Mercury Residues in Fathead Minnows, *Pimephales promelas* Rafinesque, Chronically Exposed to Methylmercury in Water

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Fathead minnows were exposed to methylmercury water concentrations ranging from 0.018 to 0.247 µg/liter. After 48 weeks of exposure (sufficient time for a life cycle), the total mercury residues in the whole fish body ranged from 1.47 µg/gram in fish from the lowest concentration tested to 10.90 µg/gram in fish from the highest. The control fish living in water with concentrations of mercury less than 0.01 µg/liter contained 0.21 µg/gram total body residue. These body concentrations are the result of a continuous water exposure and a probable intake from a portion of the food eaten.

In waters having no known man-made sources of mercury, residues of total mercury in fish flesh have exceeded the 0.5 µg/gram level set by the U. S. Food and Drug Administration as being safe for human consumption (BACHE et al. 1971, SUMMER et al. 1972). It has been documented that fish can concentrate mercury in tissues either directly from water or via the food chain (JOHNELS et al. 1967, HANNERZ 1968), but available data indicate that fish obtain most of the mercury from the water (JOHNELS et al. 1967, JERNOLOV 1971). Furthermore, mercury compounds present in the water or sediments can be biologically converted into the highly toxic methylated form, methylmercury (WOOD et al. 1968, JENSEN and JERNOLOV 1969). Long-term exposure to low mercury concentrations in water results in tissue accumulation by ingestion (JERNOLOV 1971), absorption into the external mucus (MCKONE et al. 1971), and absorption through the gill membrane during respiration (DRUMMOND et al. 1974) possibly because the ability of aquatic organisms to degrade or excrete methylmercury is either minimal or nonexistent (MIETTINEN et al. 1969, UNLU et al. 1970).

The pronounced uptake of methylmercury and a limited ability to excrete it suggest that even very low concentrations of methylmercury in water, concentrations not detectable by usual analytical methods, might result in unacceptable tissue levels in fish. Because data from controlled, long-term exposure are lacking, we

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report here the mercury residues found in fathead minnows exposed to low concentrations of methylmercury for 48 weeks.

MATERIALS AND METHODS

Biological

Fathead minnows, an omnivorous food chain species, were exposed to methylmercury chloride in water at nominal concentrations of 0.015, 0.03, 0.06, 0.12, and 0.24 µg/liter, expressed as mercury. Unfiltered Lake Superior water, having a pH of approximately 7.5 and hardness of 45 mg/liter (as $CaCO_3$), was used for the test after it was heated to 25° C ± 1°. Test water was aerated after heating but before addition of the toxicant because of its volatility. Mercury was added as a water solution by use of a proportional diluter dosing system (MOUNT and BRUNGS 1967).

Embryos from laboratory stocks of fathead minnows were incubated in the test tanks, and the newly hatched fry were released into 0.5 by 2 feet test chambers. All fish were fed live Daphnia, in addition to the main diet of commercial trout chow. After 48 weeks of exposure, the minnows were killed and the whole body residue of total mercury was determined.

Chemical

Total mercury in test water. The concentrations were usually measured weekly by the flameless atomic absorption method described by KOPP et al. (1972). The method was slightly modified so that 0.015 µg/liter could be measured reliably. Samples of 150 milliliters were measured into 250 milliliter flat bottom boiling flasks which were previously cleaned with boiling nitric acid and rinsed with distilled water. Samples were acidified with 2 milliliter HNO_3 (concentrated) within 30 minutes. To keep the total air volume of the aeration system at a minimum, all connections were made with 1/16 inch I.D. Tygon tubing. The absorption cell was constructed of quartz tubing 2.0 centimeters in diameter and 18 centimeters in length with quartz end windows. All tubing was replaced monthly and the cell was cleaned every 2-3 weeks with hot soapy water, diluted nitric acid, distilled water rinse, and air dried. The absorbance readings were expanded ten times. The sensitivity of the method was increased to a detection limit of 0.020 to 0.011 µg/liter of total mercury by using chemical reagents with minimal amounts of mercury contamination, thereby reducing blank values. Precision and accuracy were checked by adding 0.05 and 0.10 µg/liter

of methylmercury to control experimental water and determining the recovery. Recovery water samples and reagent blanks were analyzed each time samples from the test system were analyzed, and the resulting recovery data were 101 percent ± 2 percent during the experimental period.

Inorganic mercury in test water. It was found that MMC added to Lake Superior water could not be measured by the water method unless the procedure includes addition of $K_2S_2O_8$ and heat. This was not required for the recovery of mercuric chloride ($HgCl_2$). As shown in Table 1 total mercury (MMC and $HgCl_2$) could be measured by the addition of $K_2S_2O_8$ and heat. Also, $HgCl_2$ in the presence of MMC, could be determined by omission of $K_2S_2O_8$ and heat. Therefore, by making one measurement with $K_2S_2O_8$ and heat (total mercury) and one measurement without $K_2S_2O_8$ and heat (inorganic mercury), the amount of organic mercury present can be determined by difference.

TABLE 1.

Standard added MMC (ppb)	Standard added $HgCl_2$ (ppb)	$K_2S_2O_8$ milliliters	Boil Yes or No	Concentration (ppb) found	Concentration (ppb) expected	Percent recovered
1		2	Yes	1.00	1.00	100
3		2	Yes	2.93	3.00	98
1		0	No	0.06	1.00	6.0
3		0	No	0.08	3.00	2.7
	1	2	Yes	1.06	1.00	106
	3	2	Yes	2.89	3.00	96
	1	0	No	1.03	1.00	103
	3	0	No	2.90	3.00	97
1	1	2	Yes	2.12	2.00	106
1	1	2	Yes	2.14	2.00	107
3	1	2	Yes	4.00	4.00	100
1	1	0	No	1.06	2.00	53
3	1	0	No	1.16	4.00	29

Each of the above samples contained 150 milliliters of Lake Superior water plus 2 milliliters HNO_3 (concentrated), 5 milliliters H_2SO_4 (concentrated), 1 milliliter $KMnO_4$ (6 percent) and reduced by 1 milliliter $(NH_2OH)_2 \cdot H_2SO_4$-NaCl (12 percent) and 5 milliliters $SnSO_4$ (10 percent).

Using this procedure, control experimental waters were spiked with 0.25 and 0.12 µg/liter of MMC and no detectable inorganic mercury was observed. Concentrations at the 0.06 µg/liter level and lower could not be measured for inorganic mercury because of the limit of detection.

Total mercury in whole fish. The whole fish was homogenized, a 0.1 to 0.5 gram sample was weighed

and washed into a flask with 5 milliliters of distilled water. Concentrated nitric (5 milliliters) and sulfuric (10 milliliters) acids were added to all flasks including reagent blanks and standards and heated in a water bath for 1 hour at 95° C. $KMnO_4$ (10 milliliters) was added and heated for 1 hour at 95° C and if any sample lost the purple color, additional $KMnO_4$ was added. Then 5 milliliters $K_2S_2O_8$ was added and the sample boiled. One hundred milliliters of distilled water was added to all samples and the reduction reaction was completed as stated in the method of KOPP et al. (1972). Calculations of all fish muscle was based on wet weight of sample and expressed as µg/gram of tissue. Accuracy for total mercury in fish tissue was checked by addition of methylmercuric chloride (MMC) to one duplicate sample from the same homogenate. To four homogenates 0.1 µg of MMC was added and a mean recovery of 100.2 percent was obtained.

RESULTS AND DISCUSSION

The results are shown in Table 2. The mean mercury concentrations in the test water were close to, but slightly above, the nominal concentrations, excepting at 0.120 µg/liter. The mean whole body residue concentrations of mercury varied from 1.47 µg/gram in fish from the lowest concentration tested (0.018 µg/liter) to 10.90 µg/gram in fish from the highest concentration (0.247 µg/liter). The percentage of organic mercury ranged from 30 to 79 percent in the highest concentration and from 0.1 to 100 percent in the second highest concentrations. The test water flowing into the test chambers was found to be 95-98 percent organic mercury. Therefore, it was concluded that some form of demethylation was occurring in the test chambers.

TABLE 2

Water exposure and whole body concentrations of total mercury

Nominal concentration in test water (µg Hg/liter)	Measured concentration in test water (µg Hg/liter (N=37))		Concentration in fish (µg Hg/gram of tissue (wet weight))		
	Mean	95 percent confidence interval	Mean	95 percent confidence interval	Number of fish analyzed
Control water	<0.01		0.21	0.17-0.25	17
0.015	0.018	0.015-0.021	1.47	1.20-1.74	10
0.030	0.036	0.031-0.041	2.50	2.02-2.98	11
0.060	0.063	0.055-0.071	4.48	3.46-5.52	10
0.120	0.114	0.102-0.126	7.06	5.37-8.75	10
0.240	0.247	0.225-0.269	10.90	9.24-12.56	8

The relationship between these concentrations is nonlinear but monotonically increasing with magnification factors of from 8.2×10^4 to 4.4×10^5 for the lowest and highest water concentration, respectively. The nonlinear nature of this relationship is clearly shown in Table 2. This observed relationship can be explained by either a whole body saturation effect or a decrease in the percentage of methylmercury to total mercury at higher concentrations, coupled with a higher uptake rate for the methyl form of mercury. No effects from the mercury exposure were observable on survival, behavior, growth, or general appearance of the fishes.

The body residues resulting from these exposure levels may be due in part to food uptake, since fathead minnows are foragers and they were observed feeding on the periphyton that grew on the bottoms and sides of the tanks. JOHNELS et al. (1967) found high concentrations of mercury in algae. Muscle tissue alone was not analyzed for mercury residues because the fathead is used as food by other fishes and is not consumed directly by man. Total body concentration was, therefore, biologically more important.

It would be helpful to know what muscle concentrations would result in edible fish species from exposures such as those used in this experiment. MCKIM (communication 1974) exposed brook trout to methyl mercuric chloride and found the µg/g of total Hg in the muscle to be within 90-100% of the µg/g of Hg found in the whole fish. Muscle makes up a large portion of the total body weight, and methylmercury has been shown to concentrate selectively in muscle (HANNERZ 1968, JERNOLOV 1970). Therefore, it seems reasonable that even those fish at the lowest test concentration, 0.018 µg/liter, ending with a total body concentration of 1.47 µg/gram, would have a muscle concentration greater than 0.5 µg/gram, the action level established by the U.S. Food and Drug Administration and above which fish should be rejected for human consumption. These data point out the potential significance of mercury concentrations in natural waters at ng/liter concentrations.

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