Behavior of Mercury in Biosystems
I. Uptake and Concentration in Food-chain

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In recent years, attention has been focused on the binomical implications of mercurial compounds in the aquatic environment and on the mechanisms by which these toxic materials enter the various trophic levels of the food-chain. A study of mercury uptake, its biotransformation and excretion processes is, therefore, valuable in understanding the ecological significance of this heavy metal. Bacteria are well documented as agents in the uptake and the biotransformation of mercury through the processes of transmethylation (WOOD 1974, HAMDY and NOYES 1975) and volatilization (SUMMERS and LEWIS 1973, NELSON and COLWELL 1972, BRUNKER and BOTT 1974, BEN-BASSAT and MAYER 1975). These two processes may also be significant in the bioaccumulation and distribution of mercury in fish. SAYLER et al. (1975) reported that the mechanism(s) by which Hg^{2+} is accumulated in higher organisms is not completely understood. Mercury concentration in coastal marine organisms may be several orders of magnitude greater than in the surrounding sea water (KLEIN and GOLDBERG 1970, GIBLIN and MASSARO 1975). However, the level of Hg^{2+} accumulated in fish appears to be related both to the size and the species of fish (WEISS et al. 1971, BARBER et al. 1972) as well as to the time of exposure and the concentrations of Hg^{2+} compound (FANG 1974). This study was conducted to measure both the rate of uptake of two mercurial compounds in a simple model food-chain consisting of three trophic levels (bacteria, larvae and fish) and to ascertain the Hg^{2+}-concentration factor in each. These three organisms represent a unique food-chain system that can be reared easily under controlled laboratory conditions. They are also able to establish themselves within a short period of time independently of the presence or absence of a very low concentration of radioactive mercury in either the organic or inorganic form. Factors affecting both bioaccumulation and concentration factor (CF) were also examined.

Materials and Methods

Trophic systems. Species from three different trophic systems were used. The 1st level is represented by a Hg^{2+}-resistant bacterial culture isolated in our laboratory from the Savannah River sediment (HAMDY and NOYES 1975) and identified as Bacillus licheniformis; the 2nd is depicted by the larvae of the mosquito Aedes aegypti and the 3rd is an omnivore, the guppy Lebistes reticulatus.

Counting systems. All experiments were performed using $^{203}Hg^{2+}$

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isotope as mercuric nitrate, $^{203}Hg(NO_3)_2$, or phenylmercuric acetate, $C_6H_5{}^{203}HgOOCCH_3$, obtained from New England Nuclear Corp. (Boston) and International Chemical and Nuclear Corp. (Cleveland), respectively. The $^{203}Hg(NO_3)_2$ was dissolved in 0.5 N HNO_3, had a specific activity of 11.3 mci/mg Hg^{2+} and a total solids content of 8.8 mg Hg^{2+}/ml. The $C_6H_5{}^{203}HgOOCCH_3$ was prepared in 0.02 M acetic acid, had a specific activity of 3.13 mci/mg Hg^{2+} and a total solids content of 0.41 mg Hg^{2+}/ml. Aliquots of either ^{203}Hg-solution were utilized for the preparation of the working ^{203}Hg-standard solutions used.

A known weight of media, water or sample (bacterial cells, larvae or tissue homogenate of guppies) was placed in a standard scintillation vial containing 10 ml of toluene-based scintillation fluor and counted in a Beckman LS 100 C liquid spectrometer. The samples were counted to $\pm$ 1% error and all 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 halflife.

Isotopic labeling of the trophic system. Bacterial system. An 18 h active culture of B. licheniformis grown in fluid thioglycollate medium was centrifuged and the cells were washed three times with sterile saline. Approximately 2.5 g of bacterial cells were resuspended in 60 ml glucose basal salts broth (GBSB) containing 0.11 µg $^{203}Hg^{2+}$ per g medium as $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$ (HAMDY and NOYES 1975). The uptake of $^{203}Hg^{2+}$ as a function of incubation time and temperature was determined. The concentration factor (CF) of the bacterial cells was ascertained as follows:

$$CF = \frac{\text{specific activity of bacterial cells (cpm/g)}}{\text{specific activity of media (cpm/g)}}$$

Larval system. Eggs of A. aegypti were secured from the Entomology Department, University of Georgia. The eggs were surface washed with dilute Liqui-Nox^R and sterilized by immersion in 75% ethanol for 5 min (LEA et al. 1956). Eggs (12) were then transferred to a beaker of sterile distilled water containing the sterile diet (Purina rabbit chow). The eggs were hatched at 27C yielding the sterile larvae. The direct uptake of $^{203}Hg^{2+}$ (organic or inorganic form) by the larvae was measured by adding the desired isotope to the water (0.022 µg $^{203}Hg^{2+}$/g water) containing either 3, 5 or 7 day old larvae. Following exposure, the larvae were harvested, washed at intervals and their radioactivity determined. Aliquots of the water were also obtained, counted and the CF of the larvae was determined using the following equation:

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

Fish system. The guppies (Lebistes reticulatus) were obtained from a local pet shop and reared in aquarium tanks (27C). The lethal concentration of $^{203}Hg^{2+}$ was determined for the guppies (WALLACE et al. 1971) by exposure to increasing levels of mercury. To determine direct uptake of $^{203}Hg^{2+}$ by guppies, the isotope was

again added directly to the water to give a final Hg^{2+} concentration of 0.0022 μg $^{203}Hg^{2+}$/g water and the guppies incubated for 24 to 96 h at 23C. This temperature was selected as one in which fish would survive without mortality in the absence of acclimatization. Random-sized guppies were used and samples of water and fish were obtained at fixed intervals for measurement of their radioactivity. Each guppy was rinsed with water, blotted dry, weighed and immediately homogenized in a mortar and pestle. Aliquots of the homogenate were counted and the CF determined as follows:

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

Results

Factors affecting uptake and CF by bacteria

Effect of exposure time, temperature, and form of $^{203}Hg^{2+}$. The data on the uptake of $^{203}Hg^{2+}$ by B. licheniformis cells following incubation at different temperatures in GBSB containing $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$ is presented in Fig. 1. The maximal uptake at

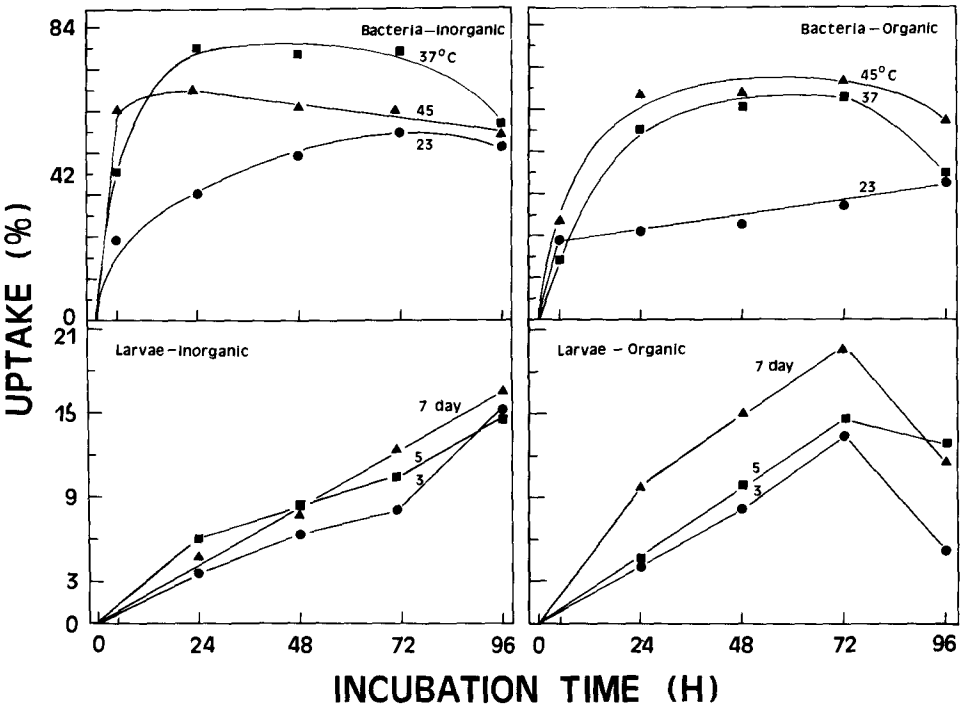

Figure 1. Factors affecting $^{203}Hg^{2+}$ uptake by bacteria and by mosquito larvae: Effect of $^{203}Hg^{2+}$-form; exposure time, incubation temperature of bacteria and age of larvae.

23C with inorganic form of $^{203}Hg^{2+}$ (i.e. ^{203}Hg-nitrate) was reached (53.6%) after 72 h incubation with little change thereafter whereas at 37 or 45C, this was achieved at 24 h (77.7 and 66.3%, respectively). It was also noted that the uptake at 45C decreased steadily while that at 37C was maintained until 72 h incubation. At 96 h, the uptake at 37 and 45C was 55.8 and 54.8%, respectively. When the GBSB contained organic form of $^{203}Hg^{2+}$ (i.e. phenyl-^{203}Hg-acetate), $^{203}Hg^{2+}$ uptake by the bacterial cells at 23C increased slowly but steadily to a maximal level of 38.7% at 96 h. In contrast, the uptake at both 37 and 45C achieved maximum value at 72 h (63.7 and 68.0%, respectively). It was also noted that for the 37C incubation, the uptake decreased rapidly to 42.0% at 96 h, but at 45C the decrease was more gradual (56.9% at 96 h).

The data for the CF of $^{203}Hg^{2+}$ by the B. licheniformis cells are summarized in Table 1 and reveal that the CF increased with time of incubation for both inorganic and organic forms of $^{203}Hg^{2+}$ and that the highest values were reached within 24 to 72 h. Higher CF values were also noted for the inorganic as compared to the organic-$^{203}Hg^{2+}$ at all incubation temperatures and that the values at 23C were always lower than those at 37 or 45C.

Factors affecting uptake and CF by larvae

Effect of exposure time, age of larvae, and form of $^{203}Hg^{2+}$. When 3-day old larvae, kept at 27C were exposed to $^{203}Hg(NO_3)_2$ in water, their uptake of $^{203}Hg^{2+}$ increased gradually during incubation from 3.7% at 24 h to 15.4% at 96 h (Fig. 1). Five-day old larvae exhibited trends in their uptake of $^{203}Hg^{2+}$ similar, but higher in magnitude, to those noted for 3-day old larvae (6.0 and 14.7% after 24 and 96 h, respectively). The % uptake increased steadily with time of incubation for 7-day old larvae exposed to $^{203}Hg(NO_3)_2$, reaching a maximum of 16.7% after 96 h incubation. The results on the uptake of $^{203}Hg^{2+}$ by larvae incubated in water with $C_6H_5{}^{203}HgOOCCH_3$ are also summarized in Fig. 1. The % uptake by 3-day old larvae increased from 4.1% at 24 h to 13.3% at 72 h then declined rapidly to 4.9% after 96 h. Both 5- and 7-day old larvae followed similar trends, reaching a maximum uptake at 72 h (14.8 and 19.5%, respectively) and then declining. At 96 h incubation, the uptake was 12.9 and 11.9% for 5- and 7-day old larvae, resepctively. The $^{203}Hg^{2+}$ CF by larvae are reported in Table 2. Organic-$^{203}Hg^{2+}$ was concentrated to a much greater extent than was the inorganic-$^{203}Hg^{2+}$ but for both mercurials, the CF increased with age of the larvae. The organic-$^{203}Hg^{2+}$ achieved maximum CF at 72 h, however; the inorganic-$^{203}Hg^{2+}$ peaked at 96 h incubation.

Factors affecting uptake and CF by guppies

Effect of exposure time and form of $^{203}Hg^{2+}$. Guppies, exposed to Hg^{2+} in the form of $HgCl_2$, survived in the presence of 15 ppb but died within 24 h exposure to 20 ppb $HgCl_2$. Figure 2 summarizes results obtained for the % uptake and CF of $^{203}Hg^{2+}$ by guppies during incubation in water containing either $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$. A maximum uptake of 22.6% was noted at 48 h incubation in the presence of organic-$^{203}Hg^{2+}$ compared to 23.4% at 72 h incubation for $^{203}Hg(NO_3)_2$. Uptake then decreased rapidly, reaching 13.6 and 4.9% at 96 h for the organic and inorganic form of $^{203}Hg^{2+}$,

respectively. The CF of inorganic-$^{203}Hg^{2+}$ by guppies increased from 34.8 at 24 h to 168.4 at 72 h incubation, followed by a rapid decline to 17.3 (96 h). For organic-$^{203}Hg^{2+}$, the CF reached a maximum of 280.5 at 48 h followed by a considerable decrease to 165.8 at 96 h. The CF was always greater when organic-$^{203}Hg^{2+}$ was used.

TABLE 1.

$^{203}Hg^{2+}$ concentration factor[a] by bacteria as a function of incubation temperature in GBSB supplemented with either $C_6H_5{}^{203}HgOOCCH_3$ or $^{203}Hg(NO_3)_2$. Average of 6-9 experiments are reported as % of initial $^{203}Hg^{2+}$ activity[b].

Incubation time (h)	Form of Mercury					
	phenyl-^{203}Hg-acetate			^{203}Hg-nitrate		
	23	37	45C	23	37	45C
5	6.7 ± 0.5[c]	4.3 ± 0.7	8.8 ± 0.5	9.1 ± 2.0	13.6 ± 2.8	34.7 ± 6.5
24	8.1 ± 1.3	31.3 ± 3.5	42.8 ± 6.3	17.7 ± 3.1	61.6 ± 3.7	69.9 ±10.8
48	8.2 ± 1.8	53.9 ±10.7	51.4 ± 9.0	25.7 ± 5.2	70.9 ± 6.7	51.9 ± 3.0
72	9.4 ± 0.4	39.4 ±10.6	55.5 ± 5.6	33.6 ± 5.3	78.0 + 2.0	42.2 ± 5.8
96	8.8 ± 1.9	19.0 ± 3.2	22.4 ± 3.9	28.6 ± 5.7	16.8 ± 7.2	24.0 ± 3.5

[a]Specific activity of bacterial cells (cpm/g)/specific activity of media (cpm/g).

[b]1.63×10^5 cpm/g.

[c]Standard deviation.

Discussion

POLIKARPOV (1966) reported that mercury uptake by an organism in an environment can be meaningfully presented in the form of CF. This CF expresses the number of times by which this organism can accumulate more of the Hg^{2+} that is present in the surrounding medium (GETSOVA and VOLKOVA 1965). The CF in the present investigation is a parameter which includes both the size of the organism and the corrected concentration of $^{203}Hg^{2+}$ in the solution. Several factors were found to affect both the $^{203}Hg^{2+}$ uptake and the CF by B. licheniformis, such as length of exposure, temperature of incubation and form of $^{203}Hg^{2+}$ used. Again, it was also observed that both uptake and CF of $^{203}Hg^{2+}$ by cells of this organism decreased after 96 h exposure. This was due in part to the ability of the bacterial

TABLE 2

$^{203}Hg^{2+}$ concentration factor by larvae of different ages during incubation at 27C in water supplemented with either $C_6H_5{}^{203}HgOOCCH_3$ or $^{203}Hg(NO_3)_2$. Average of 6 experiments are reported as % of initial $^{203}Hg^{2+}$ activity[b].

Incubation time (h)	Form of Mercury					
	phenyl-^{203}Hg-acetate			^{203}Hg-nitrate		
	3	5	7 D[c]	3	5	7 D
24	167.3 ±20.1[d]	291.9 ± 6.7	385.3 ±23.5	20.5 ± 1.2	35.1 ± 1.8	50.1 ± 3.2
48	238.1 ±18.2	334.5 ±25.3	447.2 ±53.7	24.1 ± 2.1	46.7 ± 2.8	79.8 ± 6.1
72	381.2 ±17.1	491.8 ±31.3	579.1 ±26.4	42.7 ± 1.7	63.1 ± 4.3	102.1 ± 3.1
96	165.0 ± 4.0	325.0 ±13.0	372.1 ± 7.0	95.1 ± 4.7	114.3 ± 4.8	130.2 ± 3.3

[a]Specific activity of larvae (cpm/g)/specific activity of the water (cpm/g).

[b]6.89×10^5 cpm/g.

[c]Days.

[d]Standard deviation.

cells to volatilize the mercury as elemental (Hg^o) and/or to the capacity of the cells to detoxify the mercurials and secrete them into the environment (HAMDY and NOYES 1975). TONOMURA and KANZAKI (1969) found that a mercury-resistant strain of Pseudomonas (K62) is capable of vaporizing the mercury from phenyl mercuric acetate after trapping the mercurial compound onto the cell surface. Similar data have been reported by FURUKAWA and TONOMURA (1971 and 1972) and NELSON et al. (1973). HOLM and COX (1975) reported that bacterial cells of different genera accumulated mercury, to various levels, during growth and that the CF for accumulation of Hg^{2+} were 222, 196 and 1202 for Citrobacter, E. coli and P. fluorescens, respectively. YOSHIDA et al. (1967) suggested that the uptake of mercury by marine bacteria took place by chemical bonding but not by mere physical adsoprtion. However, BURKETT (1975) reported that the live Cladophora glomerata adsorbed more methyl mercury than the dead Cladophora, at equal exposure concentrations, which is indicative of active uptake. The higher levels of mercury detected in bacterial cells within 5 h exposure may be due to both active incorporation of the $^{203}Hg^{2+}$ at optimal growth temperature (HAMDY and NOYES 1975) and passive surface chemisorption (NOYES et al. 1976).

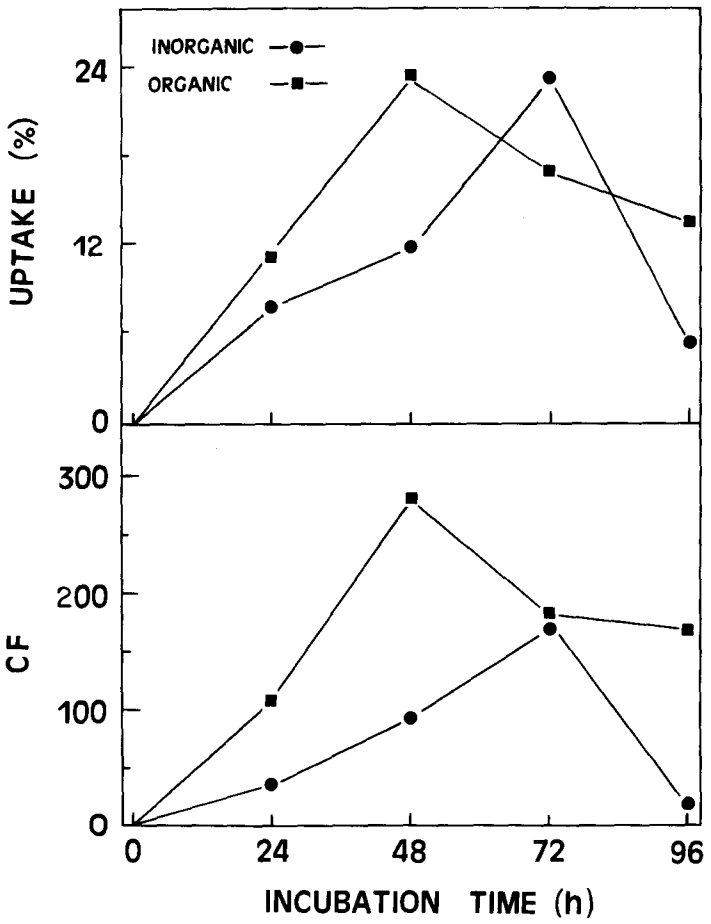

Figure 2. Effect of exposure time and $^{203}Hg^{2+}$-form on uptake and CF of $^{203}Hg^{2+}$ by guppies.

There was a significant difference ($P < 0.01$) between the uptake of $^{203}Hg^{2+}$ at the temperatures of 23 and 37C, but not between 37 and 45C. A direct interrelationship among temperature, metabolic rate and uptake may have existed between 23 and 37C but not between 37 and 45C. It is also probably that at 37C, equilibrium was reached between uptake and excretion of $^{203}Hg^{2+}$ by the bacterial cells or that $^{203}Hg^{2+}$ could have a depressed effect on the test organism above 37C.

Larvae of all ages showed a greater CF of $^{203}Hg^{2+}$ accumulation

from $C_6H_5{}^{203}HgOOCCH_3$ than from $^{203}Hg(NO_3)_2$ thus confirming the results reported by HANNERZ (1968). This author showed that water beetles larvae accumulated higher levels of mercury especially when ingested as organomercury. THOMPSON et al. (1972) proposed that the potential for mercury concentration by aquatic life is in the order of 1000X for fresh water macrophytes and phytoplankton, 100X for fish and 100,000X for fresh water invertebrates.

Guppies of all ages were able to accumulate $^{203}Hg^{2+}$ from both forms of mercurials used. However, the CF from phenyl mercury acetate was much higher than from mercury nitrate which may be attributed to the greater solubility of the organomercurial in lipid (FANG 1974). This explanation would hold if mercury gains entry into gill epithelial cells across a lipid containing cell membrane and not via water-filled pores. Several investigators (JERNELOV 1972, OLSON et al. 1973) suggested that the gills are the primary uptake pathway for non-feeding fish. In this investigation since the guppies were not fed during the 96 h exposure period to $^{203}Hg^{2+}$, the gills were probably the major portal of entry of $^{203}Hg^{2+}$ to the guppies.

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