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Accumulation and Distribution of Methylmercury in Freshwater- and Seawater-Adapted Eels

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Fish constitute the major sources of human exposure to methylmercury (MeHg) (EPA 1997). It is known that fish accumulate MeHg not only from food via a food web but also directly from the surrounding water (Olson et al. 1973). Simon and Boudou (2001) demonstrated the importance of the latter route. The capacity for trophic MeHg accumulation should be similar between freshwater and seawater fishes, since most MeHg in food binds to protein and is considered to be absorbed from the intestine through the neutral amino acid carrier (Leaner and Mason 2002). Most studies on MeHg accumulation in fish were carried out using freshwater fishes. However, information on the accumulation in seawater fishes is very limited. The direct MeHg accumulation features in fish from surrounding water may differ between freshwater and seawater fishes, since the drinking rate (Metzs and Skadhauge 1968) and permeability of water and ions in gills, digestive tract and skin must be different. Unfortunately, such a direct accumulation has not been well studied, though it is necessary to our understanding of the accumulation mechanism involved in mercury pollution of the marine environment. A euryhaline teleost, the Japanese eel (*Anguilla japonica*), is equally at home in both freshwater and seawater. We conducted a comparative examination of the accumulation and distribution rates of MeHg in the Japanese eel in its freshwater and seawater environments.

MATERIALS AND METHODS

Japanese cultured eels, *Anguilla japonica*, weighing around 200 g were obtained from a commercial source and kept in tap water or tanks of artificial seawater at 20 ºC without feeding for at least 2 weeks before use in order to allow them to acclimate to each environment.

For MeHg exposure, six eels kept in tap water were transferred to two 50 L tanks (three eels per tank) of artificial freshwater (FW; 0.5 mM NaCl, 0.05 mM KCl, 0.4 mM $CaCl_2$ and 0.2 mM $NaHCO_3$) containing 0.05 nM (10 μg Hg/L) MeHg chloride (Tokyo Kasei Co., Ltd., Tokyo, Japan). Similarly, six eels acclimated to seawater were exposed to MeHg in artificial seawater (SW) containing 462 mM NaCl, 9.39 mM KCl, 10.8 mM $CaCl_2$, 48.3 mM $MgCl_2$ and 5.95 mM $NaHCO_3$. Another six eels in each group were cultured without MeHg. The water was constantly re-circulated through a sponge filter and aerated. The room temperature was kept at 20 ºC.

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To monitor the mercury levels in the tanks, water samples of 500 mL were taken into funnel at 0, 1, 6, 24, 48 and 72 hr (single sample each) and mixed with 2.5 mL of 20 N H_2SO_4 and 1.25 mL of 0.5% $KMnO_4$. The mixture was allowed to stand for 5 min, and neutralized with 10 N NaOH (5 mL), then mixed with 1.25 mL of 10% NH_2OH-HCl. Twenty minutes after, 1.25 mL of 10% EDTA was added, and mercury was extracted into 0.01% dithizone/toluene solution (10 mL). After more than 30 min, 5 mL of toluene layer was taken into 50-mL volumetric flask, and then toluene was evaporated off. After adding HNO_3-$HClO_4$ (1:1, 2 mL), conc. H_2SO_4 (5 mL) and distilled water (1mL) in order, the flask was heated on a hot-plate at 230 to 250 °C for 20 min. After cooling, it was made up to 50 mL with distilled water. Then total mercury concentrations of the water samples thus prepared were determined by cold vapor atomic absorption spectrometry using SGI 2537 Mercury Analyzer (Sugiyama-Gen Environmental Science Co., Tokyo, Japan). Distilled water was used for a blank. MeHg chloride (#M0640, Tokyo Chemical Industry, Tokyo, Japan) was used to prepare standard solution. An aliquot of MeHg standard stock solution (0.5 mL), which contains 100 ng Hg/ml of MeHg chloride in toluene, was vigorously mixed with 5 mL of 0.1% L-cysteine in 0.1 N NaOH. Toluene layer was removed after centrifugation at 1000 rpm for 3 min. Three mL of the aqueous layer which contains 10 ng Hg/mL of MeHg was taken into 500 mL of distilled water to afford a running standard solution. Blank and standard solutions were processed in same way as samples shown above and quantified total mercury.

After three days, the eels were sacrificed under anesthesia with a 0.1% MS222 (3-aminobenzoic acid ethyl ester methanesulfonate salt, Sigma Chemical Co., St Louis, USA) solution. About 4 mL of blood samples were collected from the *bulbus arteriosus* with a heparinized syringe prior to isolation of the organs. Blood samples were then hemolised in 9 volumes of distilled water. Bile was collected from the gall bladder using a syringe. Gill filaments were isolated from the gill arch. The intestine was divided into anterior and posterior parts at the site where thickness of the muscle layer changed. Muscle and skin samples were taken from the dorsal portion of the body at the anterior end of the dorsal fin. Organ samples (0.02 - 1.5 g) were dissolved in 4 mL of 1N NaOH. The total mercury concentration in each tissue was determined as above. To control the quality of measurement, a standard reference material DORM-2, dogfish muscle, of the National Research Council, Canada, certified value of MeHg is 4.64 ± 0.26 μg/g) was used. Our quantification data was 4.50 ± 0.05 μg/g (average of 3 times repetition).

For glutathione (GSH) analysis, small pieces of liver (0.4 - 0.8 g) and kidney (0.1 - 0.3 g) were homogenized in 2 mL of ice-cold 5% perchloric acid (1 mM EDTA) immediately after sacrifice, and then centrifuged at 3000 g for 15 min. The supernatant thus obtained was stored in -80 °C until analysis. GSH levels were determined by the enzymatic recycling method according to Tietze (1969).

Statistical significance of differences in the total mercury concentration and the GSH level was evaluated by Student's *t*-test.

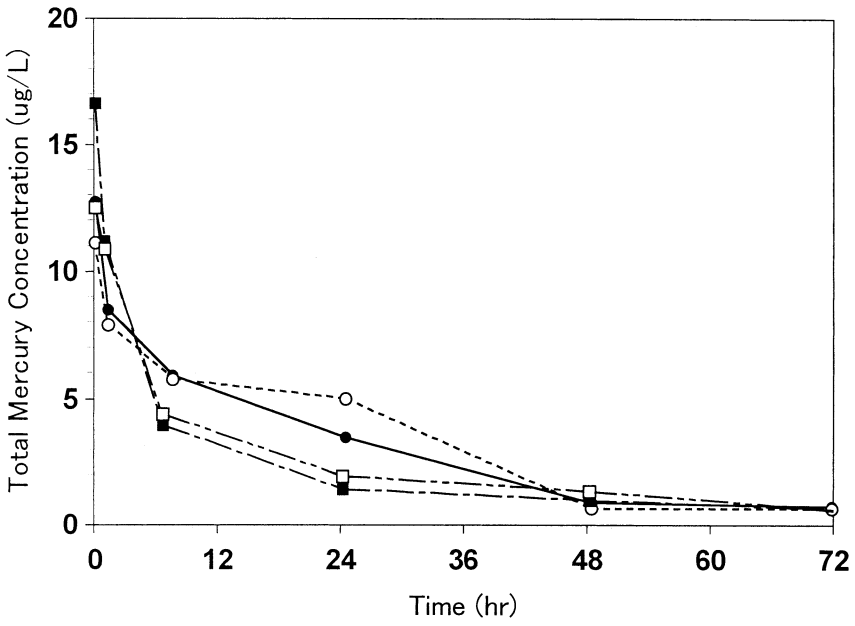

Figure 1. Total mercury concentration in FW (•) and SW (■). Each line indicates mercury concentration in each tank.

RESULTS AND DISCUSSION

The mercury concentration in both SW and FW decreased in an approximately logarithmic manner (Fig. 1). Although FW showed somewhat higher levels than SW from 6 to 24 hr, the difference was not observed beyond 48 hr. The average mercury concentrations during the experimental period in FW and SW were 3.00 and 2.50 μg Hg/L, respectively. The time-dependent reduction in the water Hg levels might possibly be due to absorption by the fish. The mercury concentrations in control tanks without added mercury remained below 0.1 μg Hg/L during experimental period.

The fact that the Hg levels were lower in SW than in FW during the initial phase (6 to 24 hr) might indicate a higher MeHg uptake rate by the fish in SW. This was confirmed by Hg levels in the eels. Figure 2 showed Hg levels in 16 tissues obtained from the eels kept in SW and FW. Higher levels were shown in various tissues of SW eels. Interestingly, although the anterior intestine of SW eels showed higher Hg levels than those of FW eels ($P< 0.01$), the levels were reversed at the posterior intestine ($P< 0.01$).

Block et al. (1997) described that a more rapid MeHg accumulation occurred in the gills at a higher chloride concentration due to the predominance of CH_3HgCl, the

Table 1. Total mercury concentration in organs of six eels (mean ± SEM).

	Total Mercury Concentration (μg/g)			
Organs	FW +MeHg	SW +MeHg	FW –MeHg	SW –MeHg
Brain	0.613 ± *0.068*	0.704 ± *0.040*	0.089 ± *0.023*	0.074 ± *0.019*
Gill	3.231 ± *0.512*	3.471 ± *0.197*	0.031 ± *0.003*	0.026 ± *0.001*
Heart	1.486 ± *0.122*	2.137 ± *0.126***	0.073 ± *0.008*	0.071 ± *0.006*
Liver	2.017 ± *0.107*	2.829 ± *0.178***	0.112 ± *0.009*	0.117 ± *0.006*
Esophagus	0.801 ± *0.158*	0.898 ± *0.071*	0.054 ± *0.011*	0.028 ± *0.005*
Stomach	0.526 ± *0.044*	1.372 ± *0.061***	0.036 ± *0.002*	0.033 ± *0.002*
Anterior Intestine	0.561 ± *0.049*	1.978 ± *0.142***	0.054 ± *0.004*	0.053 ± *0.004*
Posterior Intestine	2.011 ± *0.159*	1.374 ± *0.094***	0.054 ± *0.004*	0.054 ± *0.003*
Spleen	2.429 ± *0.237*	3.436 ± *0.243**	0.042 ± *0.001*	0.052 ± *0.003*
Kidney	1.369 ± *0.143*	1.620 ± *0.128*	0.045 ± *0.001*	0.050 ± *0.003*
Muscle	0.171 ± *0.007*	0.312 ± *0.010***	0.102 ± *0.003*	0.104 ± *0.001*
Epithelia	0.307 ± *0.022*	0.445 ± *0.023***	0.021 ± *0.002*	0.019 ± *0.004*
Pancreas	0.511 ± *0.093*	0.996 ± *0.134**	0.125 ± *0.020*	0.025 ± *0.002*
Air Bladder	0.676 ± *0.053*	1.001 ± *0.077***	0.025 ± *0.002*	0.021 ± *0.001*
Bile	0.057 ± *0.008*	0.401 ± *0.078***	0.009 ± *0.001*	0.008 ± *0.001*
Blood	1.913 ± *0.0182*	4.131 ± *0.368***	0.032 ± *0.002*	0.040 ± *0.004*

Significant differences from the FW value were shown by * ($P<0.05$) and ** ($P<0.01$).

most hydrophobic species, in the environment. Hydrophobic interaction would be important for the MeHg uptake in the gills. In the present study, however, no difference was observed in the gill-mercury level between SW and FW eels. The difference in MeHg uptake between the two water systems might be limited during the intestine uptake.

Nevertheless, we cannot rule out the possibility that the gill-mercury concentration in SW eels had begun to decrease earlier than that in FW, since the water mercury level began to decrease earlier in SW than in FW. This might be supported by previous observations that, although the gills showed a rapid absorption of MeHg, its reduction also occurred very quickly (Olson 1978; Boudou et al. 1991).

The higher mercury concentrations in the stomach ($P< 0.01$) and the anterior intestine ($P< 0.01$) of the SW eels might reflect a higher rate of water uptake by drinking than that in FW as reported by Aoki et al. (2003). Alternatively, a higher chloride concentration of SW, which would result in a higher level of hydrophobic MeHg chloride, might be responsible since the hydrophobic form may be taken up

directly through the cell membrane, bypassing the transport system. However, the mercury concentration in the digestive tract of SW eels is not simply proportionate to the chloride concentration (Smith 1930). Some ion-transport system might possibly be involved in this phenomenon.

Most mercury in the digestive tract would probably come directly from water, since the eels were not fed during the experiment. In a 30-day experiment, Simon and Boudou (2001) suggested that bile played an important role in the mercury

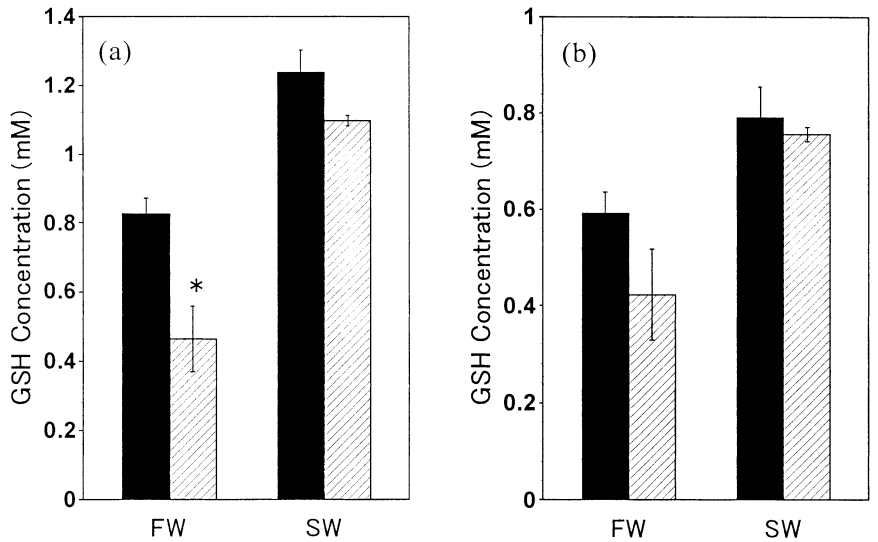

Figure 2. GSH concentrations in liver (a) and kidney (b) of six eels. Solid bars and hatched bars indicate GSH concentration in tissue of fish cultured without and with MeHg, respectively. Each bar and vertical line represents the mean ± SEM.
* Significant effect of mercury exposure ($P < 0.05$).

contamination of the fish intestine. The hepato-intestinal cycle of GSH might be responsible, since some of the MeHg in the liver was secreted in bile as a GSH conjugate (Urano et al. 1988).

However, bile in the present experiment was one of the organs showing the lowest mercury concentration in both environments. Since our experiment period (three days) was much shorter than the 30 days of Simon and Boudou (2001), bile might not be the main source of MeHg in the intestine.

The posterior intestine showed particularly higher ($P< 0.01$) mercury levels in FW, though the levels in most other organs were higher in SW (Table 1). Ando (1975) reported the higher permeability of cations due to chloride absorption in the posterior intestine of eel in seawater than in freshwater. Tissue mercury concentration is determined by influx and efflux rates. If MeHg, a cationic organic metal, acts as other cations observed by Ando (1975), efflux rate might be higher

than influx rate at the posterior intestine. Otherwise, MeHg may act in a quite different manner from other cations, since it is metabolized exclusively bound to SH compounds in animal body (Yasutake et al., 1989). Further study may be necessary to clarify it.

GSH is the major low-molecular-weight thiol in animal cells, and functions as an endogenous MeHg antidote when it is secreted following conjugation (Fujiyama et al. 1994). Accordingly, GSH secretion is suggested to be the major elimination route for tissue MeHg. Yasutake and Hirayama (1994) observed the MeHg-induced stimulation of GSH secretion in mouse liver. They suggested it might be one of the protective responses against MeHg toxicity. In the present study, GSH levels of the kidney in FW eels were lower ($P < 0.05$) than those in SW eels before MeHg exposure, and MeHg exposure caused a significant reduction ($P < 0.05$) in the liver GSH of FW eels (Fig.2). MeHg-induced stimulation of GSH efflux to the bile and/or the circulation might have occurred in the liver of the FW eels. On the other hand, MeHg-induced alterations in the GSH levels were not evident in the liver or kidney of the SW eels which had higher tissue GSH levels than those in the FW eels (Fig. 2). Thus, in the FW eels, MeHg exposure probably stimulated GSH secretion in both the liver and kidney. This might be one of the protective responses against MeHg toxicity to reduce tissue accumulation of the hazardous metal. In the SW eels, however, such stimulation might be unlikely to occur due to high GSH levels i.e., the FW eels with low tissue GSH levels may possibly be more susceptible to a severe MeHg impact than the SW eels with high tissue GSH levels.

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