Bull. Environm. Contam. Toxicol. 21, 170-178 (1979)

Behavior of Mercury in Biosystems
III. Biotransference of Mercury Through Food Chains

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The accumulation of mercury (Hg^{2+}) in aquatic food chains and especially in fish has grown to be a major environmental hazard. Numerous scientific papers have been published dealing with the toxicological and public health aspects of the Hg^{2+} problem. However, much work has also been aimed towards a better understanding of the ecological circulation of Hg^{2+} in nature. This includes microbial methylation of Hg^{2+} in bottom sludge, the paths of bioaccumulation of Hg^{2+} by predatory fishes and from these to fish-eating birds, seals, cats and man. The mechanisms by which Hg^{2+} is accumulated in higher organisms is not completely understood. It is not known as yet whether given species of fish such as tuna or swordfish acquire relatively large amounts of Hg^{2+} by unique mechanisms which are species specific or from participation in, or due to association with, a particular Hg^{2+} accumulating food web. However, it has been firmly established that fishes, shellfishes, and other aquatic organisms take up Hg^{2+} from their environment, sequester and concentrate it in their tissues (GIBLIN and MASSARO 1975, SAYLER et al. 1975, PRABHU and HAMDY 1977). Evidence for the magnification of mercury concentrations through trophic levels has been reported by many investigators (CUNNINGHAM and TRIPP 1975a, SMITH et al. 1975, and CARACCIOLO et al. 1975). However, others (JERNELÖV and LANN 1971, LEATHERLAND et al. 1973, WILLIAMS and WEISS 1973) took an opposite viewpoint. The question has not been satisfactorily answered since none of the investigations were specifically undertaken to trace mercury flow through a specific food chain under controlled conditions. Therefore, this study was conducted to follow the biotransference of radioactive mercury in a simple model food chain which consisted of four trophic levels: from mercury resistant bacteria to mosquito larvae; then from larvae to guppies (small fish); and finally from guppies to Cichlids (big fish). Factors affecting this biotransference in the various trophic levels were also examined.

MATERIALS and METHODS

Trophic systems. The following four trophic levels were used: cells of a mercury resistant bacteria (Bacillus licheniformis) representing the lowest level, a mosquito larvae (Aedes

0007-4861/79/0021-0170 $01.80

aegypti), the guppies (Lebistes reticulatus) and the Cichlids (Cichlasoma facetum), each depicting the next highest food chain systems, respectively.

Isotopic labeling. Both $^{203}Hg(NO3)_2$ and $C_6H_5{}^{203}HgOOCCH_3$ were used. Approximately 2-3 g of 18 h cells of B. licheniformis were resuspended (for 48 h) in 60 ml glucose basal salts broth (GBSB) with 110 ng $^{203}Hg^{2+}$ per g medium as $^{203}Hg(NO3)2$ or $C_6H_5{}^{203}HgOOCCH_3$ (HAMDY and NOYES 1975, HAMDY and PRABHU 1977) and incubated at 37 C. The labeled cells were then harvested by centrifugation after washing once with distilled water. The surface of A. aegypti was sterilized by immersion in ethanol for 5 min. The eggs were transferred to a beaker containing sterile diet (Purina rabbit chow) and allowed to hatch at 27 C yielding the sterile larvae. Larvae (3, 5 and 7 day-old) were placed for 48 h in sterile distilled water (23 C) containing the desired form of radioactive mercury (22 ng $^{203}Hg^{2+}$/g water). The radioactive guppies were incubated for 48 h at 23 C, rinsed once with distilled water and used.

Counting system. Aliquots of $^{203}Hg^{2+}$ water or $^{203}Hg^{2+}$ sample (bacterial cells, larvae, nitric acid digest of tissue homogenate of whole guppies, or that obtained from various tissue organs of Cichlids) were placed in a standard scintillation vial containing 10 ml of toluene-based scintillation fluor and counted in a Beckman LS 100 C liquid scintillation spectrometer. Each sample was counted to $\pm$ 1% error and all counting data were corrected for retention of $^{203}Hg^{2+}$ onto walls of containers and/or volatilization (HAMDY and NOYES 1975, NOYES et al. 1976) as well as for background and half-life.

Biotransference. From bacteria to mosquito larvae. Larvae (3,5 and 7 day-old) were placed in sterile distilled water containing bacterial cells labeled with $^{203}Hg^{2+}$ in the form of either $^{203}Hg(NO3)2$ or $C_6H_5{}^{203}HgOOCCH_3$. The mosquito larvae, kept at 27 C, were allowed to feed on the labeled bacterial cells and, at intervals, the larvae were harvested, washed and their radioactivity determined. Aliquots of radioactive water were also removed, counted and the CF for larvae then calculated using the following equation:

$$CF = \frac{\text{specific activity of larvae (cpm/g)}}{\text{specific activity of bacteria (cpm/g)}}$$

The uptake of $^{203}Hg^{2+}$ by the larvae was also determined.

From the mosquito larvae to the guppies. Guppies were exposed to three day old larvae which were previously labeled with $^{203}Hg^{2+}$ in the form of either $^{203}Hg(NO3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$. The guppies (kept at 23 C) were allowed to feed on the labeled larvae (2-4 larvae at a time). It should be pointed out that not more than five feeds were given daily over a period of 4-5 days. At the end of the specific incubation period, the $^{203}Hg^{2+}$-labeled guppies were harvested, rinsed in water, blotted dry, weighed and homogenized (whole fish). Aliquots of the tissue homogenate (nitric acid digest) as well as the water in which the guppies

were kept were counted and CF of the guppies calculated as follows:

$$CF = \frac{\text{specific activity of guppies (cpm/g)}}{\text{specific activity of larvae (cpm/g)}}$$

From guppies to Cichlids. The Cichlids (Cichlasoma facetum) were fed guppies that were previously labeled with $^{203}Hg^{2+}$ using the organic or the inorganic form of $^{203}Hg^{2+}$. Feeding was ad libitum and was carried out for three days at 23 C. The Cichlids were then harvested, weighed and dissected. The fins, liver, head, muscle, intestine and spleen were removed and weighed. Concentrated HNO_3 was then added to the whole fish or to each organ (in beaker) and digested by heating. Aliquots of the HNO_3 digest of the whole fish or each organ were assayed for radioactivity and CF of the whole Cichlids was calculated using the following equation:

$$CF = \frac{\text{specific activity of Cichlids (cpm/g)}}{\text{specific activity of guppies (cpm/g)}}$$

The % uptake of $^{203}Hg^{2+}$ by the whole fish was also determined. The CF of each organ was calculated using the following equation:

$$CF = \frac{\text{specific activity of organ (cpm/g)}}{\text{specific activity of guppies (cpm/g)}}$$

The % uptake of $^{203}Hg^{2+}$ by each organ was also determined.

RESULTS

Factors affecting the biotransference of $^{203}Hg^{2+}$ in the various trophic systems. Table 1 summarizes the changes of $^{203}Hg^{2+}$ activities as a function of time and temperature when GBSB was supplemented with $^{203}Hg(NO_3)_2$ or with $C_6H_5{}^{203}HgOOCCH_3$ and incubated in the absence of living cells (control experiments). The data indicated considerable loss of $^{203}Hg^{2+}$ from the media. This loss increased in direct proportion to time and incubation temperature, and was also affected by the form of Hg^{2+} used. Therefore, in all experiments, the % uptake of $^{203}Hg^{2+}$ by any of the trophic systems used was corrected for the $^{203}Hg^{2+}$ losses by chemisorption and volatilization.

From bacteria to mosquito larvae. When 3, 5 and 7 day-old larvae were fed $^{203}Hg^{2+}$ using $^{203}Hg(NO_3)_2$ labeled bacterial cells, % uptake (Fig. 1-A) by the larvae increased with time of incubation, regardless of age of larvae. The CF also increased rapidly with time but was affected by age of larvae.

Figure 1-B depicts the uptake and CF of $^{203}Hg^{2+}$ by larvae of different ages (3, 5 and 7 day-old) fed $^{203}Hg^{2+}$-labeled bacterial cells using $C_6H_5{}^{203}HgOOCCH_3$. The % uptake (corrected values) by the larvae showed minimal levels at 24 h and a maximal value of 72 hr for the 3 and 7 day-old larvae and at 97 h for 5 day-old larvae. The CF by the 3, 5 and 7 day-old

TABLE 1

Changes of $^{203}Hg^{2+}$ activities during incubation, in absence of organism, at different temperatures in GBSB media supplemented with $^{203}Hg(NO_3)_2$ (A) or $C_6H_5{}^{203}HgOOCCH_3$ (B). Results, representing an average of 6-9 experiments, are reported as % of initial activity.

Incubation time (h)	Incubation temperature (°C)					
	23		37		45	
	A	B	A	B	A	B
0	100	100	100	100	100	100
5	88.31 ±1.25[a]	95.19 ±1.46	38.18 ±3.41	89.04 ±2.50	33.68 ±1.46	82.53 ±1.17
24	39.04 ±11.09	78.54 ±1.13	26.83 ±2.80	70.98 ±5.22	18.78 ±1.01	64.35 ±0.84
48	27.24 ±11.04	68.47 ±2.07	21.60 ±3.30	67.32 ±1.50	10.07 ±0.41	63.08 ±1.71
72	20.79 ±7.72	65.13 ±2.07	13.28 ±1.67	61.29 ±2.56	7.05 ±0.81	60.33 ±1.04
96	12.74 ±2.10	59.56 ±2.07	12.39 ±0.46	57.42 ±2.11	7.52 ±0.75	59.60 ±2.23

[a] Standard deviation.

larvae (Fig. 1-B) increased gradually between 24 and 72 h, followed by a slight decline at 96 h. It was also evident that the increase in CF was directly related to age of larvae.

From mosquito larvae to guppies. The data for the uptake and CF of $^{203}Hg^{2+}$ by guppies fed labeled larvae are shown in Fig. 2. The larvae were labeled with either organic or inorganic form of $^{203}Hg^{2+}$. The results revealed that the % uptake by the guppies showed a maximal level after 72 hr, in case of larvae labeled with inorganic mercurial, followed by a rapid decline after 96 h. On the other hand, larvae labeled with organic mercurial did not follow the same pattern. The pattern of the CF of $^{203}Hg^{2+}$ for both types of larvae (Fig. 2) showed a low CF at 24 h reaching a maximal at 48 h (in case of organic) and at 72 hr (in case of inorganic) $^{203}Hg^{2+}$-labeled larvae. This was followed by a rapid decline at 96 h.

Analysis of variance of the data for the uptake of $^{203}Hg^{2+}$ by guppies fed $^{203}Hg^{2+}$-labeled larvae indicated that both form of mercury and time significantly affected the uptake of $^{203}Hg^{2+}$ by guppies. There was a significant interaction ($P < 0.01$) between the form of mercury, time and CF.

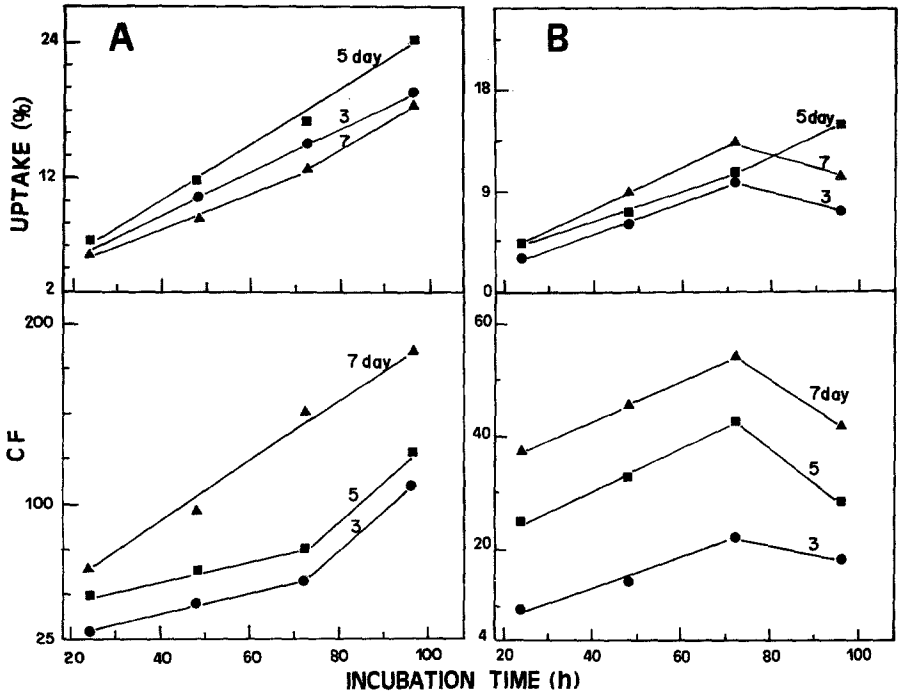

Figure 1. Percent uptake and CF of $^{203}Hg^{2+}$ by larvae as a function of incubation time when fed bacterial cells that were labeled with $^{203}Hg(NO_3)_2$ (A) or with $C_6H_5{}^{203}HgOOCCH_3$ (B).

From guppies to Cichlids. Figure 3 depicts data for the uptake and CF of $^{203}Hg^{2+}$ by Cichlids fed guppies labeled with $^{203}Hg(NO_3)_2$. The % uptake of $^{203}Hg^{2+}$ by the whole fish (excluding blood) was 83.8% of the initial activity. Muscle tissue had the highest uptake (33.3%) of the total uptake by the whole fish and the lowest uptake was in the feces (3.9% of the initial activity. Head and gut tissues exhibited high uptakes (17.3 and 15.2% of the uptake by the whole fish, respectively). The CF of the whole fish (excluding blood) was 0.046. The lowest CF's were exhibited by muscle (0.016) and head (0.019) while the highest CF was observed in the feces (0.199). The gut had a CF of 0.168 followed by the liver which had a CF of 0.110.

The values obtained for the uptake and CF of $^{203}Hg^{2+}$ by Cichlids fed guppies previously labeled with $C_6H_5{}^{203}HgOOCCH_3$ are also presented in Figure 3. The % uptake of $^{203}Hg^{2+}$ by the whole fish (excluding blood) and feces were 67.6% and

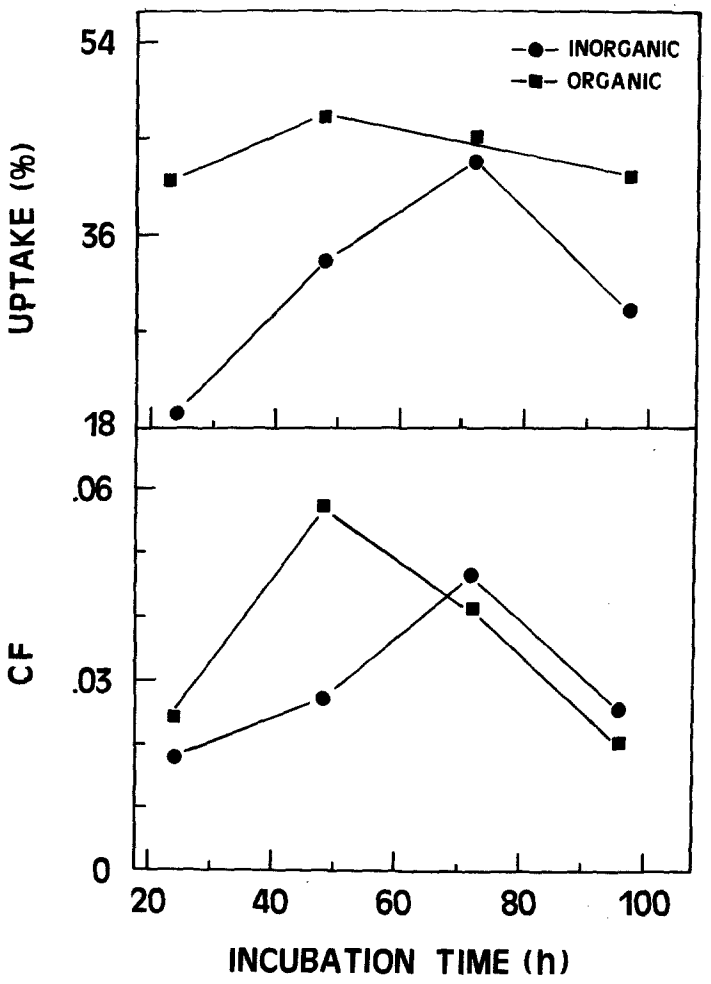

Figure 2. Data for the uptake and CF by guppies fed ^{203}Hg-labeled larvae. The larvae were previously labeled with either inorganic or organic form of $^{203}Hg^{2+}$.

25.2% respectively. Muscle tissue and gut contained 23.8 and 34.0% of the activity in the whole fish, respectively. A low uptake was noted for fins and liver, the values being 2.56% and 2.8% of the activity in the whole fish, respectively. The lowest CF was exhibited by the head (0.01) and the highest by the feces (3.12). The gut had a comparatively high CF of 0.75 followed by liver with a CF of 0.13. Muscle tissue and fins had CF's of 0.035 and 0.053, respectively. The whole fish (excluding blood) had a CF of 0.053.

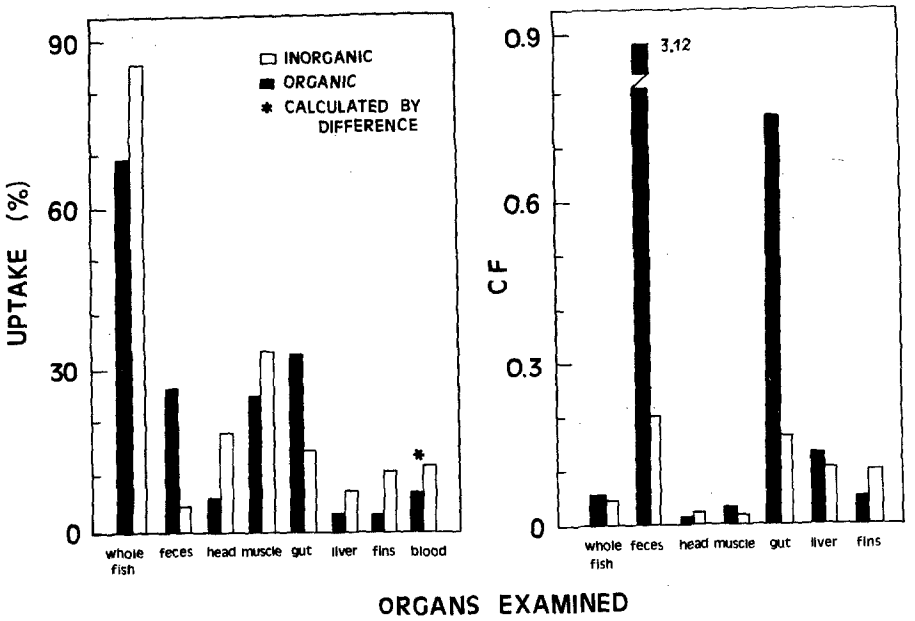

Figure 3. Data for the uptake and CF by Cichlids (whole and organs) fed labeled guppies. The guppies were previously labeled with either inorganic or organic form of $^{203}Hg^{2+}$.

When the CF's were compared for the Cichlids exposed to the two forms of mercury (organic and inorganic form), it was found that on the whole, the CF's were higher for the Cichlids exposed to $C_6H_5{}^{203}HgOOCH_3$. For example, feces of the Cichlids exposed to the organic form of mercury showed a CF of 3.12, while the feces of the fish exposed to $^{203}Hg(NO_3)_2$ showed a CF of 0.199. The % uptake of $^{203}Hg^{2+}$ was also greater (25.2%) in the feces of Cichlids exposed to $C_6H_5{}^{203}HgOOCCH_3$ as compared to 3.9% for the feces of Cichlids exposed to $^{203}Hg(NO3)_2$.

Analysis of variance of the data for the Cichlids indicated that the form of mercury did not significantly affect the CF for fins, liver, gut, muscle and head. However, the form of mercury had a significant effect ($P < 0.01$) on the concentration of $^{203}Hg^{2+}$ by the feces.

DISCUSSION

Data on the dynamics of $^{203}Hg^{+2}$ uptake (PRABHU and HAMDY 1977), its depuration (HAMDY and PRABHU 1977) and biotransference revealed that these various processes are affected by many factors, particularly those altering metabolic rates. In the specific four food chain systems used, it was observed that $^{203}Hg^{+2}$ is concentrated by two of the organisms used, namely the lower system (i.e. the bacterial cells and the

larvae). For example, it was found that the CF of $^{203}Hg^{+2}$ by B. licheniformis cells was always > 1.0 and increased with time of incubation for both inorganic and organic forms of $^{203}Hg^{+2}$ (PRABHU and HAMDY 1977). Again, feeding the labeled bacteria to mosquito larvae also resulted in an increase in CF of $^{203}Hg^{+2}$ by larvae to a value being at all times >1.0. These are the only systems that exhibited magnification of mercury in this study. Thus, this phase of investigation confirmed the results reported by PEAKALL and LOVETT (1972) and STOCK and CUCUEL (1934) who supported the biological magnification of mercury in the aquatic food chain.

On the other hand, evidence against the magnification of mercury was documented in the higher food chain system used (i.e. feeding labeled larvae to guppies and then labeled guppies to Cichlids), thus confirming other investigations (JERNELÖV and LANN 1971, LEATHERLAND et al. 1973, and WESTÖÖ 1973). When $^{203}Hg^{+2}$-labeled larvae were fed to guppies and then the latter fed to Cichlids, the CF in each of the two food chain systems were low, in all cases being < 1.0, indicating no magnification. YOSHIDA et al. (1967) found that the total rate of transference from $^{203}HgCl_2$ to bacteria to shellfish was 100:14.5:1. These investigators stated that brine shrimp was probably not the preferred food of the shellfish. In this study, it is also possible that mosquito larvae are not the desired food of the guppies, but during the four day feeding period, the guppies were not fed any food other than the $^{203}Hg^{+2}$-labeled larvae.

The distribution pattern of $^{203}Hg^{+2}$ in Cichlids fed labeled guppies showed that the highest CF were in the feces and gut. This may be due to the fact that $^{203}Hg^{+2}$ was provided to Cichlids by direct feeding on guppies, hence the major pathway for the uptake of $^{203}Hg^{+2}$ was the digestive tract. ÜNLÜ (1972) showed that when Tapes decussatus were fed $^{203}Hg^{+2}$-labeled Phaeodactylum tircornutum, the digestive system contained 70% of all body mercury. CUNNINGHAM and TRIPP (1975b) established that 57% of all the body mercury in Crassostrea virginica was detected in digestive tissue. DEFRIETAS and HART (1975) showed that smaller fish accumulate mercury in their body tissues at a faster rate per unit weight of body tissue than larger fish.

The results obtained in the present investigation also showed that the CF of the inorganic and organic mercury compounds via biotransference were different in the various trophic levels used. However, it was also evident that at the highest food chain system (Cichlids), the magnitude of the difference in the CF diminished regardless of the chemical form of $^{203}Hg^{+2}$ used and that most of the radioactivities were detected in the digestive tract. The total rate of transference through the food chain of $^{203}Hg(NO_3)_2$ to bacteria to 3-day-old larvae was 100:46.1:0.9, which again showed that the transference of inorganic $^{203}Hg^{+2}$ from water to bacteria is of greater magnitude than the transference from bacteria to

mosquito larvae. This is in contrast with GOLDWATER (1971) who reported that the aquatic food chain is a primary mechanism by which mercury is concentrated and that at each trophic level, less mercury is excreted than ingested, so that there is more mercury in organisms at higher trophic levels.

ACKNOWLEDGEMENTS

This research was supported in part by Grant B-069-Ga. from the Office of Water Resources Research, Department of the Interior, Washington, D.C. We thank Mrs. Diane Whitt for her technical assistance.

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