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ESD #1106

Reprinted from
International Conference on Heavy Metals in the Environment
Toronto, Ontario, Canada Oct. 27-31, 1975
pp. 199-216.

METHYLMERCURY IN A FRESHWATER FOODCHAIN

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ABSTRACT

Mercury body burdens of aquatic organisms are contributed to by mercury uptake from the ambient water and mercury uptake from the food. To assess the relative importance of each route, we conducted a series of laboratory experiments with the natural foodchain *Daphnia pulex* (water flea) and *Gambusia affinis* (mosquito fish). The organisms were tagged with radioactive (^{203}Hg) methylmercury amended to their water and food. Uptake tests were run at 0.2, 0.1 and 0.05 ng/ml. Uptake rates, assimilation efficiency, and elimination rates for the fish and uptake and elimination rates for *Daphnia* were determined for methylmercury. A simple model was developed to clarify methylmercury transfer. *Daphnia pulex* acquired methylmercury from ambient water much faster than *Gambusia*, but uptake rate was not linear with respect to water methylmercury concentration. *Daphnia* eliminated methylmercury at a biological halflife (T_b) of 3.2 days. *Gambusia* assimilated methylmercury from water and food at similar rates, but did not show a detectable elimination rate. Food was shown to be a significant source of methylmercury in *Gambusia*, but not in *Daphnia*.

RÉSUMÉ

Les concentrations de mercure dans les organismes aquatiques proviennent de l'absorption du mercure contenu dans l'eau ambiante et dans la nourriture. En vue d'évaluer l'importance relative de chaque voie d'absorption, on a réalisé une série d'expériences en laboratoire avec la chaîne alimentaire naturelle de *Daphnia pulex* et de *Gambusia affinis*. Les organismes ont été marqués avec du méthyl-mercure radioactif (^{203}Hg) ajouté à l'eau et à la nourriture. On a mesuré le taux d'absorption aux

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concentrations de 0.2, de 0.1 et de 0.05 ng/ml. On a déterminé pour le méthyl-mercure le taux d'absorption, l'efficacité d'assimilation et le taux d'élimination dans le cas du poisson, ainsi que le taux d'absorption et d'élimination dans le cas de *Daphnia*. On a élaboré un modèle simple pour clarifier le mécanisme de transfert du méthyl-mercure. *Daphnia pulex* a absorbé le méthyl-mercure de l'eau ambiante beaucoup plus rapidement que *Gambusia*, mais le taux d'absorption n'était pas linéaire par rapport à la concentration du méthyl-mercure dans l'eau. Dans le cas de *Daphnia*, la période T_b pour l'élimination du méthyl-mercure était de 3.2 jours. *Gambusia* a assimilé à un même rythme le méthyl-mercure contenu dans l'eau et la nourriture, mais le taux d'élimination chez ce poisson n'était pas décelable. Il a été démontré que la nourriture constituait une source importante de méthyl-mercure chez *Gambusia*, mais non chez *Daphnia*.

INTRODUCTION

Organisms are adapted to certain concentration ranges of those chemical elements that they ordinarily encounter in their environments through the evolution of homeostatic regulating mechanisms. Excesses of almost any element can be toxic, but even low-level effluents of biologically active chemicals are ecologically hazardous because of the potential for exposing organisms to substances or to concentrations of substances for which their evolutionary experience has not prepared them.

Homeostatic regulation is quite efficient for the common macronutrients (i.e., Na, K, N, etc.) and elevated concentrations of these are seldom found in healthy organisms. The ability to depurate certain micronutrients (i.e., Se, Zn, Mn, etc.) is tuned to much smaller concentrations; these elements are ordinarily much scarcer in the environment. Metabolic defenses are effective against only very small quantities of some very rare, but biologically active, trace elements (i.e., Hg, Pb, Cd, etc.).

Of the toxic heavy metals, mercury is perhaps the most ubiquitous; it can be detected almost anywhere in the ecosphere, but almost always at very low (ng/g) concentrations. Naturally high levels of mercury such as ore bodies and volcanic emissions are very unusual, in the ecological sense. One could make a similar case for cadmium; it is the unusual chemistry of mercury that renders it unique. It is not within the scope of this work to discuss the mercury cycle or mercury toxicity, but it is relevant to point out that mercury vapor (Hg°), ionic mercury (Hg^{2+}), and organic mercurials, all of which occur in nature, have long been recognized as toxic, notwithstanding that organisms have always been exposed to low levels of mercury.

It is an oversimplification to assert that organisms are protected against mercury toxicity by being adapted to levels of it that are seldom exceeded in nature. In fact, an elaborate buffer system exists that greatly reduces the

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biological availability of mercury. This system operates because of a chemical quirk; ionic mercury reacts avidly with sulfide ligands, which abound in all biological material. Closely related is the affinity of mercurials for Se, which is also common, although in much lower quantities than sulfide ligands in biological tissue and most waters. Any mercury present will be bound by these ligands and thereby rendered less harmful. Additionally, it has recently been suggested that non-saturated hydrocarbons in natural waters may be important reducing agents for mercury salts, resulting in the depuration of the water by Hg volatilization (Defilippis and Pallaghy 1975).

Concern over cultural releases of mercury to the environment has waxed and waned in recent years, but there have been serious consequences—both direct toxic effects and detrimental economic effects resulting from governmental edicts, even in the absence of overt toxic effects from mercury effluents (Goldwater 1974). These effluents have now been curbed, and the rhetoric seems to be at a low ebb.

What can we say with certainty about the ecological significance of mercury in the aquatic environment? As previously mentioned, it is almost everywhere and is toxic in excess. The most toxic mercurial is methylmercury, which is the most prominent (usually greater than 80%) mercurial present in fish tissue (Bache *et al.* 1972; Kamps *et al.* 1972; Westoo 1973; Huckabee *et al.* 1974). Methylmercury can be produced under aerobic and (to a lesser extent) anaerobic conditions in sediments, but it is very seldom found in sediments or in water (Jensen and Jernelov 1969; Gillespie 1972; Andren and Harriss 1973; Chau and Saitoh 1973). The uptake of methylmercury by fish is rapid and the elimination rate is slow (Tillander *et al.* 1969; Jarvenpaa *et al.* 1970; Miettinen *et al.* 1970; Lockhart *et al.* 1972; Huckabee and Goldstein 1973; Olson *et al.* 1973; Reinert *et al.* 1974).

Benthic organisms also contain methylmercury, but at somewhat lower percentages (50-76%) (Huckabee and Hildebrand 1974). The question then arises, where do fish get their methylmercury body burdens; from the water, from their food, from their own bacterial flora independent of the sediments, or any combination of these.

Hard evidence exists only for the food chain route (Hannerz 1968). Food organisms do contain methylmercury and it is transferred from prey to predator. This evidence must be regarded as incomplete. However, just because we have not found methylmercury in the water does not mean it is absent; our analytical capabilities may be insufficient. Given the measured uptake rates of methylmercury, a water concentration below presently detectable limits can account for the observed body burdens in many fish (Fagerstrom *et al.* 1974). It is therefore reasonable to ask what is the relative contribution of

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each route—water uptake and food uptake—to a fish's body burden of mercury?

We conducted a series of laboratory experiments with *Gambusia affinis* (mosquito fish) and a prey organism, *Daphnia pulex* (water flea), to help clarify the foodchain transport and accumulation of methylmercurials. We measured uptake and elimination rates of (^{203}Hg) CH_3Hg in both species exposed to the mercurial in the ambient water at concentrations of 0.2, 0.1, and 0.05 ng/ml and the assimilation efficiency and the elimination rate of the mercurial in the *Gambusia* after feeding them mercury-tagged *Daphnia*.

MATERIALS AND METHODS

The mercury uptake from water experiments were conducted in environmental chambers with spring water at 20°C and a 12 hour light-12 hour dark cycle. The zooplankton experiments were in 1 liter beakers, and the fish experiments were in 2 liter plastic tubs. All containers had been pre-treated ("seasoned") with diluted mercury solutions to minimize the effects of added mercury adsorbing on the sides. The methylmercury was labeled with ^{203}Hg, giving a water activity of 300 to 1000 dpm/ml, depending upon the specific activity and age of the radioisotope.

Previous experiments had shown that inorganic mercury concentrations at 1 μg/l in spring water were stable for one week. Even with fish present, it was not necessary to add more mercury because the concentration in the water did not change. However, the accumulation of organic matter will eventually cause a lowering of the concentration of ionic mercury because of the affinity of the mercury ion for thiol ligands. It was therefore necessary to filter out any organic detritus present and add mercury based on measurements of filtered water. All mercury water concentrations were based upon 0.4 μ Nuclepore filtered water samples. A test was conducted to detect changes in mercury retention on the filter because of inorganic chemical changes in the filtered water over time. Methylated mercury with ^{203}Hg was added to 1 liter of filtered spring water (20°C) to a concentration of 0.2 ng/ml. Samples were removed by pipette and filtered through 0.4 μ Nuclepore filters periodically. Counts were taken for ^{203}Hg activity in unfiltered and filtered water and in the filter.

The *Daphnia pulex* mercury uptake experiments were run in triplicate. Adult animals from laboratory cultures taken at or near the end of the log-phase of the population cycle were used. Counts for ^{203}Hg activity were made each 1.5 hour for the first 6 hour post-tag, then each 6 hours for 24 hours, and then daily for 3 days (unfed) or for 6 days (fed). Ten organisms

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were caught at random, placed in a spring water rinse for 2-5 minutes, and then transferred to spring water in a counting tube. The organisms were lyophilized, radioassayed, and then weighed.

For determination of the elimination rate of mercury from *Daphnia*, 10 organisms were taken that had been exposed to the mercurial exactly as in the uptake experiments. These 10 animals were held in separate beakers, but were all placed in the same counting tube for radioactivity measurements. After counting, the organisms were again separated. Counts were taken daily for 5 days; water in the beakers was changed daily and newborn individuals removed. At the termination of the elimination rate experiment, the animals were pooled, lyophilized, and weighed. The data, expressed as percent activity remaining, were fitted to a straight line by standard linear regression techniques. The elimination rate, expressed as the biological half-life (T_b), was then calculated by

$$T_b = \frac{\ln 0.5}{y}$$

where y = slope of the regression line.

Weights of the *Daphnia* were expressed on a dry-weight basis because live-weight determinations were so time-consuming. It was necessary to first determine the weight of the moisture lost from the organisms by the drying (lyophilization) process, and to determine if methylmercury was lost by the drying. Ten replicate samples of between 5 and 8 ^{203}Hg-tagged animals were counted live and weighed wet. Each sample was then lyophilized, and the dried animals were counted and weighed again. Comparison of the two counts and weights showed if any mercury was lost and how much moisture was lost.

Ten *Gambusia* held in separate 2 liter buckets were used for each test of uptake of mercurial from ambient water. Methylated mercury with ^{203}Hg was added to each container and the fish introduced. The buckets were covered with 1 mm^2 mesh screen to provide the fish with cover and to prevent them from jumping out. At 6 hours, 24 hours, and then daily for 7 days post-tag, the fish were netted and placed in water in counting tubes for measurement of the ^{203}Hg activity. The fish were counted for 1 minute and returned to their appropriate container. Mercury concentration in the water was monitored as described above and mercury added when necessary. After the uptake rate of methylated mercury was determined, the fish were transferred to clean water, fed, and counted for ^{203}Hg activity until an elimination rate could be measured.

The transfer of mercury from *Daphnia* to *Gambusia* was determined by feeding ^{203}Hg-tagged *Daphnia* to the fish at a rate of 5 *Daphnia*/fish/day and

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50 *Daphnia*/fish/day. The zooplankters were allowed to equilibrate in each solution of mercurial under identical conditions as in the uptake experiments. The plankters were dipped out of the tagged solution, rinsed, and fed to the fish once daily. The fish were then counted, as described above, once daily just before feeding. After equilibration, the fish were fed non-radioactive *Daphnia* and freeze-dried brine shrimp (*Artemia salina*) and counted periodically until an elimination rate could be determined as described above. The assimilation efficiency for each feeding regime was calculated by finding the y-intercept of the slope of the elimination rate regression line. This intercept represents the methylmercury that the fish assimilated through the wall of the gut and into the circulatory system.

The coefficients measured in these experiments were used in a simple model to calculate the relative contribution of water and food to the mercury body burden in *Gambusia*.

RESULTS AND DISCUSSION

Maintenance of Test Solutions. Extremely dilute solutions of mercury such as those used in these experiments are difficult to maintain at a stable concentration (Feldman 1974). Losses of mercury from solution are the result of adsorption on the walls of the container, binding with ligands and precipitation, and reduction to Hg° with consequent out-gassing to the atmosphere. Adsorption can be reduced by treating the containers with dilute mercury solutions prior to use. This seasoning process tends to saturate the available sites on the container walls, thus preventing further adsorption. Care must be taken to thoroughly rinse the test vessel, or excess mercury adsorbed on the walls will pass back into solution and raise the concentration.

Proper seasoning was established by treating the containers with ^{203}Hg-tagged mercury test solutions and spiking in mercury as it disappeared from solution. For the methylmercury tests at 0.2, 0.1, and 0.05 ng/ml, this process usually required a few hours until equilibrium was established.

With distilled water, the seasoning process is uncomplicated. However, the experiments were conducted in spring water which contained low concentrations of some substances that bound mercury ions. It would seem that this problem could be overcome simply by saturating these ligands the same as the available sites on the container walls. In the fish experiments, this situation indeed held, but in some of the smaller volume zooplankton cultures, the maintenance of stable low concentration mercury solutions proved more difficult. We hypothesized that as the water stood for the several

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days duration of an experiment, the pH changes could have been causing the precipitation of dissolved solids with a concomitant mercury loss.

The test for this possibility showed that there was a general but erratic rise in pH from 6.5 to 7.5, but there was no change in the Nuclepore filter's ^{203}Hg retention. We conclude that the difficulties in maintaining the mercury concentration were the result of ligands excreted by the plankton, the reduction of Hg^{2+} to Hg°, and the absorption of methylmercury by the animals, which is much faster than elimination (discussed later).

***Daphnia pulex* Experiments.** The *Daphnia* uptake experiments showed a non-linear increase in methylmercury tissue concentration with increasing methylmercury water concentration (Fig. 1). Care was taken to compare animals of similar sizes among all three experiments to avoid uptake rate differences due to size rather than methylmercury concentration.

An obvious possibility that would explain these uptake rate differences would be errors in maintaining the desired experimental water methylmercury concentrations. The measured values for the concentrations were 0.20 ± 0.07 ng/ml (1 S.E.), 0.09 ± 0.003 ng/ml (1 S.E.), and 0.048 ± 0.003 ng/ml (1 S.E.). Even when measurements were taken as rapidly as possible and

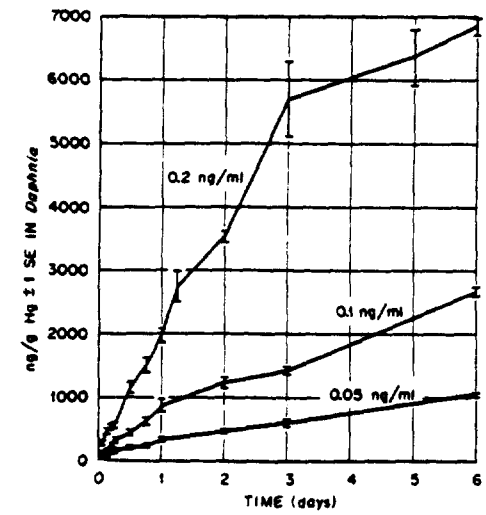

Fig. 1. Uptake of [^{203}Hg] methylmercury by *Daphnia pulex* at 0.2, 0.1, and 0.05 ng/ml of [^{203}Hg] methylmercury in culture solution. Concentration of [^{203}Hg] Hg in organisms is ng Hg/g organism ± 1 S.E.

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appropriate quantities of methylmercury spiked in, the concentration changes were seen to be very pronounced. However, the zooplankton uptake curves do not reflect even the greatest water concentration differences, indicating that the spiking procedure was adequate to maintain an even uptake rate by the zooplankton. Theoretical considerations of changing uptake rates due to changing water concentrations are treated more fully under the discussion of the food chain model.

A second possibility to explain non-linear differences in the zooplankton uptake rate is errors in the addition of food to the water or differences in rates of food consumption by the zooplankton. The first case is unlikely because food (blended trout chow) was pipetted in systematically; the second case is unlikely because the same number of plankters were present in each culture, and all were at the same environmental conditions.

The uptake experiments simulated natural conditions in that the methylmercury was assimilated by the zooplankters both from ambient water and from their food. (The food accumulating methylmercury from the water also.) Previous tests had shown that the animals begin to die of starvation after 3 days, which is well before the equilibrium concentration of methylmercury is acquired (> 6 days).

The test in which *Daphnia* were not fed for 3 days during methylmercury accumulation resulted in plankters with 13% less activity at 72 hours than the fed animals. Methylmercury uptake appears to be so rapid that ingestion of mercury-contaminated food is a minor contribution to body burden.

A third possibility to explain the differences in uptake rate is that the organisms tested at the higher concentrations may have started to suffer deleterious effects after a few days exposure which altered the uptake rate. The highest concentration we used (0.2 ng/ml) is less than the minimal concentrations shown by Biesinger and Christensen (1972) (3.4 ng/ml) and Baudoin and Scoppa (1974) (5.5 ng/ml) to be toxic to *Daphnia*.

The T_b of methylmercury by *Daphnia* was about 3 days, regardless of the concentration of methylmercury that they were exposed to or if they were fed or starved during the uptake phase (Fig. 2). No error terms are given for the unfed 0.2 ng/ml experiment *Daphnia* because all 10 organisms were maintained separately but counted together in one tube. The curve for the fed (during uptake) *Daphnia* has error estimates because this experiment was done in triplicate using randomly sampled organisms. Although it appears to make no difference, this latter case did not provide an unequivocal elimination rate because of the birth of new organisms that had been exposed to methylmercury as eggs still in the exposed adults.

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This result—a fairly rapid elimination rate of methylmercury independent of body burden and route of uptake—differs from previously reported elimination rates of methylmercury from fish (Tillander 1969; Miettinen *et al.* 1970; Lockhart *et al.* 1972). These data also indicate that zooplankton cycle methylmercury much more rapidly than other animals. The significance of this idea is discussed later more fully, when the food chain model is developed.

The weight loss and potential activity loss of methylmercury by the lyophilization process showed that *Daphnia* are 91% water, and that practically all of the methylmercury is bound in tissue. There was no detectable difference in ^{203}Hg activity after the lyophilization.

***Gambusia affinis* Experiments.** The methylmercury uptake data for *Gambusia* are presented in Fig. 3. Since the objective of the experiment was to carefully examine the transfer of methylmercury from zooplankton to *Gambusia*, the

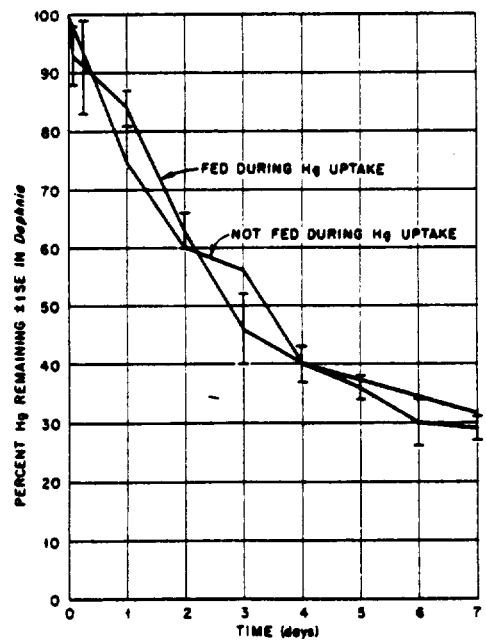

Fig. 2. Elimination of [^{203}Hg] methylmercury by *Daphnia pulex* after exposure to 0.2 ng/ml of [^{203}Hg] methylmercury. One test culture was fed and one was not fed during [^{203}Hg] methylmercury uptake. Concentration of [^{203}Hg] in organisms is ng Hg/g organism ± 1 S.E.

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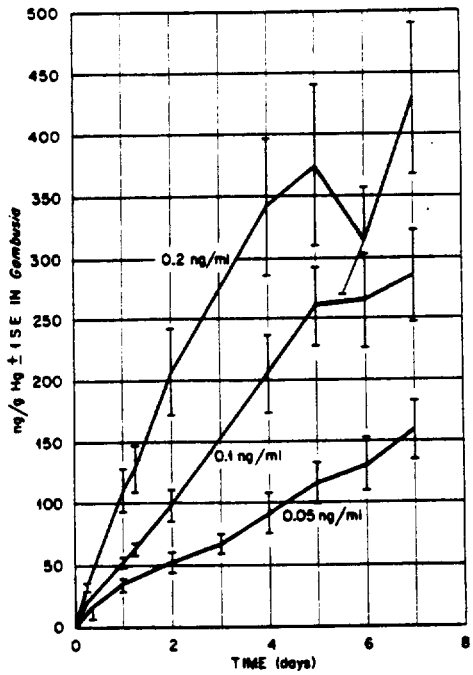

Fig. 3. Uptake of [^{203}Hg] methylmercury by *Gambusia affinis* at 0.2, 0.1, and 0.05 ng/ml of [^{203}Hg] methylmercury in ambient water. Concentration of [^{203}Hg] Hg in fish is ng Hg/g fish ± 1 S.E.

fish were not fed during the water uptake tests. Healthy *Gambusia* easily survive 7 days without food. Individual fish varied considerably in uptake rate, even among those within 5% or less of the same weight. It is therefore important to use several fish for each experiment in order to minimize the statistical error of each estimate. The importance of this point is also emphasized by Fagerstrom *et al.* (1974).

Unlike the zooplankton, the *Gambusia* showed a near linear relationship between water concentration of methylmercury and uptake rate. At the two higher concentrations (0.1 and 0.2 ng/ml), the uptake curves showed a tendency toward saturation as the uptake rate appeared to be slowing. MacLeod and Pessah (1973) demonstrated that Hg^{2+} and phenylmercury at increasing concentrations depressed the active metabolic rate of rainbow trout. It is certainly plausible that methylmercury would exert the same effect.

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The elimination experiments showed that following a rapid first-component loss, methylmercury is retained in the tissues of the fish for so long that an elimination rate cannot be determined with 46-day half-life ^{203}Hg (Fig. 4). Therefore, no differences in elimination rate could be attributed to the different exposures (0.2, 0.1 and 0.05 ng/ml). The fish that accumulated high specific activity (no carrier added) ^{203}Hg-tagged methylmercury until they had body burdens of >400 ng/g (concentrations often found naturally in fish) had lost less than 30% of the accumulated activity in 60 days. At this level, the count rate would have dropped too low for accurate measurements by about 200 days. Since >50% of the activity would remain at 200 days and the elimination curves are nearly flat, the biological half-life of the methylmercury must exceed ~400 days. This value for biological half-life of methylmercury is in agreement with all of the other values reported for many different species of cold water fish (Tillander 1969; Jarvenpaa *et al.* 1970; Lockhart *et al.* 1972; Giblin and Massaro 1973). However, it is not in agreement with Burrows and Krenkel's (1973) report on bluegill (*Lepomis macrochirus*). They found an elimination rate of about 150 days in fish kept at 24°C. In an experiment with largemouth bass (*Micropterus salmoides*) at 25°C, Blaylock (personal communication) found no discernable elimination of methylmercury, even after 145 days, from bass that had fed upon (^{203}Hg) methylmercury-tagged *Gambusia*, and a loss of about 15% after 130 days of (^{203}Hg) methylmercury from bass that had accumulated the mercurial from ambient water.

Food Chain Experiments. The feeding experiments were arbitrarily terminated at 10 days. Figure 4 represents the elimination rate of the *Gambusia* fed

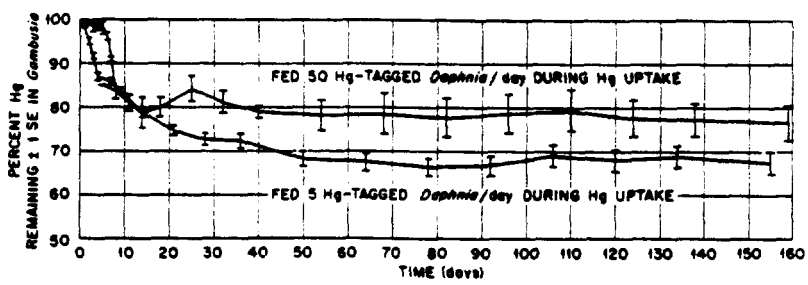

Fig. 4. Elimination of [^{203}Hg] methylmercury by *Gambusia affinis* after feeding on [^{203}Hg] methylmercury-tagged *Daphnia pulex* for 10 days. One group was fed (*ad libitum* up to) 50 tagged *Daphnia*/day, the other group was fed 5 tagged *Daphnia*/day. Concentration of [^{203}Hg] Hg in fish is ng Hg/g fish ± 1 S.E.

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(^{203}Hg) methylmercury-tagged *Daphnia*. The T_b of methylmercury obtained from food is indistinguishable from the T_b of methylmercury obtained from ambient water; both are too long to be measured with 46-day half-life ^{203}Hg.

The assimilation efficiency of methylmercury in food was seen to increase with an increase in ingestion rate. The fish that ate 5 *Daphnia*/day acquired 79.5 ± 4.5% of the (^{203}Hg) methylmercury that the *Daphnia* contained, while the fish that ate *ad libitum* ($\bar{X}$ = 38) *Daphnia*/day acquired 89.2 ± 6.0% of the (^{203}Hg) methylmercury present in *Daphnia*. A t-test showed that this difference is significant at the 5% level. This difference may simply be a function of more complete digestion as a result of an increase in residence time of the several-fold larger food mass in the gastrointestinal tract. The implication remains, however, that feeding rates significantly influence assimilation efficiencies.

Model Development. The three-compartment trophic model developed to calculate the transfer of methylmercury in this food chain is shown schematically in Fig. 5. The water compartment is composed of both water and suspended materials. It is assumed that over the time span of the experiments the concentration of methylmercury in the water remains constant. The zooplankton population takes up methylmercury from the water and eliminates methylmercury to the water. The minor uptake of methylmercury from food is ignored. The *Gambusia* population accumulates mercury from both the water and the zooplankton population.

Let X_1 designate the concentration of methylmercury in the zooplankton population and X_2 the concentration of methylmercury in the *Gambusia* population. Then if we assume that the biomasses of the two compartments remain constant over the time of the experiments,

$$\frac{dX_1}{dt} = a_1 - K_1 X_1 \tag{1}$$

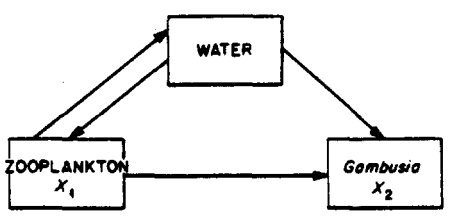

Fig. 5. Schematic diagram of the methylmercury uptake experiments. The elimination rate of methylmercury by *Gambusia* is effectively zero.

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$$\frac{dX_2}{dt} = a_2 + iX_1\epsilon \tag{2}$$

where a_1 and a_2 are the uptake rates (ng/g/day) of methylmercury from the water compartment by the zooplankton and *Gambusia*, respectively; K_1 (day^{-1}) is the elimination rate coefficient of methylmercury by the zooplankton; i is the consumption rate (grams zooplankton/grams *Gambusia*/day) of zooplankton by *Gambusia*; and ϵ is the assimilation efficiency (percent methylmercury ingested that was absorbed through wall of gut) of the *Gambusia*.

The uptake rates, a_1 and a_2, are functions of the concentration of methylmercury in the water compartment (Table 1). In the 0.05 to 0.1 ppb range a_1 and a_2 appear to increase linearly. Between 0.1 and 0.2 ppb a_1 appears to increase at a greater than linear rate while a_2 appears to be equilibrating. In the following analysis, we assume that the concentrations in the water compartment, and hence a_1 and a_2, remain constant during the time span of each experiment. We also assume that the ingestion rate and assimilation efficiency are constant.

The solutions to equations (1) and (2) are

$$X_1 = \frac{a_1}{K_1}(1 - e^{-K_1 t}) \tag{3}$$

$$X_2 = a_2 t + \frac{iea_1}{K_1}(t + \frac{1}{K_1}(e^{-K_1 t} - 1)) \tag{4}$$

TABLE 1

Uptake rates of methylmercury (ng MeHg/g/day) for *Gambusia* and *Daphnia* and calculated equilibrium concentrations for *Daphnia* (ng MeHg/g) for different water concentrations.

Parameter		Concentration of MeHg in Water (ng/ml)		
		0.05	0.1	0.2
a_1	(*Daphnia* uptake rate from water)	418	809	2000
a_2	(*Gambusia* uptake rate from water)	26.4	52.1	85.2
X_1^*	(*Daphnia* equilibrium concentration)	1930	3730	9220
b_3^*	(*Gambusia* uptake from *Daphnia*)	4.4	8.5	21.0
b_{3s}^*	(*Gambusia* uptake from *Daphnia*)[1]	39.0	74.0	184.0

[1] Fish fed *ad libitum* up to 50 *Daphnia* per day. Average consumed was 38 *Daphnia* per day.

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respectively. The assumption has been made that at some initial time, $t = 0$, both X_1 and X_2 are zero.

For a long exposure time, $t >> 1/K_1$, to a constant methylmercury concentration, the zooplankton population will reach an equilibrium concentration, $X_1{}^*$ (Table 1). The elimination coefficient for the zooplankton population was measured to be 0.217 day^{-1}, which is equivalent to a half-life of 3.2 days. For long exposure times, the *Gambusia* concentration, X_2, becomes a linear function of time.

$$X_2 = (a_2 + \frac{i\epsilon a_1}{K_1})\, t - \frac{i\epsilon a_1}{K_1^2} \tag{5}$$

The general shapes of equations (3) and (4) are shown in Fig. 6.

Clearly the concentration of methylmercury in the *Gambusia* cannot increase indefinitely. We can speculate as to what might eventually occur. The duration of our experiments may have been too short to detect an elimination

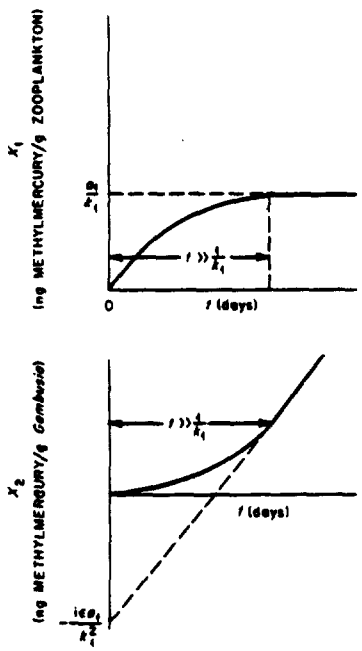

Fig. 6. General shape of the equations for methylmercury uptake as a function of time by *Daphnia* and *Gambusia*.

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that would eventually produce an equilibrium situation or there may be a threshold concentration above which a lethal reaction occurs.

We will now compare the relative contributions of the water and zooplankton pathways to the accumulation of methylmercury by the *Gambusia*. The experiments indicated $\epsilon = 0.775$ when the ingestation rate is 5 zooplankters per day and $\epsilon = 0.89$ when the ingestion rate is *ad libitum* (~38 zooplankters per day). Let us assume that the average dry weight for a *Gambusia* is 0.085 g and the average dry weight of an ingested zooplankter is 0.05 mg. Then

$$b_n = \frac{n\overline{Z}}{\overline{g}} X_1 \epsilon_n = .00059nX_1\epsilon_n \qquad (6)$$

where b_n (ng MeHg/g *Gambusia*/day) is the amount of methylmercury accumulated per gram *Gambusia* via the consumption of zooplankton; n is the number of zooplankton consumed per *Gambusia* per day; $\overline{Z}$ is the average dry weight of the consumed zooplankton; and $\overline{g}$ is the average weight of *Gambusia*. Table 1 lists the calculated b_5 and b_{38} for zooplankton having the equilibrium concentration of methylmercury, X_1^*. For diets consisting of 38 zooplankters per day, the *Gambusia* uptake rates for methylmercury *via* the food chain are about 10% the uptake rates from the water compartment. Fewer zooplankters consumed, or zooplankters having lower concentrations, would result in lower food chain uptake rates. Conversely, more zooplankters consumed would result in higher uptake rates. Theoretically, it should not be possible under natural circumstances to have zooplankters that exceed the equilibrium tissue concentration. The rapid uptake and elimination of methylmercury by the zooplankton means that these organisms can serve as a very sensitive indicator of methylmercury in the ambient water, concentrating from water levels below detection up to tissue levels easily detectable.

CONCLUSIONS

As expected from surface area-volume relationships, zooplankton accumulate methylmercury at least 10-15 times faster than fish. A similar relationship does not seem to hold for elimination rates. A biochemical difference, such as a lower content of thiol and other mercury-binding ligands in zooplankton or simply a physiological difference, such as faster metabolism in zooplankton, should be investigated to explain these data. Ingestion of mercury-contaminated food accounts for less than 15% of the zooplankton

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uptake. For zooplankton the food chain appears to be much less important than ambient water as the source of methylmercury.

For the fish, the relative importance of the ambient water and the food chain is mostly a function of ingestion rate. Clearly the food chain can be a contributor to methylmercury body burden in *Gambusia*.

At the measured concentrations of mercurials in nature and the uptake rates of methylmercury from food and water, *Gambusia* (and other fish) would seem to be living perilously close to mercury poisoning. The fact that mercury poisoning, at least overt poisoning, is so rare attests to the efficiency of the buffering capacity of the environment. The vulnerability of the aquatic ecosystem to methylmercury, as demonstrated by the lack of a metabolic regulating mechanism in fish, augurs well for conservatism in the question of release of mercurials to aquatic ecosystems.

ACKNOWLEDGEMENTS

Research was sponsored by the National Sciences Foundation–RANN Environmental Aspects of Trace Contaminants Program under NSF Interagency Agreement with the U.S. Energy Research and Development Administration. Publication of the Environmental Sciences Division.

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