Importance of Water pH in Accumulation of Inorganic Mercury in Fish

by SHAN-CHING TSAI, G. MALLORY BOUSH, and FUMIO MATSUMURA

Department of Entomology
University of Wisconsin
Madison, Wis. 53706

In spite of worldwide concern about mercury contamination of fish (WEIR and HINE 1970; UCHIDA et al. 1961), relatively little effort has been expended on determining the mechanisms involved in bioaccumulation (HANNERY 1968). It has been found that bottom sediments in aquatic environments contain higher mercury levels than the associated water phase (BURTON and LEATHERLAND 1971; LEATHERLAND et al. 1971; KONRAD 1971), due largely to mercury's tendency to be readily absorbed by particulate matter (U.S. GEOLOGICAL SURVEY 1970). However, the relationship between mercury content of sediment and water and the associated flora and fauna is not clear. For example, mercury levels in fish do not always parallel those in adjacent sediment. In Wisconsin, mercury content in river bottom and flowage silt varies greatly (0.05 to 684.0 ppm) and appears dependent upon proximity to mercury cell chlorine-caustic soda plant activity, or paper pulp plants where phenyl mercuric acetate (PMA) was likely used at one time for slime control (KONRAD 1971). A similar situation has been shown to exist in Sweden (HASSELROT 1968). However, mercury content in the edible portions of fish from these areas often bears little similarity to sediment mercury levels. Fish from the Fox River from Neenah to Menasha, (where the pH of the water was 7.8 and the silt contained 2.0 ppm Hg), contained 0.36 ppm Hg. However, fish from the Wisconsin River from Rhinelander to Tomahawk, (where the pH of the water was 6.7 and the silt contained 1.5 ppm Hg), contained 0.95 ppm Hg (KONRAD 1971). Other similar examples have been noted; generally the mercury levels in fish increased as the pH of the water decreased, where mercury in silt was comparable.

Of interest, recent studies have shown that mercury levels in water in a water-sediment partition system of high pH value were higher than those in a comparable system of low pH (MATSUMURA et al. 1972). Thus, we have the enigma of lower mercury content in fish inhabiting waters of higher pH and comparably higher mercury concentration. It appears that mercury concentration in water is not the only factor controlling the amount of mercury in fish.

Our study was designed to evaluate the factors which we thought might effect the translocation of mercury from water into forage fish on the lower level of the food chain.

We used two bait fish as test animals, the fathead minnow, Pimephales promelas and the emerald shiner, Notropis atherinoides.

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Both were obtained from local bait dealers. The juvenile fathead minnows were approximately 45 mm long and weighed about 0.9 g whereas the shiners were approximately 70mm long and weighed about 3.1 g. All were held two weeks in the laboratory prior to testing. Our mercury concentrations of water were 1.5 ppm (7.5×10^{-6} M) which was obtained from a 240 ppm mercuric chloride stock solution prepared fresh with distilled water for each test. Phosphate buffer solutions were prepared in 0.5 pH increments from pH 5.5 to 9.0 utilizing sodium dihydrogen phosphate and disodium hydrogen phosphate at a concentration of 12 mM in distilled water. The acetate buffer solutions were prepared at a similar concentration in 0.5 pH increments from pH 4.0 to 6.5 using sodium acetate and glacial acetic acid in distilled water. For the study, we placed 50 ml of mercuric chloride stock solution in 8000 ml of the appropriate buffer solution at 22°C in a 2 gal (U.S.) glass fish bowl. Four fish of comparable size were then placed in the bowl and allowed to remain for 15 min. after which they were removed and weighed. An exposure period of 15 min. was used as preliminary experiments had demonstrated that an equilibrium between mercury in water and fish was established within this time. After weighing, the entire fish was digested with a 4:1 mixture of sulfuric and nitric acid (JOINT MERCURY RESIDUE PANEL 1961), and the mercury content measured by the flameless atomic absorption method (HATCH and OTT 1968; UTHE et al. 1970).

In the experiment shown in Fig. 1, we utilized two different buffer systems to examine the effects of pH. It is evident that in either the acetate or phosphate buffer solution, the higher the pH, the less the mercury moved from the water into either the fathead minnow or emerald shiner. The uptake rate of mercury was not linear with pH, but increased rapidly when the pH decreased below 7.0.

The distribution of mercury in the tissue of adult fathead minnows (body weight approx. 3.0 g, total body length approx. 65 mm) is shown in Table 1. The Hg content in the mucus increased to 90 ppm from a Hg solution of pH 5.0. The effects of pH were noted not only on the mercury content of the external mucus but also in the blood (Table 1). About one-half of the mercury in the entire fish can be recovered from the mucus of the external body surface and gills. Another 25% of the mercury was found in the head, which also contained considerable unrecoverable mucus in the mouth cavity. These data are in agreement with the theory that the accumulation of mercury is most probably initiated by a complexing reaction taking place at the exposed external surface of the organism (GOLDBERG 1957). It is speculated that some amino acids on the protein-carbohydrate mucus (ENOMOTO et al. 1961) act as strong ligands for the mercury (II) ions. The Hg content of fathead minnow decreased from 1.43 ppm to 0.25 and 0.18 ppm in the presence of 2 ppm cystein-Hydrochloride and 5 ppm disodium edetate respectively. Probably, the competition of sulfhydryl groups on cystein makes the mercury (II) ions less available to the mucus ligands.

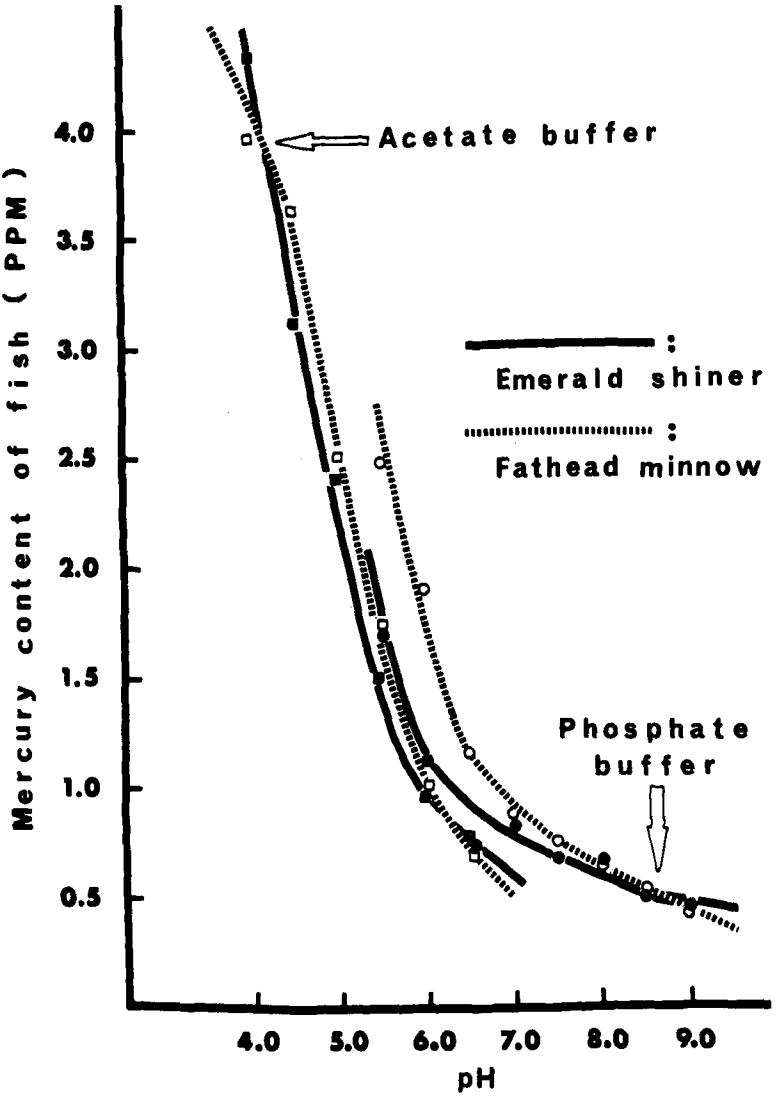

Fig. 1 The effect of different pH levels on the mercury content of the fathead minnows, Pimephales promelas and emerald shiner, Notropis atherinoides after exposure to 1.5 ppm Hg from mercuric chloride solutions.

The mechanisms of how pH affect mercury uptake in fish have been studied. In our studies, we maintained a pH of 5.75, and changed the concentrations of acetate. No significant changes in the mercury content of fish were found in response to the variatios of acetate ion concentrations within the range of 0.5-10 meq/l. Therefore, the changes in the concentration of hydroxide ions are likely the cause of the accumulation difference. In our water system, the concentration of mercury was 7.5×10^{-6} M, which is smaller than the Ks2 (2.4×10^{-4}M) of mercuric hydroxide (SILLENS and MARTELL 1964), and it is expected that no $Hg(OH)_2$ would precipitate under this experimental condition. The OH^- ion in the solution may form complexes with Hg (II). The mercury may be present as Hg^{++}, $Hg(OH)^+$, or $Hg(OH)_2$ as a function of pH (HAHNE and KROONTJE 1973). It is probable that at high pH, complexes of low reactivity are formed and such a shift in the form of mercury (II) affected the extent of accumulation by fathead minnows.

TABLE 1.

Mercury content in tissue of fathead minnows exposed to $HgCl_2$ solution of different pH values (in ppm).

Tissue:	approx. weight (g/8 fish)	pH 5.0	pH 6.5	pH 7.5
Mucus (Extern. sfc.)	0.12	90.0	45.5	13.9
Blood	0.40	1.79	0.93	0.59
fin	0.49	3.62	2.93	0.36
mucus (gill)	0.38	25.90	13.20	5.47
gill (filaments, rakers & arches)	0.50	9.58	5.01	1.78
head (gills removed)	2.53	19.50	1.37	0.48
viscera	12.33	3.31	0.36	0.24
meat, skin, bone & adipose	3.35	7.75	0.29	0.07
whole fish		2.7	1.8	0.40

In contrast to acetate buffer, the concentration of phosphate buffer affected mercury uptake rate in fish. At pH 7.0, mercury in fathead minnows in 0.10, 0.50, 2.5, and 12 mM of phosphate buffer was 3.09, 2.75, 1.79, and 1.10 ppm respectively. In this system, a pH increase raised the concentration of $HPO_4^{=}$ as well as OH^-. Both of these may contribute to decreasing Hg (II) accumulation in fish.

In the 12 mM phosphate buffer of pH 7.0, the fish accumulated more mercury if sodium halides were present. The extent in increase of mercury uptake is parallel to the order of stability for the anionic complex, $I^- > Br^- > Cl^- > F^-$ (SILLENS and MARTELL 1964). When 2 ppm of sodium halide is added to the solution, the Hg content of fish increased to 18 and 6.0 ppm for iodide and bromide

respectively. Chloride and fluoride effect no increase in mercury accumulation at such low concentrations. Mercury content of fish rose to 1.6 and 1.3 ppm after the addition of 300 ppm of sodium chloride and sodium fluoride respectively (Fig. 2). The halides may form complexes which are more readily accumulated by fish than the one originally existing in the buffer. However, it is not known which species of the complex aid in Hg accumulation. In agreement with our data, field tests have shown that a mercuric compound was more toxic to fish when the natural water contained more chloride (AMEND et al. 1969).

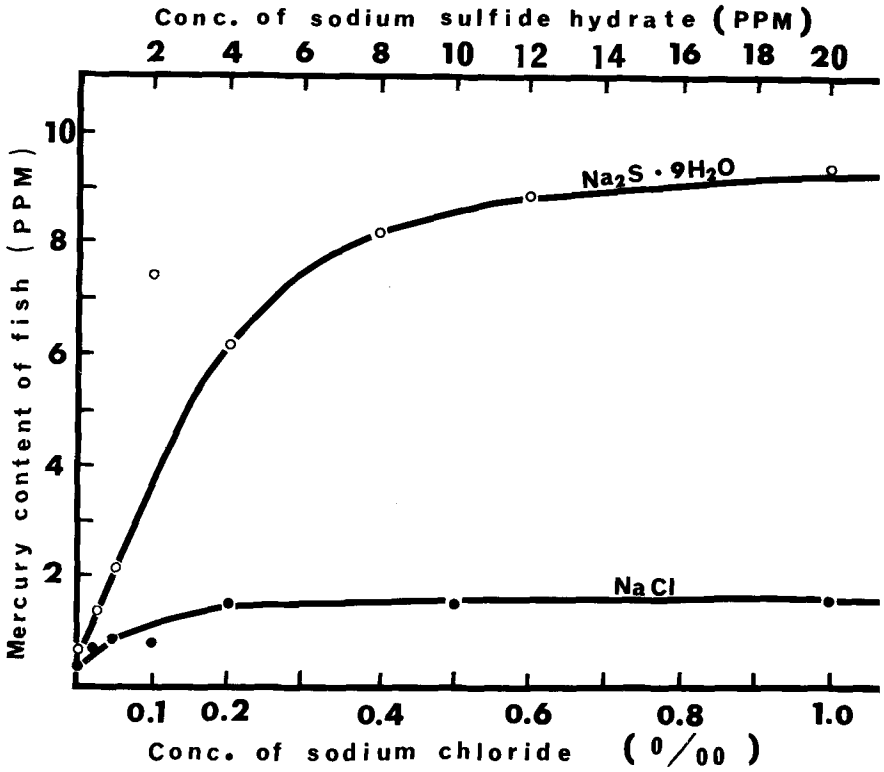

Fig. 2 The effect of different concentrations of sodium sulfide and sodium chloride in water on mercury uptake by the fathead minnow, Pimephales promelas.

Besides halides, sulfide is a strong ligand for mercuric ions (SILLENS and MARTELL 1964). Addition of a trace amount ($<$ 10 ppm) of sodium sulfide into the phosphate buffer solution also increased the uptake rate of mercury by the fathead minnow (Fig. 2). Since the hydrogen sulfide in the deep bottom waters of stratified lakes may be brought up to the surface water when the overturn of lake water takes place in the autumn, it is of interest to checkwhether

this laboratory phenomenon takes place in natural waters.

In summary, inorganic mercury under high pH values was not as readily translocated from water into fish as under low pH conditions, even though abundant in high pH water. The translocation of mercury into fish is not dictated by analytical concentration of mercury (II) in water only. It is evident that pH of ambient water has great effect on translocation. It is important to note that this finding is closely in agreement with data obtained in the field (KONRAD 1971). While under alkaline conditions, more inorganic mercury is released into water (MATSUMURA et al. 1972), but the rate of pickup by fish is reduced. The cause of such a reduction might be related to those unreactive forms of mercury complexes formed under alkaline conditions.

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