg(I)$ found in the phytoplankton cytoplasm. These differences are in agreement with the copepod assimilation model put forward by Reinfelder & Fisher (1991) i.e. fractionation within the algae determines copepod assimilation. These results suggest that trophic transfer efficiency will be higher for contaminated systems, or systems with low pH, which both result in higher loadings of mercury within phytoplankton.

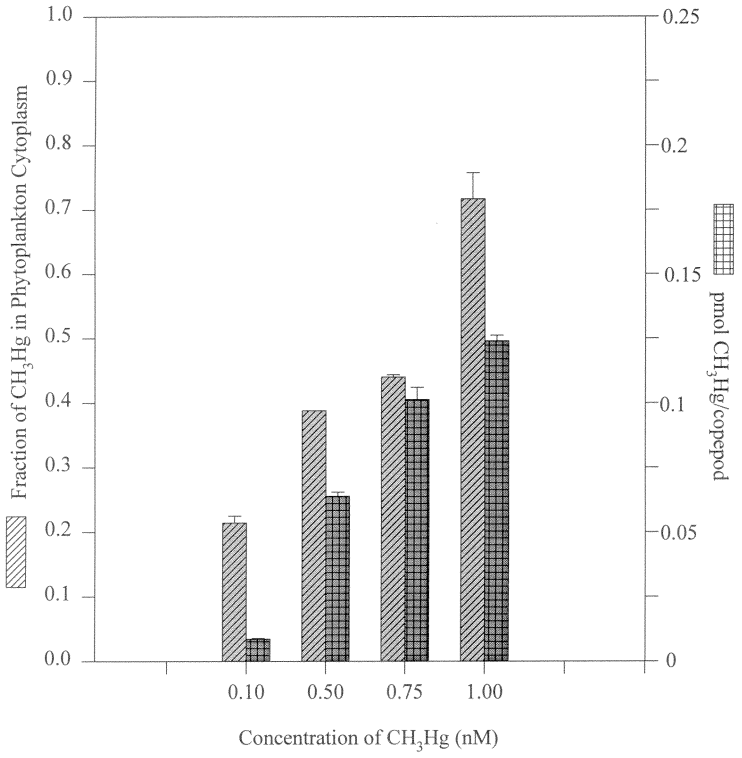

Figure 5. The fraction of $CH_3Hg(I)$ present in the cytoplasm of *T. weissflogii* and the concentration of $CH_3Hg(I)$ found in the copepod *E. affinis* fed this phytoplankton.

Assimilation of Hg(II) and $CH_3Hg(I)$ by fish

Copepods fed on mercury-laden algae were fed to *C. variegatus.* The assimilation efficiency of mercury by the fish was compared to the percent of the Hg(II) or $CH_3Hg(I)$ present in the soft tissues of the ingested copepods. Reinfelder & Fisher (1994) have recently suggested that there is a linear relationship between assimilation efficiency of an element by fish and the fractionation of the element between the copepod tissues and the exoskeleton. As seen in Table 2, assimilation by the fish was highest for $CH_3Hg(I)$, in concert with a larger percent of $CH_3Hg(I)$ being associated with the non-exoskeleton tissues of the food. Assimilation of $CH_3Hg(I)$ by fish was greater than assimilation of Hg(II) by a factor of 3. These results concur with field data (Watras & Bloom 1992; Lindqvist et al. 1991; Mason & Sullivan, 1997) that indicate that $CH_3Hg(I)$ is preferentially accumulated over Hg(II) at all levels of the food chain by a factor of 3 to 5 per trophic transfer. The data for $CH_3Hg(I)$ fit the Reinfelder & Fisher (1994) model. However, for Hg(II), the percent

Table 2. Percent of ingested mercury associated with soft tissues of copepods and the corresponding assimilation efficiencies (AE) of mercury by fish.

Parameter	Hg(II)	$CH_3Hg(I)$
% in soft tissues of copepods	85	100
AE by *C. Variegatus*	37	76

associated with soft tissues is much higher than would be expected based on the assimilation efficiency. The fractionation method used here does not distinguish between intracellular material and cellular material and thus it is possible that Hg(II) is strongly bound to cell membranes or in the gut lining of the food, for example, and is therefore relatively unavailable for assimilation.

Conclusions

Methylmercury uptake by phytoplankton was examined in the presence of mercury-binding ligands. While uptake decreased as the portion of the mercury bound as the CH_3Hg-bisulfide complex increased, the opposite was found for the CH_3Hg-thiourea and -cysteine complexes. However, the percent of the CH_3Hg-ligand complex which was stored in the cytoplasm relative to the membrane did not depend upon which ligand was present and as a result, when these contaminated phytoplankton were supplied as food to copepods, assimilation efficiency of the $CH_3Hg(I)$ was unaffected. In comparative experiments, it was found that assimilation efficiency of $CH_3Hg(I)$ exceeded that of Hg(II) for both amphipods and copepods, and copepods were less able to assimilate $CH_3Hg(I)$ from a culture subjected to microbial decay. Even after 33 days of senescence, however, amphipods were able to assimilate a large amount of $CH_3Hg(I)$ from their food. Exposing phytoplankton to different concentrations of $CH_3Hg(I)$ demonstrated that at low exposures, a higher fraction of the $CH_3Hg(I)$ resided in the cell membrane than the cytoplasm. Greater $CH_3Hg(I)$ concentrations were measured in the copepods which fed on the algae exposed to higher concentrations, i.e. under conditions where a larger fraction of $CH_3Hg(I)$ was found in the cell cytoplasm. Fish feeding on copepods assimilated $CH_3Hg(I)$ more efficiently than Hg(II). This difference is explained by the fact that a larger fraction of the $CH_3Hg(I)$ was found in the soft tissues of the copepod as opposed to its exoskeleton.

Acknowledgements

This research has been funded by Sea Grant through the CBEEC program and formed part of the thesis research of Nicole L. Morgan. We thank Bud Millsaps, John Magee, and others at CBL for their assistance in this research.

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