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Bioaccumulation kinetics and exposure pathways of inorganic mercury and methylmercury in a marine fish, the sweetlips *Plectorhinchus gibbosus*

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ABSTRACT: We experimentally determined the assimilation efficiency (AE) of ingested prey, the uptake-rate constant from the aqueous phase, and elimination-rate constant of both inorganic Hg (Hg[II]) and methylmercury (MeHg) in a marine predatory fish, the sweetlips *Plectorhinchus gibbosus*, using radiotracer techniques. The AE of Hg(II) and MeHg ranged between 10 and 27 % and between 56 and 95 %, respectively, for 3 different prey (copepods, silverside, and brine shrimp). The ingestion rate of the fish did not significantly affect the AE of Hg(II). Uptake of both species of Hg proceeded in a linear pattern, and the calculated uptake-rate constant was 0.195 and 4.515 l g^{-1} d^{-1} for Hg(II) and MeHg, respectively. Most of the accumulated Hg(II) was distributed in muscle tissues, whereas the accumulated MeHg was distributed evenly between gills and muscle tissues. The calculated elimination-rate constants for MeHg were 0.0103 and 0.0129 d^{-1} following dietary and aqueous uptake, respectively, whereas the elimination-rate constant of Hg(II) following dietary uptake (0.0547 d^{-1}) was 1.9 times higher than the elimination following aqueous uptake (0.0287 d^{-1}). These experimentally determined values were incorporated into a kinetic model to predict the exposure pathways and the relative contribution of Hg(II) and MeHg to the sweetlips. At the high end of the bioconcentration factor for both species of Hg in the prey, dietary ingestion is likely to be the main channel for their accumulation in the fish. The relative contribution of Hg(II) vs MeHg to the overall Hg bioaccumulation is largely controlled by the relative concentration of MeHg dissolved in seawater. Similar to the results of numerous field studies, the kinetic model predicted a potential trophic transfer factor of <0.6 for Hg(II) and >1 for MeHg under conditions likely to be experienced by the fish in its natural environment.

KEY WORDS: Mercury · Methylmercury · Exposure · Fish · Kinetic modeling

INTRODUCTION

Concern about mercury (Hg) in marine food webs is mainly related to the biomagnification of methylmercury (MeHg) with increasing trophic levels (Monteiro et al. 1996, Boudou & Ribeyre 1997, Bargagli et al. 1998, Jackson 1998, Mason 2002, Wang 2002). There are numerous examples of the biomagnification of MeHg toward the upper end of the marine food chain, raising considerable concern about potential human exposure through seafood consumption. In Hong Kong, some reports have pointed to the possible links between local human male subfertility and the consumption of seafood containing Hg (Dickman et al. 1998, 1999). There is now substantial concern about the potential human parental transfer of mercury in Hong Kong, because fishes comprise the major seafood in the region. Understanding the bioaccumulation and bioavailability of different Hg species in marine fishes can thus aid the prediction of the environmental fate of Hg (Morel et al. 1998) and realistic risk-assessment of Hg exposure and toxicity. Many studies have examined the bioaccumulation of inorganic Hg [Hg(II)] and MeHg in freshwater fishes (Boudou & Ribeyre 1984, 1997, Ribeyre & Boudou 1984, Boudou et al. 1991, Jackson 1998, Bowles et al. 2001). The biokinetics and

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bioavailability of these Hg species in marine fishes are less well studied (Pentreath 1976a,b, Honda et al. 1978, Barkay et al. 1997, Rouleau et al. 1998).

Most previous studies measured the concentration of different Hg species in different tissues of fishes (Leah et al. 1991, Monteiro et al. 1996, Joiris et al. 2000). The routes of Hg accumulation, including the relative importance of different Hg species (inorganic and organic) and exposure pathways (aqueous vs dietary), are not yet well understood. Whereas it has generally been assumed that fishes accumulate MeHg mainly from dietary sources through very efficient assimilation of this compound (Mason 2002), it is quite possible that, because of its highly efficient absorption properties, they accumulate it from the aqueous phase also. Experimental studies on the routes of methylmercury exposure in fishes are indeed rather controversial (Honda et al. 1978, Phillips & Buhler 1978, Fujiki 1980, Hall et al. 1997). Kinetic modeling now offers a powerful approach for delineating the exposure of different metal species and pathways (Wang et al. 1997, 1998, Wang & Fisher 1999). Within the framework of the kinetic model, however, some important parameters need to be quantified, including the assimilation efficiency from the ingested prey, the uptake-rate constant from the aqueous phase, and the elimination-rate constant, as well as some physiological and geochemical parameters such as the feeding rate of the animals and the concentrations of metals in the water and in the dietary sources. Although the biokinetic parameters of metals have been extensively quantified in several species of marine animals, many of these parameters are not yet available for marine fishes (Baines et al. 2002, Xu & Wang 2002).

The relative importance of Hg(II) and MeHg in the overall Hg bioaccumulation in fishes has been little studied. Whereas numerous studies have demonstrated that MeHg is the predominant Hg form (Bowles et al. 2001, Kannan et al. 1998) in fish muscle, it is not known whether it is directly accumulated from the ambient environment (including both aqueous and dietary sources) or is methylated within the fish body. In natural oxic water, the proportion of MeHg in the total Hg concentration is generally very low (Fitzgerald & Mason 1997, Watras et al. 1998); thus, whether MeHg can contribute considerably to the overall Hg bioaccumulation in fishes is a matter of speculation. The speciation of Hg in other fish tissues (e.g. gills, brains, viscera) has not been extensively quantified. There is some evidence of considerable methylation and demethylation in fish muscle (Burrows & Krenkel 1973, Simon & Boudou 2001), although some studies have also indicated that fishes are unlikely to methylate the inorganic Hg to MeHg within their tissues *in vivo* (Pennacchioni et al. 1976).

In this study, we examined the biokinetics of both Hg(II) and MeHg in a marine predatory fish (the sweetlips *Plectorhinchus gibbosus*) during both the aqueous and dietary phases. Sweetlips is important fish species widely cultured in China, and is used either as fish food or for direct human consumption. We measured a few kinetic parameters (assimilation efficiency, aqueous uptake-rate, and elimination-rate) of both Hg species in the fish. A simple kinetic model (Thomann 1981) was then constructed to predict the trophic transfer factor of Hg and the exposure pathways of both Hg species in the fish under different environmental conditions. Despite the considerable ecological interest in the trophic transfer of mercury in marine food chains, the exposure pathways and the relative importance of different mercury species in mercury bioaccumulation in marine animals are not well known. Such information is critical for the assessment of the environmental fate of mercury in marine ecosystems as well as for determining the transfer of mercury in marine planktonic food chains.

MATERIALS AND METHODS

Fish and radioisotopes. Marine sweetlips *Plectorhinchus gibbosus* (3.0 to 3.5 cm) were purchased from a fish farm in Hong Kong, and were maintained in aerated artificial seawater (Instant Ocean®; 23°C, 30 psu) and fed fresh clam and/or frozen shrimp (obtained from a local supermarket) twice a day. All experiments were performed at this temperature and salinity. The uptake and elimination by the fish were studied using radiotracers: ^{203}Hg(II) ($t_{1/2}$ = 46.9 d, in 0.1 N HCl, specific activity = 10.8 GBq g^{-1}; purchased from Riso National Laboratory, Denmark) and Me^{203}Hg. Me^{203}Hg was synthesized from ^{203}Hg(II) using a well-established method (Rouleau & Block 1997). Radioactivity of ^{203}Hg(II) and Me^{203}Hg was measured by a gamma counter at 279 keV, and was corrected for counting efficiency and geometry.

Assimilation efficiency (*AE*). The *AE* of ^{203}Hg(II) and Me^{203}Hg were measured using the pulse-chase technique, described in Ni et al. (2000) and Xu & Wang (2002). Experiments were conducted to quantify the *AE* in fish feeding on 3 different prey and at different densities of copepod prey. In the prey-type experiment, 3 prey diets were used: the brine shrimp *Artemia* sp. (hatched under laboratory conditions for 1 d), the copepod *Acartia spinicauda* (collected from Port Shelter, Hong Kong), and small fish (silverside *Atherion elymus* collected from Port Shelter, Hong Kong: tissues only were used). The brine shrimp were radiolabeled with ^{203}Hg(II) and Me^{203}Hg, respectively, in 200 ml 0.22 µm filtered seawater. Radioactivity additions were 3.7 and

1.85 kBq for ^{203}Hg(II) and Me^{203}Hg, respectively. The fish prey were radiolabeled in 1.5 l filtered seawater spiked with 3.7 and 1.85 kBq for ^{203}Hg(II) and Me^{203}Hg, respectively. The copepods were radiolabeled in 400 ml filtered seawater spiked with 3.7 and 7.4 kBq of ^{203}Hg(II) and Me^{203}Hg, respectively. After 36 h exposure to radiotracers, the prey (copepods and brine shrimp) were removed from their exposure medium, rinsed thoroughly with seawater, and fed to the fish naturally. The silverside prey was dissected, and only the muscle tissues were fed to the sweetlips naturally. The radioactive pulse feeding lasted 60 min, during which no radioactive feces were produced. There were 5 replicate individuals in each treatment. After the radioactive feeding, the fish were placed in nonradioactive water (10 l) and depurated of their ingested metals for 48 h. The radioactivity remaining in the fish was quantified non-destructively at 3, 6, 12, 18, 24, 36, and 48 h. Water was renewed every 12 h to ensure that the amount of radioactivity in the water was negligible within the 48 h depuration period. The fish were fed with nonradioactive shrimp meat twice a day during the depuration period. The *AE* was calculated as the percentage of metal retained in the fish at 24 h.

In the prey-density experiment, the copepods were radiolabeled by exposure to 7.4 kBq of ^{203}Hg(II) in 400 ml filtered seawater for 36 h. Considering the very efficient assimilation by the fish [see 'Results'], we did not examine the influence of prey density on the AE of MeHg). The prey was then rinsed and fed to the fish at different densities for 30 min: 50, 100, 500, 1000 individuals l^{-1}. There were 4 to 5 individual fish in each treatment. Prey was added every 10 min to maintain a constant density. After feeding, the radioactivity of each individual fish was immediately measured. A subsample of copepod prey remaining in the feeding beakers was collected and the number of copepods as well as the amount of ^{203}Hg(II) radioactivity were counted and measured. The ingestion rate of each individual fish was calculated by dividing the total amount of radioactivity ingested (measured by gamma counting) by the radioactivity of each individual copepod. The fish were subsequently returned to nonradioactive water and allowed to depurate the ingested food materials for a period of 48 h. Shrimp meat was fed to the fish during the depuration period, and the seawater was changed every 12 h. Feces produced during the depuration period were removed every 8 h. At the end of the experiment, the fish were dried at 90°C overnight, and their dry weight measured. Ingestion rate was expressed as a percentage of the dry weight on a daily basis.

^{203}Hg(II) and Me^{203}Hg uptake from dissolved phase. Preliminary experiments suggested that the fish exhibited a linear pattern of uptake over a 24 h exposure period. We thus used a shorter term of exposure (4 h) to quantify mercury unidirectional flux into the fish, in order to avoid a decline in radioactivity in the exposure medium as well as to maintain optimum conditions for the fish. ^{203}Hg(II) and Me^{203}Hg uptake by the fish was studied at different dissolved concentrations. No stable Hg was added to the water, thus different concentrations were achieved by addition of ^{203}Hg(II) and Me^{203}Hg of different radioactivities. The concentrations of Hg(II) resulting from radioisotope addition were 0.109, 0.421, 2.105, 10.90 µg l^{-1}, and those of MeHg were 0.020, 0.105, 0.361, 1.832 µg l^{-1}. Higher concentrations than background concentrations were employed because of the availability of the ^{203}Hg isotope with a relatively low specific activity (including carrier). The radioisotopes were equilibrated with the seawater for 8 h before the uptake measurements. We placed 5 replicate fish in 1.5 l of 0.22 µm filtered seawater containing the radioisotopes. At 1, 2, 3, and 4 h exposure, the fish were removed from the radioactive medium and transferred to another beaker containing filtered non-radioactive water to remove weakly bound radioisotopes. The radioactivity of the fish was then measured non-destructively using a gamma detector. A seawater sample was also taken, and its radioactivity was measured at each time point. The exposed seawater was renewed after 2 h of exposure. In general, the decrease of radioactivity in the medium due to uptake by the fish was negligible for ^{203}Hg(II), and <27 % for Me^{203}Hg within the first 2 h of exposure. After the radioactivity measurement, the fish were returned to the exposure medium. After a further 4 h exposure, the fish were dissected into 3 body parts: gills, viscera, and the remainder of the tissues, including bones (hereafter referred to as 'tissues'). The radioactivity of each body part was measured. The fish were then dried at 90°C for >1 d and their dry weights were measured.

The dry weight concentration factor (DCF, l kg^{-1}) of both ^{203}Hg(II) and Me^{203}Hg was calculated as the ratio of the radioactivity in the fish (counts min^{-1} kg^{-1}) to the radioactivity in the water (cpm l^{-1}, calculated as the mean before and after exposure for each time point). The uptake-rate was calculated as the slope of the linear regression between the DCF and the time of exposure multiplied by the dissolved Hg concentrations.

^{203}Hg(II) and Me^{203}Hg elimination after aqueous and dietary exposure. Fish were exposed to ^{203}Hg(II) and Me^{203}Hg in the aqueous phase or the dietary phase. In the aqueous exposure, 10 individual fish were exposed to dissolved ^{203}Hg(II) or Me^{203}Hg for 7 d, as described above. Radioisotope additions were 0.617 and 0.123 kBq l^{-1} for ^{203}Hg(II) and Me^{203}Hg, respectively (in 3 l seawater). Each day, the fish were exposed to radioactive water for 4 h before they were removed

and placed in non-radioactive water and fed with shrimp meat. In the food exposure treatment, 10 fish were placed in 3 l seawater and fed radiolabeled brine shrimp for 4 h each day before being returned to non-radioactive water and fed with shrimp meat. The radioactivity of all fish was assayed, and 2 individuals were immediately dissected to determine the distribution of metals in their different body parts (gills, viscera, and tissues). The remaining 8 fish were rinsed with filtered non-radioactive water, and the radioactivity of each individual was measured immediately. They were subsequently depurated in natural seawater (10 l) for 28 d. The seawater was renewed on a daily basis, and the fish were fed with frozen shrimp meat twice a day. The radioactivity in these individuals was measured at frequent time intervals (every 1 to 3 d). At the end of the elimination period, all fish were dissected to determine the distribution of metals in the gill, viscera, head, and tissues.

Modeling exposure and food-chain transfer. Under steady-state conditions, Hg and MeHg accumulation in fishes can be calculated by the following equation (Thomann 1981, Wang & Fisher 1999):

$$C_{ss} = (k_u \times C_w)/k_{ew} + (AE \times IR \times C_f)/k_{ef} \quad (1)$$

where C_{ss} is the Hg or MeHg concentration in the fish (µg g^{-1}), k_u is the metal net-uptake rate-constant from the aqueous phase (l g^{-1} d^{-1}), C_w is the metal concentration in the dissolved phase (µg l^{-1}), k_{ew} is the elimination-rate constant following uptake from the dissolved phase (d^{-1}), AE is the metal-assimilation efficiency, IR is the fish feeding rate (in fraction of body weight d^{-1}), C_f is the metal concentration in the ingested prey (µg g^{-1}), and k_{ef} is the elimination-rate constant following uptake from food (d^{-1}). The growth-rate constant was ignored in the calculation.

Assuming that C_f can be predicted based on the bioconcentration factor of metals (BCF, under assumption of equilibrium) in the prey and on C_w ($C_f = BCF \times C_w$), the fraction of Hg or MeHg (f) coming from the aqueous phase can be calculated from Eq. (1) (Wang & Fisher 1999):

$$f = [(k_u/k_{ew})]/[(AE \times IR \times BCF)/k_{ef} + (k_u/k_{ew})] \quad (2)$$

To predict the fraction of total Hg accumulation arising from Hg(II) or MeHg, we first calculated the concentration factor (CF) of both Hg species using Eq. (1):

$$CF = k_u/k_{ew} + (AE \times IR \times BCF)/k_{ef} \quad (3)$$

Thus, the fraction of total Hg in the fish due to accumulation from Hg(II) (R) can be calculated as (Wang et al. 1998, Wang & Fisher 1999):

$$R = CF_{Hg}/[CF_{Hg} + (CF_{MeHg} \times C_{MeHg}/C_{Hg})] \quad (4)$$

where CF_{Hg} is the concentration factor of Hg(II) in fish, CF_{MeHg} is the concentration factor of MeHg, and C_{MeHg}/C_{Hg} is the ratio of MeHg concentration to Hg(II) concentration in water.

The food-chain transfer factor (TTF), or the ratio of metal concentration in fish to metal concentration in prey, can be calculated as (Reinfelder et al. 1998, Wang & Fisher 1999):

$$TTF = (AE \times IR)/k_{ef} \quad (5)$$

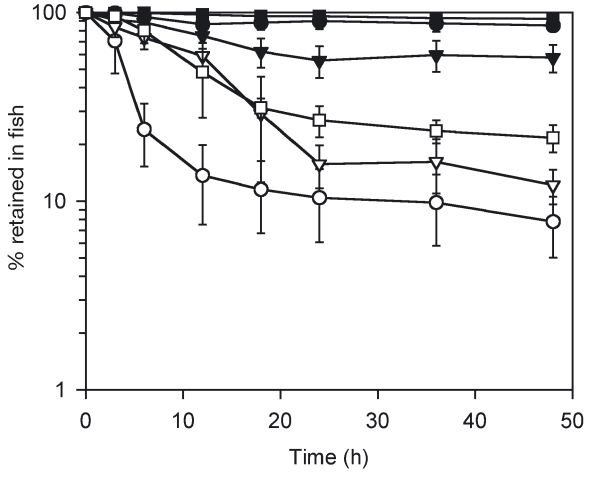

Fig. 1. *Plectorhinchus gibbosus*. Retention of Hg(II) and MeHg following pulse ingestion of different radiolabeled prey. (○) Hg(II) bound to *Artemia* sp.; (●) MeHg bound to *Artemia* sp.; (▽) Hg(II) bound to fish prey; (▼) MeHg bound to fish prey; (□) Hg(II) bound to copepod *Acartia spinicauda*; (■) MeHg bound to copepod *A. spinicauda*. Values are means ± SD (n = 7)

RESULTS

Assimilation of Hg(II) and MeHg

Retention of ingested Hg(II) and MeHg by the fish following short-term pulse radioactive feeding is shown in Fig. 1. Significant depuration of ingested Hg(II) occurred during first day of depuration, whereas there was very little loss of MeHg over the 2 d depuration period. MeHg associated with both

Table 1. *Plectorhinchus gibbosus*. Calculated assimilation efficiency mean ± SD (n = 5) of Hg(II) and MeHg by sweetlips feeding on different prey

Prey	Hg(II)	MeHg
Brine shrimp *Artemia* sp.	10.4 ± 4.4	89.8 ± 8.2
Copepod *Acartia spinicauda*	26.9 ± 5.1	95.4 ± 1.2
Silveside *Atherion elymus*	15.8 ± 4.0	55.6 ± 10.7

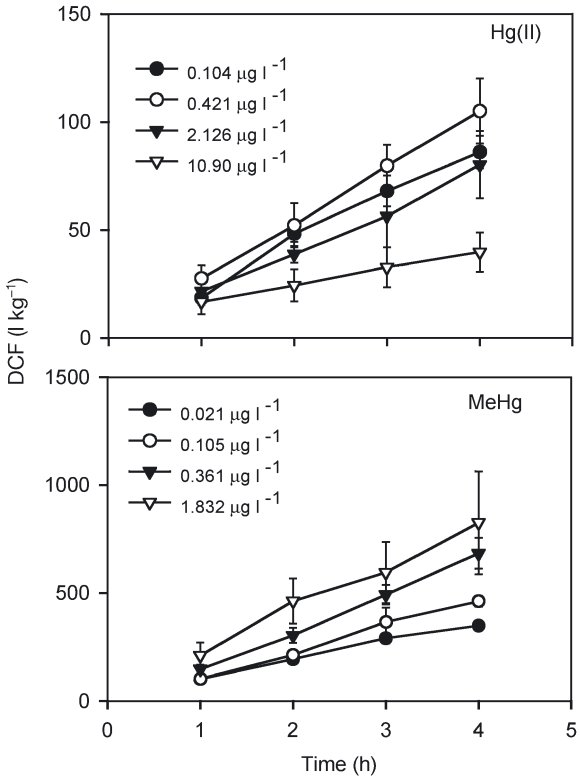

Fig. 2. *Plectorhinchus gibbosus*. Calculated dry weight concentration factors (*DCF*) of Hg(II) and MeHg as a function of exposure time at different dissolved concentrations. Values are means ± SD (n = 3)

brine shrimp and copepods was retained very efficiently by the fish over the 2 d period. Because there was little further loss of either Hg(II) or MeHg after 24 h depuration, the assimilation efficiency (*AE*) was calculated as the percentage of metal retained in the fish after 24 h (Table 1). *AE* ranged between 10 and 27% for Hg(II) and between 56 and 95% for MeHg. In general, both Hg(II) and MeHg associated with the copepods were assimilated by the fish at a higher efficiency than when associated with brine shrimp or silverside prey.

In the prey-density experiment, the ingestion rate of the fish on copepods varied considerably among different experimental individuals, even within the same density treatment. It was therefore not possible to calculate the *AE* for each food-density treatment. Instead, *AE* was calculated for each individual fish at each specific ingestion rate. The calculated ingestion rate of the fish ranged from 0.2 to 10.3% of their tissue dry weight each day. The measured *AE* of Hg(II) among the different individuals was 8 to 30%. There was no significant relationship between *AE* and the ingestion rate of fish when all individuals were considered (data not shown), suggesting that the *AE* of Hg(II) did not vary significantly over the ingestion-rate range quantified in this study (0.2 to 10.3%).

Uptake of Hg(II) and MeHg from aqueous phase

Between 1 and 4 h of exposure, the quantified dry weight concentration factor (*DCF*, radioactivity in fish divided by radioactivity in the water) exhibited an approximately linear uptake pattern. There was however a significant difference in the calculated *DCF* among the 4 experimental concentrations. A much lower uptake of Hg(II) was documented with increasing Hg(II) concentration, whereas uptake of MeHg increased with increasing MeHg concentration (Fig. 2). By the end of the exposure period, the *DCF* of Hg(II) at the highest concentration (10.90 µg l^{-1}) was 2.64 times lower than that at 0.421 µg l^{-1}, whereas the DCF of MeHg(II) at the highest concentration (1.832 µg l^{-1}) was 2.4 times higher than that at the lowest concentration (0.021 µg l^{-1}). After 4 h, the *DCF* of MeHg was much higher than the uptake of Hg(II) at all concentrations.

The calculated uptake-rate at different ambient concentrations is shown in Fig. 3. There was a log-log

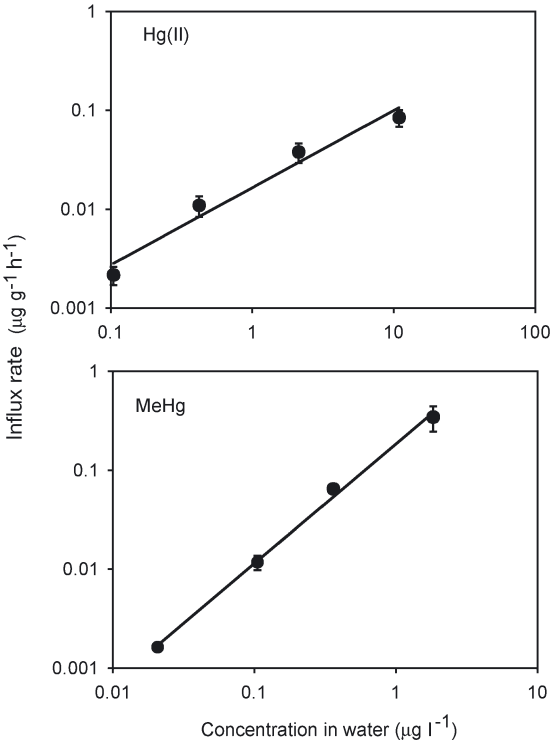

Fig. 3. *Plectorhinchus gibbosus*. Calculated uptake-rate of Hg(II) and MeHg at different dissolved concentrations. Values are means ± SD (n = 3)

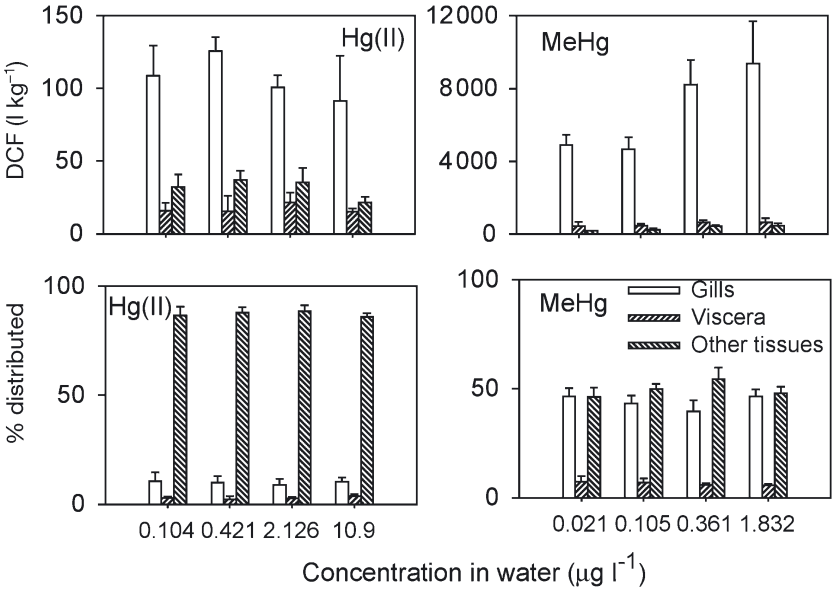

Fig. 4. *Plectorhinchus gibbosus*. Calculated dry weight concentration factors (*DCF*) and distribution of Hg(II) and MeHg in different tissues after 4 h exposure to different dissolved concentrations. Values are means ± SD (n = 3)

linear relationship between the uptake-rate and the ambient dissolved concentrations. The *b* coefficient of this relationship was 0.781 for Hg(II) and 1.210 for MeHg. Because the *b* coefficients deviated from 1.0, we calculated the uptake-rate constant by direct linear regression between uptake-rate and dissolved concentration without log-log transformation, with the assumption of a zero intercept. Thus, the uptake-rate constant of Hg(II) was 0.195 l g^{-1} d^{-1}, and was 23.1 times lower than the uptake-rate constant of MeHg (4.515 l g^{-1} d^{-1}). By the end of the 4 h exposure period, Hg(II) and MeHg were also distributed differently in the fish tissues (Fig. 4). Most of the accumulated Hg(II) (86 to 89%) was in the tissues, with only a small proportion in the gills and the viscera. In contrast, 43 to 46% of MeHg was distributed in the gills and 46 to 55% in tissues. There was little difference in the distribution among the different concentration treatments. The calculated DCF was highest for the gills (Fig. 4). The DCF of Hg(II) in tissues was 1.4 to 2.4 times higher than that in the viscera, whereas the DCF of MeHg in tissues was 1.4 to 2.6 times lower than that in the viscera. The DCFs of MeHg in the gills were somewhat higher at the 2 highest concentrations.

Elimination of Hg(II) and MeHg

The elimination of radioisotopes from the fish following 7 d exposure to dissolved or dietary Hg(II) and MeHg is shown in Fig. 5. Over the 28 d elimination period, only 20% of the accumulated MeHg was lost by the fish, whereas 73 to 81% of the accumulated Hg(II) was lost from the fish. There was little difference in the loss of accumulated MeHg between aqueous and dietary uptake. Hg(II) was retained more efficiently when accumulated from the aqueous phase than when accumulated from the dietary phase. Depuration can be approximately modeled by a 2 step loss, first during a 7 d depuration period and then during a 9 to 28 d depuration period. The calculated efflux-rate constant (the loss between the 9th and 28th day of depuration) and the biological retention half-lives are shown in Table 2. Thus, MeHg was lost at a much lower rate (0.0103 to 0.0129 d^{-1}) than Hg(II) (0.0287 to 0.0548 d^{-1}) by the fish. Hg(II) associated with the dietary source was lost 1.9 times faster than the Hg(II) associated with the aqueous source.

After 7 d exposure, about 4 to 17, 5 to 40, and 55 to 78% of both Hg species was distributed in the gills, viscera and tissues, respectively (Fig. 6). Much higher fractions of Hg(II) and MeHg were found in the viscera

Table 2. *Plectorhinchus gibbosus*. Compartmental analysis of elimination mean ± SD, (n = 8) of Hg(II) and MeHg following exposure to dietary and aqueous metals for 7 d. %: percent of mercury in compartment; *k*: elimination-rate constant; $t_{1/2}$: biological retention half-lives

Compartment		Hg(II)		MeHg	
		Water	Food	Water	Food
1 (0–7 d)	%	59.2 ± 16.2	65.3 ± 18.0	2.2 ± 5.0	11.1 ± 5.1
	k (d^{-1})	0.0722 ± 0.0066	0.0957 ± 0.0179	0.0096 ± 0.0062	0.0116 ± 0.0059
	$t_{1/2}$ (d)	9.67 ± 0.89	7.51 ± 1.52	40.2 ± 69.9	72.8 ± 27.4
2 (9–28 d)	%	38.8 ± 16.0	36.2 ± 12.2	97.8 ± 5.6	89.5 ± 5.0
	k (d^{-1})	0.0287 ± 0.0071	0.0547 ± 0.0093	0.0129 ± 0.0026	0.0103 ± 0.0020
	$t_{1/2}$ (d)	25.4 ± 4.9	13.1 ± 2.45	55.7 ± 9.5	69.8 ± 13.4

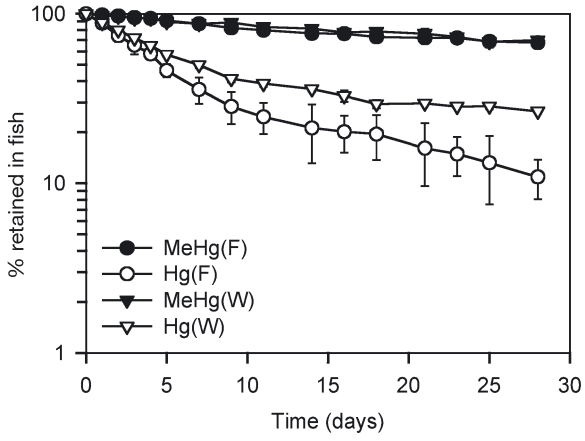

Fig. 5. *Plectorhinchus gibbosus.* Elimination of Hg(II) and MeHg following 7 d exposure to aqueous (W) or dietary (F) Hg(II) and MeHg. Values are means ± SD (n = 8)

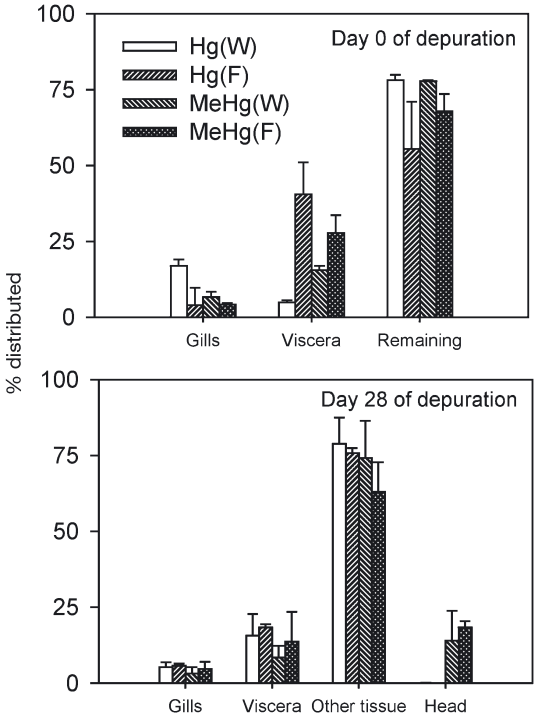

Fig. 6. *Plectorhinchus gibbosus.* Distribution of Hg(II) and MeHg following 7 d exposure to aqueous (W) or dietary (F) Hg(II) and MeHg (Day 0) and after 28 d elimination (Day 28). Values are means ± SD (n = 2 for Day 0, and 4 for Day 28)

when accumulated from the dietary source than when accumulated from the aqueous phase. After 28 d elimination, the distribution of Hg(II) and MeHg in the head was also examined. Only a small percentage of both mercury species was found in the gills (<6%); about 8 to 18% of the metal was in the viscera, and most was distributed in tissues. About 14 to 18% of MeHg was found in the head, whereas no Hg(II) was detected in the head.

Modeling exposure and trophic transfer

Modeling analysis of the exposure and trophic transfer factor of Hg and MeHg in fishes requires measurements of several of the parameters in Eq. (1), including *AE*, k_u, k_{ew}, k_{ef}, *IR* and *BCF* of Hg(II) and MeHg in the prey. Values of *AE*, k_u, k_{ew}, and k_{ef} have been taken from this study (Table 3). In our calculations we used a single mean value for these parameters, because we did not examine their variability under diverse environmental conditions (except for *AE*, which was within a relatively narrow range for different prey types and at different prey densities). Instead, we modeled the exposure of metals within a range of *IR* and *BCF* because these 2 parameters vary greatly under natural conditions and we are less certain about them. A range of *IR* (1 to 10% of body weight per day) (Garnier-Laplace et al. 2000) and *BCF* for both Hg(II) (2000 to 200 000) and MeHg (5000 to 500 000) (ranging over 2 orders of magnitude) was used in the analysis. The range of *BCF* for both invertebrate and fish prey was

Table 3. *Plectorhinchus gibbosus*. Parameters used in modeling exposure of Hg(II) and MeHg. Assimilation efficiency calculated as % metal retained. Numbers in parentheses: mean of bioconcentration factor

Parameter	Hg(II)	MeHg	Source
Assimilation efficiency (*AE*, %)	20	80	This study
Ingestion rate (*IR*, in % body weight d^{-1})	0.01–0.1	0.01–0.1	Garnier-Laplace et al. (2000)
Bioconcentration factor in prey (includes copepods and fishes; *BCF*, l kg^{-1})	2000–200 000 (20 000)	5000–500 000 (50 000)	IAEA (2000)
Uptake rate constant (k_u, in l g^{-1} d^{-1})	0.195	4.515	This study
Elimination rate constant from water (k_{ew}, in d^{-1})	0.0287	0.0129	This study
Elimination rate constant from food (k_{ef}, in d^{-1})	0.0547	0.0103	This study

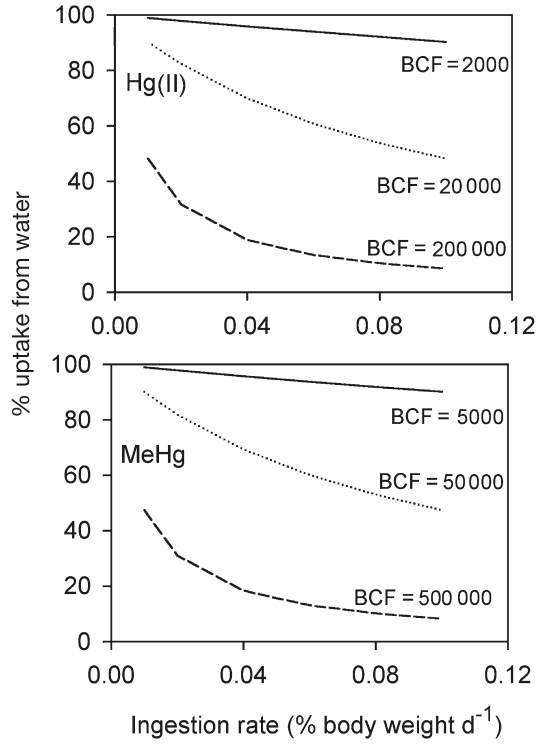

Fig. 7. *Plectorhinchus gibbosus.* Model-predicted percentage of Hg(II) and MeHg uptake from aqueous phase as a function of ingestion rate. *BCF*: bioconcentration factor in prey (l kg^{-1})

taken from the literature (IAEA 2000). Our calculation, using Eq. (2), indicates that the relative importance of dissolved vs diet uptake varies greatly at different *IR*s and *BCF*s for both species of mercury (Fig. 7). At the low end of *BCF* (2000 for Hg[II] and 5000 for MeHg), uptake is dominated by the aqueous phase, irrespective of the change in *IR*. At the high end of *BCF* (200 000 for Hg[II] and 500 000 for MeHg), dietary uptake dominates the accumulation of both Hg(II) and MeHg (>50%).

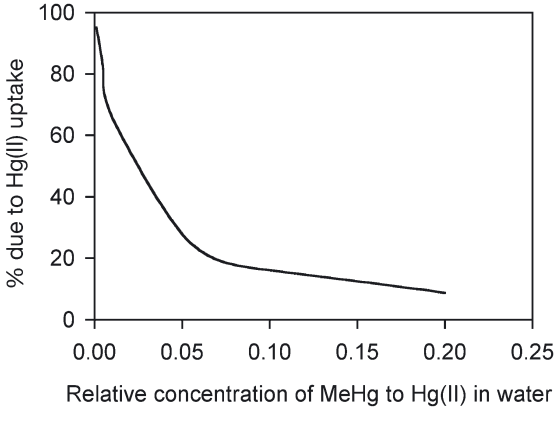

Fig. 8. *Plectorhinchus gibbosus*. Model-predicted percentage of contribution of Hg(II) to overall Hg accumulation as a function of relative concentration of dissolved MeHg to dissolved Hg(II) in seawater

To further model the relative importance of Hg(II) and MeHg species in the overall Hg bioaccumulation in the fish, we used the mean values of *AE*, k_u, k_{ew}, k_{ef}, and *BCF* for both Hg(II) and MeHg in our calculations (Table 3; Eq. 4). Variation in *IR* will have little influence, because uptake from both Hg(II) and MeHg depends on *IR*. Methylation of inorganic Hg in the water column is possible (Topping & Davies 1981). Generally, MeHg comprises a small fraction of the total Hg in oxic aquatic environments. Kannan et al. (1998) found that MeHg accounted for 0.03 to 52% of the total Hg in canals and creeks that discharged into the Florida Bay. Fitzgerald & Mason (1997) indicated that MeHg concentration in the Patuxent River was about 10% of the total Hg concentration. We therefore modeled the likely contribution of Hg(II) accumulation with a dissolved MeHg to Hg(II) concentration ratio of 0.001 to 20% (Fig. 8). Thus, about 16 to 95% of total Hg in the fish may have been due to the uptake of Hg(II).

The potential trophic transfer factor of both Hg(II) and MeHg was modeled using Eq. (5), assuming a range of *IR* for the fish (Fig. 9). The calculation shows that Hg(II) is unlikely to be biomagnified due to the rel-

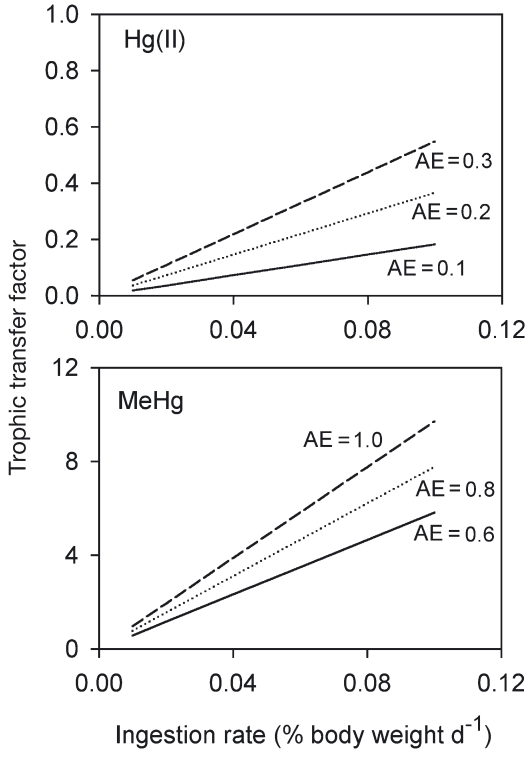

Fig. 9. *Plectorhinchus gibbosus*. Model-predicted food-chain transfer factor of Hg(II) and MeHg as a function of ingestion rate. *AE*: assimilation efficiency

atively low *AE* as well as the relatively high elimination-rate constant. The highest potential *TTF* of Hg(II) in the fish is about 0.6 using the highest *AE* and *IR*. In contrast, the *TTF* for MeHg is >1 and <10 under conditions likely to be encountered by the fish.

DISCUSSION

The assimilation efficiency of Hg(II) and MeHg measured in the marine sweetlips *Plectorhinchus gibbosus* was quite comparable to that in previous studies on other fishes (Pentreath 1976a, Mason 2002). MeHg appears to be very efficiently assimilated by fishes from dietary sources, with an *AE* of >56% in our study, although some earlier studies only found an *AE* of 20% in freshwater fishes (Ribeyre & Boudou 1984, Boudou & Ribeyre 1997). The *AE* of MeHg was 3.5 to 8.6 times higher than that of Hg(II) for sweetlips feeding on 3 different prey species. The high assimilation of MeHg by this fish may have been due to the lipophilicity of this compound and/or the digestive physiology of the sweetlips. Mason (2002) proposed the likelihood of rapid desorption of MeHg within the digestive fluids of animals leading to a much higher assimilation. Digestive fluid desorption may control the assimilation of metals such as Cd, whereas for other metals, these 2 processes (desorption and assimilation) are likely to be decoupled (Wang et al. 2002, Yan & Wang 2002). Lawson & Mason (1998) suggested that fishes feeding on copepods assimilate MeHg more efficiently than Hg(II), as they found a larger fraction of MeHg in the soft tissues of the copepods. Ni et al. (2000) however did not find any relationship between the *AE* of Cd, Cr, and Zn in mudskippers *Periophthalmus cantonensis* and glassy fish *Ambassis urotaenia* and their distribution in the soft tissues of the copepod prey. The difference in trophic transfer between Hg(II) and MeHg may also be caused by their differential partitioning in phytoplankton cells (e.g. surface-bound and cytoplasm-bound); thus, speciation and uptake at the base of marine foodchains are critical for the food chain transfer of these 2 mercury species (Mason et al. 1995).

In our experiments, both mercury species associated with the copepods were generally assimilated at a higher efficiency than those associated with brine shrimp and silversides. Among the many elements studied so far, the *AE* of MeHg and radiocesium in fishes are the highest (Ni et al. 2000, Zhao et al. 2001, Baines et al. 2002, Xu & Wang 2002). For example, the *AE* of radiocesium range between 78 and 95% in the marine mangrove snappers *Lutjanus argentimaculatus* (Zhao et al. 2001). Such a high *AE* from a dietary prey source contributes greatly to the potential biomagnification of these 2 compounds in marine predatory fishes.

Few studies have examined the influence of various food conditions on the assimilation of metals by fishes (Xu & Wang 2002). In our study, a range of typical ingestion rates (0.2 to 11%) of marine fish in natural environment had no significant influence on their *AE* of Hg(II), presumably because of the relatively low range of ingestion rates examined. The sweetlip fish appears to process the ingested dietary Hg comparably in different feeding conditions. In a previous study, we found that the *AE* of Cd, Se, and Zn decreased significantly with increasing ingestion rate of the fish, but at a much higher *IR* (>28% of body weight d^{-1}) than found in this study (Xu & Wang 2002). At the 2 lower *IR*s examined by Xu & Wang (2002) (5 to 7% of body weight d^{-1}), there was no major difference in the *AE* of these metals. Because the *AE* of Hg(II) was fairly independent of the feeding rate under typical conditions, the uptake-rate constant for dietary sources (a combination of *AE* and *IR*) would be expected to increase proportionally with increasing *IR*.

The uptake of both Hg species was linear over time of exposure at a relatively constant dissolved concentration, generally agreeing with earlier studies (Pentreath 1976a, Ribeyre & Boudou 1984). The uptake-rate exhibited a log-log relationship with the dissolved concentration for both Hg species, indicating that uptake was primarily a Freundlich absorption type dominated by passive diffusion or a facilitated transport process (Golding et al. 2002). Whether the dissolved uptake is active, however, cannot be concluded from this study. We calculated a *b* coefficient of the power relationship between uptake-rate and dissolved concentration of 1.21 for MeHg and 0.761 for Hg, suggesting that the uptake of MeHg increased whereas the uptake of Hg(II) decreased disproportionally with increasing dissolved concentration. Accordingly, a higher MeHg concentration resulted in a higher DCF in the fish, whereas the DCF of Hg(II) decreased with increasing Hg(II) concentration.

The calculated uptake rate constant (with the assumption that uptake is a first-order process) of MeHg was about 23 times higher than that of Hg(II). Ribeyre & Boudou (1984) found that the accumulation of MeHg by the fish *Salmo gairdneri* was about 6 times greater than the accumulation of Hg(II) over a 30 d exposure period. Because both species displayed comparable lipophilicity (Mason et al. 1995, Mason 2002), lipophilicity cannot solely account for the difference in their uptake. As in previous studies (Xu & Wang 2002), the DCF of the gills in this study was the highest among the different fish parts examined, primarily because the gills were the primary site for metal uptake from the aqueous phase and have a relatively large surface area. A marked difference between Hg(II) and MeHg distribution in the tissues was also

found. MeHg was evenly distributed between the gills and tissues, whereas most Hg(II) was in the tissues, suggesting that Hg(II) was in fact rapidly transported to the tissues after being taken up by the gills.

Our elimination rate measurements demonstrated a major difference between the 2 Hg species, with Hg(II) being lost at a faster rate than MeHg. For comparison, the elimination rate constants (k_e) in mangrove snappers were 0.025–0.047 d^{-1}, 0.020–0.023 d^{-1}, 0.027–0.031 d^{-1}, and 0.015 d^{-1} for Cd, Cs, Se, and Zn, respectively (Zhao et al. 2001, Xu & Wang 2002). Dietary Hg(II) was lost by the sweetlips about 2 times faster than aqueous Hg(II), whereas there was no major difference in the elimination between dietary and aqueous MeHg. Consistently, a much higher fraction of dietary Hg(II) was found in the viscera compared with aqueous Hg(II) at the end of the exposure pereiod. Digestive processes may have thus facilitated the elimination of ingested Hg(II) from the sweetlips. Despite the greater distribution of dietary MeHg in the viscera, there was no major difference in its elimination, implying that the elimination of MeHg may not necessarily be controlled by the digestive process.

Trudel & Rasmussen (1997) summarized the elimination rate of Hg(II) and MeHg in fishes, mostly freshwater species. They found that the elimination rate of Hg was highly dependent on several factors, including radiolabeling period, body size and temperature. For example, short-term experiments (<90 d) overestimated the elimination rate of mercury, and Hg(II) was excreted 3 times faster than MeHg. We radiolabeled both Hg species for 7 d, and the elimination rate was quantified using the radiotracer technique. Whether the elimination rate constant determined after a relatively short exposure period overestimates that in nature is unknown. It should be noted that elimination rate measurements quantified under field conditions using stable-metal analysis may underestimate the elimination rate because stable-metal analysis does not necessarily quantify the unidirectional flux of metals across fishes. Trudel & Rasmussen (1997) further showed that both Hg(II) and MeHg excretion are negatively correlated with body size, but that only MeHg elimination is significantly correlated with water temperature. The MeHg elimination rate in the present study was independent of mercury burden and concentration, indicating that this is a first-order process. Marine teleost fishes must drink seawater to counteract loss of water from their tissues. Active elimination (as opposed to passive uptake) to remove salt from tissue to seawater against the concentration gradient may have contributed to the higher elimination of metals compared to freshwater fishes.

Using the autoradiography technique, Rouleau et al. (1999) demonstrated the specificity of the accumulation sites of Hg(II), in which waterborne Hg was taken up by water-exposed receptor cells of sensory nerves and subsequently transferred to the brain by axonal transport, a normal physiological process for the transport of organelles and dissolved neuronal constituents along nerve axons. After 28 d elimination, we found a significant fraction of MeHg in the head of sweetlips probably associated with the brain nervous system or fatty tissues. In contrast, no Hg(II) was found in the head after 28 d elimination. Riisgaard & Hansen (1990) showed that flounder *Platichthys flesus* force-fed with contaminated mussels accumulated MeHg in blood cells, liver, kidney and muscle tissues, while Hg(II) only accumulated in measurable amounts in the liver and kidney.

We used the radiotracer technique to study the biokinetics of Hg(II) and MeHg in the sweetlips. Whether there was any biotransformation (methylation and demethylation) within the fish body was not revealed by our study. Pennacchioni et al. (1976) suggested that fishes are unable to methylate Hg *in vivo*, whereas other studies indicated that methylation and demethylation are possible (Burrows & Krenkel 1973, Simon & Boudou 2001). We found a significant difference in the *AE*, the uptake rate constant, and the elimination rate constant between Hg(II) and MeHg. There was also a marked difference in the distribution of Hg(II) and MeHg in the head of the fish. About 14 to 18% of MeHg was detected in the head at the end of the 28 d elimination period, whereas Hg(II) in the head was undetectable. Clearly, biotransformation in the tissues of *Plectorhinchus gibbosus* needs to be further examined.

Our calculation using experimentally derived *AE* and an elimination rate constant showed a trophic transfer factor (*TTF*) of <1 for Hg(II), and >1 for MeHg under all conditions, consistent with the results of numerous field studies on the potential biotransfer of both species of Hg in marine and freshwater food chains (Hill et al. 1996, Jackson 1998). Our model identified a few important processes responsible for the potential transfer of Hg(II) and MeHg. Despite the major differences in the *AE* and in elimination between MeHg and Hg(II), the biomagnification of MeHg (*TTF* > 1) is probably caused principally by very efficient assimilation of the prey, even though the elimination rate constant is consistently low. Similarly, the potential biomagnification of radiocesium with a predicted mean *TTF* of 2.0 (Zhao et al. 2001) resulted from a very high *AE*, although its elimination rate constant was fairly comparable to that of metals such as Cd, Se, and Zn.

Although most bioaccumulation studies on the biomagnification of MeHg assume the Hg species is accumulated in fishes mainly in the dietary phase (e.g. by trophic transfer), there is little experimental evidence on the relative importance of aqueous vs dietary uptake under environmentally relevant exposure regimes (Hall et al. 1997). Hall et al. (1997) conducted a field

experiment exposing the freshwater finescale dace *Phoxinus neogaeus* to different concentrations of aqueous and dietary MeHg. They provided the first experimental evidence that food was the dominant pathway for MeHg bioaccumulation in fishes at natural levels of MeHg. In their study, uptake from water made up at most 15% of the total accumulation of Hg in the fish. A much earlier study, however, found that dissolved MeHg in seawater was the critical factor for MeHg accumulation (Fujiki 1980). Our kinetic model demonstrated that at the high end of the bioconcentration factor ($>5 \times 10^5$), especially for fish prey, dietary uptake dominates MeHg accumulation. At the low end of *BCF* (5×10^3), however, aqueous uptake dominates MeHg accumulation. It thus appears that the importance of dietary ingestion is strongly related to the type of prey ingested and the feeding rate of the individual fish. In a recent study, dietary MeHg decreased the reproduction of adult fathead minnows at a dietary concentration encountered by predatory fishes in aqueous systems (Hammerschmidt et al. 2002).

A vast number of studies have shown that the majority of Hg in fish muscle is in the methylated form (Lasorsa & Allen 1994, Wagemann et al. 1997), but the percentage of MeHg in fishes is also highly dependent on the trophic status of the fishes (Kidd et al. 1995, Mason et al. 2000). We are not aware of any previous experimental studies attempting to separate the relative importance of both species of Hg to the overall Hg bioaccumulation in marine fish. Our model simulation demonstrates that the relative contribution of the 2 different Hg species varies as a function of relative concentration of MeHg in the water. With a low fraction of dissolved MeHg in the water, Hg(II) uptake still dominates the overall Hg accumulation, even though the bioconcentration factor (or uptake rate constant) of MeHg is much higher than that of Hg(II). Of course, this calculation assumes that there is no methylation/demethylation in the fish. It should also be pointed out that we used a mean value of *AE*, k_u, k_{ew}, k_{ef}, and *BCF* in our calculation. Any change in these parameters (particularly in *BCF*) could alter the relative contribution of the 2 mercury species. A further challenge will be to verify our modeling prediction on the significance of both species of Hg to the total Hg accumulation in fishes. Validation of the kinetic model has been conducted for a few marine invertebrates (e.g. Wang et al. 1996, 1998), and it is equally important to validate this model for fishes.

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