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MERCURY BIOACCUMULATION IN THE DETRITUS-FEEDING BENTHIC INVERTEBRATE, HYALELLA AZTECA (SAUSSURE)[1]

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Uptake and retention of ingested methylmercury and inorganic divalent mercury from the gastrointestinal tract of the freshwater amphipod, *Hyalella azteca* (Saussure) were estimated by radiotracer techniques. The mercury-contaminated food items consisted of 3 species of algae *(Scenedesmus quadricauda, Anabaena flos-aquae,* and *Navicula pelliculosa)* and pulverized autumn maple leaves. The net assimilation of methylmercury into body tissues was 70-80% of the amount present in the ingested diet compared to 3-8% for inorganic divalent mercury. Clearance of methylmercury from the body tissues of *Hyalella azteca* was a first-order process with a halflife of about 30 da. A positive relation was observed between ingestion rate and whole-body retention. Clearance rate did not increase with temperature, over a range in temperature of 10-20°C. The assimilation from water strongly favors the preferential bioaccumulation of methylmercury compared to divalent mercuric ion. The efficiency of methylmercury removal from water by the amphipod is equivalent to approximately 15-20% of the efficiency of oxygen uptake from water. Inorganic mercury is taken up from the water by *Hyalella azteca* at a slower rate (2-3 times more slowly) than methylmercury. There was no evidence of an appreciable "in vivo" conversion of inorganic mercuric ion, present in tissues, to organic forms of mercury. However, slow "in vivo" conversion of methylmercury to inorganic mercury may be an important factor in elimination of methylmercury from tissues.

Les taux d'absorption du méthyl-mercure et de mercure inorganique divalent dans le système gastrointestinal de l'amphipode dulcicole *Hyalella axteca* (Saussure) ont été estimés à l'aide de traceurs radioactifs. Trois espèces d'algues *(Scenedesmus quadricauda, Anabaena flos-aquae* et *Navicula pelliculosa)* et des feuilles automnales pulvérisées d'érable constituaient les aliments contaminés par le mercure. De 70% à 80% du méthyl-mercure présent dans l'ingesta est assimilé dans les tissus contre seulement de 3 à 8% du mercure inorganique divalent. L'élimination du mercure des tissus d'*Hyalella azteca* est un processus du premier ordre et a une demi-vie d'environ trente jours. On a observé une relation positive entre le taux d'ingestion et la rétention dans le corps dans son ensemble. Le taux d'élimination n'augmente pas avec la température pour des températures comprises entre 10 et 20°C. L'assimilation à partir de l'eau favorise fortement la bio-accumulation du méthyl-mercure par rapport à celle de l'ion mercurique divalent. L'efficacité de l'absorption du méthyl-mercure de l'eau par l'amphipode équivaut à environ de 15 à 20% de son efficacité à absorber l'oxygène de l'eau. Le mercure inorganique est absorbé de l'eau par *Hyalella azteca* à un taux 2 à 3 fois inférieur à celui de l'absorption du méthyl-mercure. On n'a trouvé aucun signe d'une transformation significative "in vivo" de l'ion mercurique inorganique présent dans les tissus en mercure organique. Toutefois, il se peut qu'une lente transformation "in vivo" du méthyl-mercure en mercure inorganique soit un facteur important dans le processus d'élimination du méthyl-mercure des tissus.

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Introduction

Benthic invertebrates can be expected to mediate mercury flux by 3 distinctly different mechanisms. The most obvious mechanism involves mechanical agitation of sediments by movement of the organisms (Boddington et al. 1979). The second mechanism is by direct uptake of mercury from water as is common to all aquatic organisms. The third mechanism is by ingestion of mercury-contaminated detrital material and other food items.

Uptake rate constants for inorganic (mercuric chloride) and organic mercury (methylmercuric chloride) and whole-body clearance rates have been reported for various species of fish (Jarvenpaa et al. 1970; de Freitas et al. 1977; Sharpe et al. 1977). These rate constants have been used to explain: 1) the levels of mercury observed in wild populations or organisms (Fagerstrom & Asell 1973; Norstrom et al. 1976) and 2) the relative magnitude of the food and water vector (de Freitas et al. 1974; Fagerstrom et al. 1974; Norstrom et al. 1976). Corresponding parameter values for mercury dynamics in benthic organisms are not available, and the question arises, then, as to the similarity between invertebrates and fish in their ability to assimilate and eliminate mercury.

In order to help clarify the role of amphipods in the mobilization and transport of mercury to benthophagous fish, we worked with the freshwater amphipod *Hyalella azteca* (Saussure) fed defined algal food material contaminated with ^{203}Hg-labelled methylmercuric chloride (CH_3HgCl) and mercuric chloride ($HgCl_2$). The efficiency of mercury assimilation from the gastrointestinal tract and subsequent clearance of both forms of mercury was measured. Also measured was the amount of mercury absorbed directly from water by amphipods exposed to water contaminated with CH_3HgCl and $HgCl_2$.

Methods

Maintenance of Experimental Animals

The amphipod, *Hyalella azteca*, was obtained from Carolina Biological Supplies[1] and maintained in a 45-*l* aquarium half-filled with dechlorinated tap water under constant aeration. The aquarium contained a layer of sediment, approximately 1 cm deep, from the Ottawa River and the aquatic plants, *Myriophyllum, Elodea* and *Lemna*. Approximately half of the water in the aquarium was replaced every 2 weeks with fresh dechorinated tap water. The system was maintained on a 14:10-h light:dark cycle at a water temperature of 19 ± 1°C. In all of the experiments the head length of the amphipods was measured to minimize differences in results due to body size, but amphipods were not separated by their stage of intermoult.

Handling of ^{203}Hg-labelled Compounds

Mercury-203 is a gamma-emitting isotope allowing easy measurement of mercury taken up by amphipods from contaminated food and water. The amount retained in subsequent periods is estimated from whole-body ^{203}Hg counts of live amphipods. Compounds labelled with ^{203}Hg were purchased from New England Nuclear[2] as aqueous solutions of $CH_3{}^{203}HgCl$ and $^{203}HgCl_2$, with specific activities at time of purchase of 2.7 to 5.2 mCi mg^{-1} and 4 mCi mg^{-1} mercury respectively. The ^{203}Hg-labelled material, as supplied, was diluted with distilled water and stored in 15-ml vacutainer tubes[3]. The initial concentration of radioactivity

[1]Carolina Biological Supply Company, Burlington, North Carolina

[2]575 Albany Street, Boston, Massachusetts, 02118, U.S.A.

[3]Evacuated glass tubes, 127x16 mm., Becton-Dickinson, Division of Becton, Dickinson and Company, Rutherford, New Jersey.

in the diluted stock was usually 20 μCi mg^{-1}. At this concentration, methylmercury does not undergo radiation-induced chemical breakdown, and can be used for up to 6 months after purchase without a significant increase in the level of ^{203}Hg-labelled impurities (Sharpe et al. 1977).

The counting efficiency of the deep well scintillation counter[4] was determined by the use of a standard containing a ^{203}Hg-labelled $HgCl_2$ solution in a sealed glass ampoule within a standard scintillation vial. This standard was used to determine the day-to-day variations in counting efficiency observed during the extended periods of measurements.

A modification of the Westoo procedure (Westoo 1967), was used for separating organic mercury from inorganic mercury. Exposure medium was analysed as follows: a 10-ml sample was placed in a 50-ml stoppered graduated cylinder to which was added 0.5 ml of an aqueous solution containing 250 μg of CH_3HgCl and 250 μg of $HgCl_2$; aliquots (0.25 ml) of 1000 μg ml^{-1} solutions of CH_3HgCl and $HgCl_2$ were added as carrier material; NaCl (2.0 g), concentrated HCl (3.5 ml) and benzene (20 ml) were added and the mixture stoppered and shaken for 30 seconds; the mixture was allowed to stand until the phases separated; the benzene layer was removed and placed in a 100-ml graduated cylinder; the benzene extraction was repeated 2 more times and the extract transferred to the 100-ml graduated cylinder; final volumes of the aqueous phase and benzene phase were recorded; a 5-ml sample of each phase was placed in separate sample vials for measurement of radioactivity.

A similar procedure was used for separating the organic and inorganic mercury fractions present in individual amphipods and mercury-contaminated foods. The amphipod was first homogenized in a 15-ml glass centrifuge tube modified to function as an all-glass homogenizer with matching ground glass rod. To analyse food, approximately 10 mg wet weight of food was added directly to the centrifuge tube, followed by 2 ml distilled water, 2 ml benzene, 0.25 ml each of CH_3HgCl (1000 μg Hg ml^{-1}) and $HgCl_2$ (1000 μg Hg ml^{-1}), 0.7 ml concentrated HCl and 0.4 g NaCl. The contents were mixed on a vortex mixer for 30 seconds, then centrifuged. The benzene layer was removed and placed in a scintillation vial and the benzene extraction repeated twice. The entire benzene fraction and aqueous fraction (each in separate scintillation vials) were then assayed for radioactivity.

Mercury Dosing Procedures

Foods contaminated with mercury were: the green alga, *Scenedesmus quadricauda* (Indiana University Collection[5] No. 77); the bluegreen alga, *Anabaena flos-aquae,* (I.U. No. 1444); and the diatom, *Navicula pelliculosa,* (I.U. No. 668). The background level of mercury in these algae prior to addition of ^{203}Hg-labelled solutions, was 315 ppb on a dry-weight basis, using adaptation of the Magos (1971) procedure for atomic absorption determination of total mercury, as described by Norstrom and Peter (1972). These background levels were less than 5% of the mercury levels in the mercury-contaminated foods used in the feeding experiments.

To prepare amphipods for a feeding experiment, 15 to 30 amphipods were removed from their culture tank and placed in a 2-ℓ glass beaker containing 1800 ml dechlorinated tap water and the uncontaminated version of the diet to be used in the experiment. After a 24-hour acclimation period, the amphipods were placed in another 2-ℓ glass beaker containing 1800 ml dechlorinated tap water

[4]Ino-tech 5100 multichannel analyzer coupled to a 5-cm diameter NaI doughnut crystal detection unit. Counting efficiency for ^{203}Hg was approximately 40%.

[5]Culture Collection of Algae, Dept. of Botany, Indiana University, Bloomington, Indiana.

but no food and then held for 2 hours before addition of the mercury contaminated diet.

Mercury-contaminated food was prepared by adding approximately 350 mg (wet weight) of live algae to a 20-ml glass test tube containing dechlorinated tap water and ^{203}Hg-labelled CH_3HgCl or $HgCl_2$. The tube was shaken for 2 hours, centrifuged, the water layer decanted off, and fresh dechlorinated tap water added. To remove mercury that was not firmly bound, the tube was mixed for 30 seconds on a vortex mixer and centrifuged again. This procedure was repeated 4 to 6 times until only 2% or less of the mercury was lost per rinse. Distribution of the firmly bound mercury was not examined, but it has been reported that both methylmercury and inorganic mercury are absorbed into plant cells, and remain firmly bound to intracellular components (Czuba & Mortimer 1980). An excess of food (approximately 30 times the amount required) was added to the feeding beaker.

In order to correct for any uptake of mercury by the amphipods from the water, a 200-ml sample of water in the feeding exposure beaker was removed by pipet halfway through the exposure period, centrifuged to remove any food particles and the supernatant assayed for ^{203}Hg. Amphipods which had been acclimated with the amphipods used in the feeding experiment, were then added to the supernatant for the same time of exposure as experienced by the feeding group.

At the end of exposure the amphipods were removed from the feeding beaker, using a 5-ml pipet with a tip modified to allow an opening sufficiently large for the passage of amphipods. The amphipods were rinsed by transferral through 3 separate beakers, each containing 150 ml dechlorinated tap water (total time: 30 s). The rinsed amphipods were placed in separate beakers each containing another 150 ml dechlorinated tap water for a period of 6 minutes. At the end of this time individual amphipods were transferred to glass counting vials along with 1 ml of water and then assayed for body content of ^{203}Hg. Each amphipod was then transferred to 2-ℓ beakers containing 1800 ml dechlorinated tap water and maintained there at a density of 2 amphipods per beaker. Measurements of the ^{203}Hg content of each amphipod were taken at post-exposure times of 2, 4, 8, and 24 hours, and then once daily for a maximum of 4 weeks. Water was changed in the clearance beakers each time the amphipods were assayed. Any moults found during clearance were also assayed for their ^{203}Hg content. During the clearance period, amphipods were fed the same diet but uncontaminated with ^{203}Hg. Water temperature during all feeding experiments and subsequent clearance periods was maintained at $19 \pm 1°C$ except when the effect of temperature on clearance of CH_3HgCl was being investigated.

Dechlorinated tap water was used in all water uptake experiments, except for the study on the effect of water quality where unfiltered Ottawa River water was also used. Mercury-contaminated water was prepared by adding a measured portion of the stock solution of ^{203}Hg-labelled CH_3HgCl (approximately 4 μg Hg ml^{-1}) or $HgCl_2$ (approximately 5 μg Hg ml^{-1}) to a glass beaker containing 1800 ml dechlorinated tap water. The water was stirred for 60 seconds and left to stand for 2 hours before addition of the amphipods.

Throughout the various exposure periods, the concentration and chemical form of mercury in the exposure water was determined as described previously. At the end of the exposure period, amphipods were removed from the contaminated water, rinsed and assayed for their whole body content of ^{203}Hg as described in detail for the feeding experiments.

Data Handling

All individual whole-body measurements of live organisms taken during the post-exposure period after either water or food uptake demonstrated biphasic clearance with a fast-clearing initial phase followed by a much slower phase as illustrated by the results in Figure 1. The data were treated as an exponential curve described by the general equation:

$$y = ae^{-bt}$$

where y is the body burden of ingested mercury, a is compartment size, t is the time, in days of clearance, and b is the fractional clearance rate per day. The

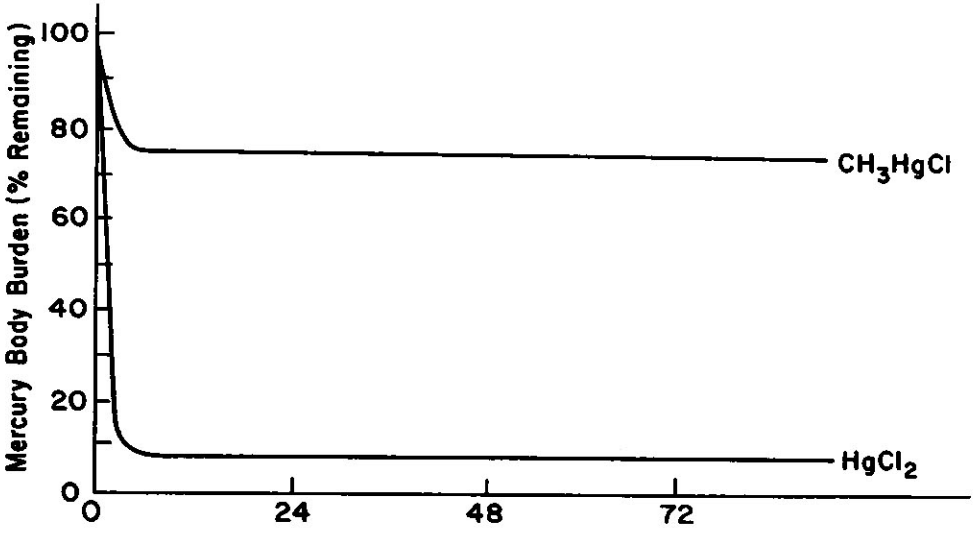

Fig 1. The effect of chemical form of mercury on its assimilation efficiency from the gastrointestinal tract into body tissue of *Hyalella azteca* after ingestion of mercury-contaminated *Scenedesmus* (40 min ingestion period).

biological half life, $t_{1/2}$, or the time required for a half of the accumulated tissue mercury to be lost from the organism as a result of biological processes, was determined by the expression:

$$t_{1/2} = \frac{\ln 2,}{b}$$

with the units being days. Data points were fitted to a straight line by least squares linear regression (Cunningham & Tripp 1973). The results in Table I represent clearance of CH_3HgCl from one amphipod, following a 40-minute period of ingesting $CH_3{}^{203}HgCl$-contaminated *Scenedesmus*. These data are used in the following example calculation of clearance rate and net assimilation efficiency.

The correlation coefficient of the least squares line that best fits the data for the slow-clearing compartment is given by the equation:

$$r = \frac{\Sigma XY - \Sigma X \Sigma Y/n}{[\Sigma X^2 - (\Sigma X)^2/n]\,[\Sigma Y^2 - (\Sigma Y)^2/n]},$$

Table I. Clearance of CH_3HgCl from *Hyalella azteca*[1] following a 40-minute period of ingesting $CH_3{}^{203}HgCl$-contaminated *Scenedesmus*.

Time Post-exposure (h)	Amphipod Body Burden (cpm/organism) Measured cpm	Corrected for Decay[2]	ln Corrected cpm
0.0	465.2	465.2	6.14236
1.2	436.7	436.7	6.07925
2.3	412.2	412.2	6.02151
5.1	412.2	412.2	6.02151
9.6	395.4	395.4	5.9799
24.0	324.3	329.1	5.79636
45.6	335.0	345.0	5.84354
101.4	297.2	315.0	5.75257
148.1	253.2	276.8	5.6233
193.2	272.4	306.5	5.72522
244.3	249.0	288.6	5.66504
289.8	245.6	297.3	5.69474
336.8	211.4	259.7	5.55953
367.3	204.6	259.0	5.55683
439.8	174.6	231.0	5.44242
489.8	187.5	255.6	5.54361

[1]Water temperature during ingestion and clearance was 19±1°C. Specific activity of $CH_3{}^{203}HgCl$ was 2.0×10^5 cpm μg^{-1} Hg.

[2]Using a ^{203}Hg decay half life of 47.2 da.

where X is the post-exposure time in hours and Y is the ln corrected cpm. For this specific example, r = 0.901. The slope, b, or fractional clearance rate is calculated using the expression:

$$b = \frac{\Sigma XY - \Sigma X \Sigma Y/n}{\Sigma X^2 - (\Sigma X)^2/n},$$

and is 0.00105 h^{-1} or 0.0252 da^{-1}. $T_{1/2}$ is ln2/0.0252 = 27.5 da. The size of the slow-clearing compartment given by the y-intercept is defined by the equation:

$$a = \frac{\Sigma Y - b\Sigma X}{n}$$

and is 380.7 cpm. Assimilation efficiency of the ingested CH_3HgCl is defined by the ratio:

$$\frac{a}{c} \times 100$$

where a is the size of the slow-clearing compartment (cpm) and c is the mercury body burden at the end of the exposure period. In this case, assimilation efficiency of CH_3HgCl is 380/465 x 100 = 81.8%. Data for uptake from water and subsequent clearance are treated similarly.

In studying uptake of CH_3HgCl and $HgCl_2$ from water by the amphipod, the term transfer coefficient (T_c), as described by de Freitas and Hart (1975), was used. This unit is defined as the amount of water (g) cleared of its mercury content by 1 g of amphipods in 1 hour. The following is an example of the calculation

Table II. Direct uptake of CH_3HgCl[1] from water by the amphipod *Hyalella azteca*.[2]

^{203}Hg Conc. in Exposure Medium (cpm ml^{-1})	Organism Number	^{203}Hg Content of Organism at 6 min Post-exposure (cpm/organism)	Transfer Coefficient for Uptake: Total (Fast- and Slow-clearing Compartments) (h^{-1})	Transfer Coefficient for Uptake: Slow-clearing Compartment[3] (h^{-1})
13000	1	3408	26.2	11.8 (45)
13000	2	3333	25.6	13.1 (51)
13000	3	2796	21.5	8.6 (40)
13000	4	3839	29.5	13.9 (47)
13000	5	2473	19.0	7.4 (39)
13000	6	4108	31.6	16.4 (52)
13000	7	3205	19.0	8.6 (45)
13000	8	1761	13.6	5.6 (41)
		$\bar{x} \pm SE = 3115 \pm 267$		

[1]Specific activity of CH_3HgCl was 2.74×10^6 cpm μg^{-1} Hg, and the level of radioactivity in exposure medium was 13000 cpm ml^{-1} dechlorinated tap water.

[2]Amphipods were exposed for a period of 2 h.

[3]Values in parentheses refer to the size of the slow-clearing compartment relative to the total body burden at 6 min post-exposure.

used to obtain transfer coefficient values for CH_3HgCl from the water to amphipods using the data in Table II.

$$T_c = \frac{3115 \text{ cpm amphipod}^{-1}}{13000 \text{ cpm g}^{-1} \text{ water}} \times 200 \text{ amphipods g}^{-1} \div 2 \text{ h} = 24 \text{ h}^{-1}$$

An amphipod body weight of 5.0 mg (wet weight) was used in calculating transfer coefficient (T_c) values. Note that this transfer coefficient value of 24 h^{-1} is based on the total body burden of mercury acquired during the exposure period. Since mercury clearance is biphasic, as noted previously, the T_c value of 24 h^{-1} includes mercury uptake into the fast-clearing as well as the slow-clearing pool. The fast-clearing pool probably results from surface adsorption desorption processes; whereas the slow-clearing pool is associated with the process of mercury assimilation into amphipod tissue and subsequent excretion. The size of the slow-clearing pool of mercury is obtained from an analysis of the clearance data, similar to the analysis described above for data from mercury-ingestion ex-

periments. The results of this type of analysis, as shown in Table II, demonstrate that uptake into the slow-clearing compartment occurs with transfer coefficient value of 10.8 h^{-1} based on a compartment size of about 45% of the initial total body burden of mercury acquired during the exposure period.

Results and Discussion

Mercury Uptake from Food

Whole-body retention data from amphipods exposed to a single dose of ingested mercury, demonstrated 2-compartment clearance as illustrated in Figure 1. Two-compartment clearance has also been shown for several species of fish by many other workers (Jarvenpaa et al. 1970; Giblin & Massaro 1973; Weisbart 1973; Suzuki & Hatanaka 1975), for the oyster (Cunningham & Tripp 1973), and for the shrimp and polychaete worm (Luoma 1977). With continuously feeding organisms like *Hyalella azteca*, the time of exposure to the contaminated diet is important in determining net assimilation efficiency, as for example, if an ingestion period of longer duration than the gut voidance time is used, erroneously high values will result. Table III shows the results of the experiment to estimate *H. azteca*'s gut clearance time (voidance time). The amphipod's mercury body burden after a 40-minute ingestion period is approximately twice that achieved after a 20-minute ingestion period. However, there is a smaller increase in the amphipod's body burden of mercury from the 40-minute to the 60-minute ingestion period, indicating that somewhere between 40 and 60 minutes of ingestion the amphipod begins to either ingest less or egest a portion of the ingested mercury not absorbed by its gastrointestinal tract. If we assume that the levelling off between 40 and 60 minutes of the mercury body burden does not represent a decrease in ingestion rate, as the amphipods had been starved for several hours before the experiment was performed, then these results demonstrate that the voidance time is $\geq$40 minutes, in general agreement with voidance times reported by Hargrave (1970). Therefore, to measure the entire size of the fast-clearing compartment, and hence net assimilation efficiency, an ingestion period no longer than 40 minutes should be used.

The results presented in Figure 2 also demonstrate that, as exposure time increases, the measured size of the fast-clearing compartment becomes less. After a 40-minute ingestion period, the fast compartment represented 17% of the ingested CH_3HgCl whereas after 24 hours of ingestion, it represented only 11%. This is expected because, as the body burden increases with increasing exposure

Table III. Gut clearance time in *Hyalella azteca* using $CH_3{}^{203}HgCl$-contaminated food.

Time of Exposure to Food (min)	Body Burden of Mercury at end of Exposure Period (cpm/amphipod)*	Number of Amphipods
20	699.3 ± 108.6	4
40	1388.0 ± 143.0	4
60	1481.7 ± 91.9	5

*Specific activity of $CH_3{}^{203}HgCl$ was 5×10^5 cpm μg^{-1} Hg.

time, the proportion of the body burden associated with the gut contents becomes less. On this basis, after 4 to 5 days of continuous exposure to methylmercury, the size of the fast-clearing compartment relative to the total body burden will be undetectable, being about 1 to 2% of the total body burden and hence lost in the scatter of the data.

A typical result for whole-body clearance following a single ingested dose (40 min dosing period) of food contaminated with CH_3HgCl or $HgCl_2$ is illustrated by the results in Figure 2. The initial post-dose series of whole-body measurements defining the fast-clearing (first) compartment coincides with the voidance time of the gastrointestinal tract and represents clearance of that portion of the ingested dose of mercury not absorbed by the intestine. The slow-clearing (second) compartment represents the proportion of the ingested dose that remains associated with body tissues, and thus its size relative to the size of the ingested dose is an effective measure of net assimilation efficiency. As stated earlier, net assimilation efficiency can be calculated by extrapolating the y-intercept of the slow curve back to zero clearance time. In the case of CH_3HgCl, the example clearance curve illustrated in Figure 1 demonstrates that 25% of the ingested dose is

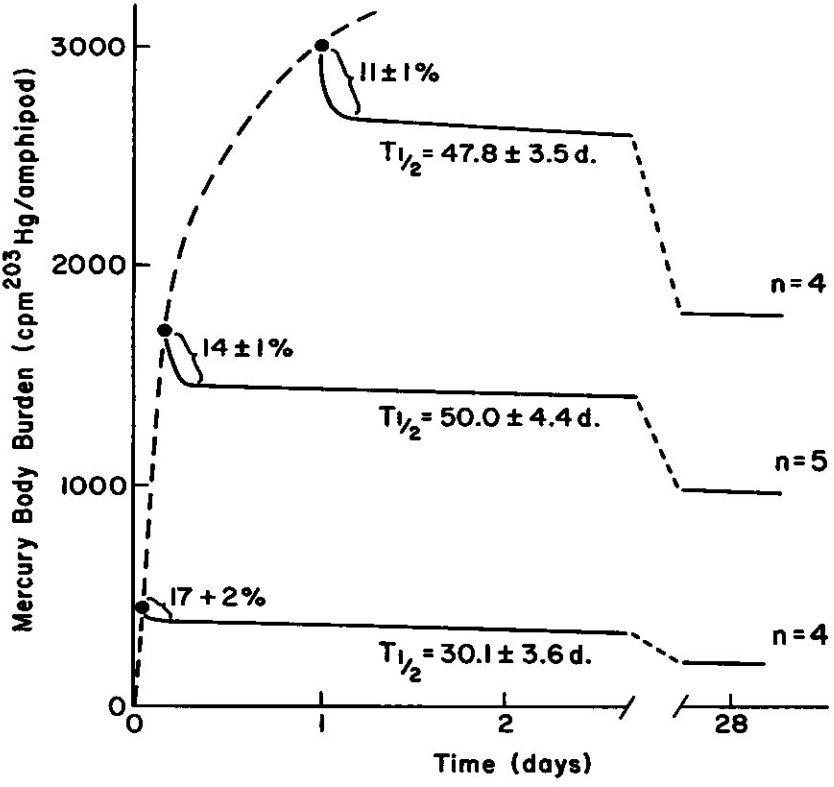

Fig 2. The influence of ingestion period on the apparent assimilation efficiency of CH_3HgCl from the gastrointestinal tract of *Hyalella azteca* and whole-body clearance of CH_3HgCl following ingestion of $CH_3{}^{203}HgCl$-contaminated *Scenedesmus*. Specific activity of $CH_3{}^{203}HgCl$ was 5.9×10^6 cpm μg^{-1} Hg.

rapidly lost and the remaining 75% becomes associated with body tissues and is lost at a very slow rate. Contrastingly, inorganic mercury is assimilated with only about 10% efficiency, as shown in Table IV. These results seem to be independent of food type.

Table IV. Influence of diet composition and chemical form of mercury on mercury assimilation from the gastrointestinal tract of *Hyalella azteca* and subsequent clearance from body tissues.

Food Type[1]	Ingested Mercury[2] (ng Hg/amphipod)		Assimilation Efficiency[2] (%)		$T_{1/2}$ for Slow Compartment (da)	
	CH_3HgCl	$HgCl_2$	CH_3HgCl	$HgCl_2$	CH_3HgCl	$HgCl_2$
Scenedesmus	1.56 ± 0.30(7)[3]	1.44 ± 0.30(11)	77.2 ± 2.4	5.0 ± 0.9	40.8 ± 3.7	6.7 ± 1.4
Navicula	1.88 ± 0.38(5)	1.44 ± 0.30(9)	86.9 ± 1.2	15.1 ± 3.4	50.0 ± 8.5	6.1 ± 1.5
Anabaena	0.68 ± 0.23(4)	6.88 ± 2.15(5)	79.6 ± 3.3	3.3 ± 1.3	55.6 ± 9.0	14.4 ± 3.8

[1]Concentration of methylmercury and inorganic mercury in contaminated foods was 340 and 160 μg Hg g^{-1} dry weight of food, respectively. An ingestion period of 40 min was used. The natural levels of mercury (Norstrom & Peters 1972) occurring in the different algal types were ≤2% of the levels used in the experiments. Analysis of the mercury content in the methylmercury-contaminated foods by the Westoo procedure (Westoo 1967) demonstrated that ≥96% mercury was in the methylmercury form.
[2]Values are given as mean ± SE.
[3]Values in parentheses refer to number of organisms.

Similar assimilation efficiencies for CH_3HgCl and $HgCl_2$ have been shown using many other species of animals: man (Miettinen et al. 1971), fish (Hannerz 1968; Matida et al. 1971, de Freitas et al. 1974), and aquatic invertebrates (Miettinen et al. 1971; Huckabee et al. 1975). The amount of mercury taken up from water during ingestion of mercury-contaminated food accounts for only a very small (3% or less) proportion of the body burden acquired during the ingestion period, as shown by the results in Table V. This can be considered a relatively insignificant error, but it should be noted that a correction for this error in estimating the amount of ingested mercury has not been applied to the data.

The results in Figure 3 on the effect of feeding rate on assimilation efficiency of CH_3HgCl-contaminated *Scenedesmus* and *Anabaena* demonstrate that with both foods there was an increase of about 10% in the assimilation efficiency of mercury with feeding rate ranging from 1 to approximately 14 μg food (dry weight)/hour/amphipod. These feeding rates are moderate compared to ingestion rates of 30 μg sediment/hour/amphipod and 25 μg bacteria/hour/amphipod (Hargrave 1970a; b). It is probable that as ingestion rate increases, so does the residence time of the larger food mass in the gut. The longer residence time may allow more complete digestion of mercury as well as ingested calories, as suggested by Huckabee (Huckabee et al. 1975). In the case of $HgCl_2$-contaminated foods, the assimilation efficiencies reported in Table IV were so low that even if they were affected by feeding rates, we were unable to detect an effect.

Results presented in Table VI on the fractionation of the mercury remaining in amphipod tissue after a post-exposure period of 28 days showed that the chemical form of mercury remains unchanged during ingestion and subsequent tissue retention. This agrees with several studies by Jernelov (1968; 1972), Pennacchioni et al. (1976) and Pentreath (1976). In contrast to this, Guarino et al.

Table V. Direct uptake from water of CH_3HgCl and $HgCl_2$ during ingestion of mercury-contaminated food by *Hyalella azteca*, due to leaching of mercury from the food.

Chemical Form of Mercury	Food Type	Total Uptake from Food and Water (40 min exposure) (cpm/amphipod)	Direct Uptake from Water[1] (40 min exposure) (cpm/amphipod)	Error due to Direct Uptake from Water (%)
CH_3HgCl[2]	*Scenedesmus*	310.0 ± 60.2(7)[4]	3.0 ± 2.0(3)	1.0
	Anabaena	131.3 ± 45.0(9)	8.0 ± 4.7(3)	6.1
	Navicula	334.7 ± 70.4(11)	8.0 ± 3.0(2)	2.4
$HgCl_2$[3]	*Scenedesmus*	3510.0 ± 804.9(15)	29.0 ± 2.7(4)	0.8
	Anabaena	14800.0±3954.3(7)	383.0±287.3(2)	2.6
	Navicula	3560.0 ± 902.9(9)	96.0 ± 10.5(2)	2.7

[1]Amphipods were exposed for 40 min to filtered water samples removed from the beakers containing ^{203}Hg-contaminated foods.
[2]Specific activity of $CH_3{}^{203}HgCl_2$ was 1.92×10^6 cpm μg^{-1} Hg.
[3]Specific activity of $^{203}HgCl_2$ was 2.7×10^6 cpm μg^{-1} Hg.
[4]All values represent the mean ± SE; values in parentheses refer to the number of organisms.

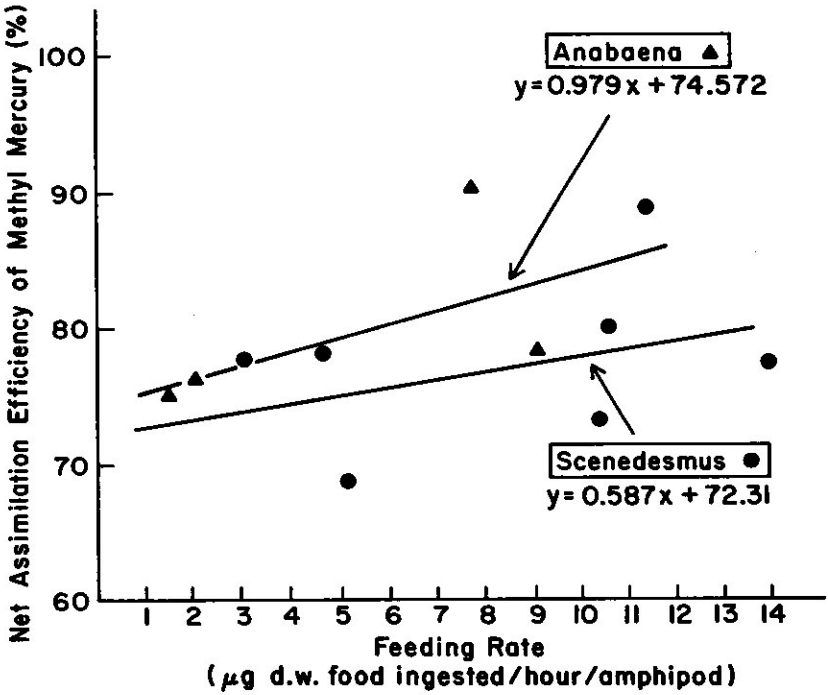

Fig 3. Effect of feeding rate and type of food on assimilation efficiency of CH_3HgCl from the gastrointestinal tract of *Hyalella azteca*.

Table VI. Mercury concentration in water and its chemical stability during exposure of *Hyalella azteca*.[1]

Chemical Form of Mercury	Duration of Exposure (h)	Mercury Concentration in Water (μg Hg ml^{-1})	Mercury Loss from Water during Uptake by Amphipods (%)	Mercury Composition % organic	% inorganic
CH_3HgCl[2]	0	9.04×10^{-4}	0	98	
	8	8.96×10^{-4}	0.8 (5%)[4]	90	
	24	8.78×10^{-4}	2.9 (10%)	93	
	28	8.48×10^{-4}	6.2 (10%)	83	
$HgCl_2$[3]	0	6.09×10^{-3}	0		91.3
	4	5.94×10^{-3}	2.5 (10%)		89.8
	8	5.57×10^{-3}	8.5 (13%)		—
	24	4.86×10^{-3}	20.2 (40%)		87.8

[1]An average density of 5 amphipods 100 ml^{-1}.
[2]Specific activity of $CH_3{}^{203}HgCl$ was 1.06×10^6 cpm μg^{-1} Hg.
[3]Specific activity of $^{203}HgCl_2$ was 1.66×10^6 cpm μg^{-1} Hg.
[4]Values in parentheses show mercury loss from water in absence of amphipods. These results were obtained at CH_3HgCl conc. of 4.2×10^{-3} μg Hg ml^{-1} and $HgCl_2$ conc. of 2.3×10^{-3} μg Hg ml^{-1}, at 24°C in dechlorinated tap water (D.C. Mortimer, in verb.).

(1976) reported biotransformation rates ≤ 3%/day in lobsters and as high as 6%/day in rats.

The clearance times for both CH_3HgCl and $HgCl_2$ after consuming mercury-contaminated foods are reported in Table IV. The half life of CH_3HgCl ranged from 40 to 55 days. In contrast, $HgCl_2$ was cleared much more rapidly with a $t_{1/2}$ of only 6 to 14 days. These results agree with other studies showing that CH_3HgCl is excreted more slowly than $HgCl_2$ (Berlin & Ulberg 1963; Jarvenpaa et al. 1970; Smith et al. 1975). Clearance time of $HgCl_2$ has been found to be 25 days for crabs (Sloan et al. 1974) and 5 to 10 days for molluscs (Unlu et al. 1972).

Mercury Uptake from Water

The maintenance of essentially steady state conditions with respect to both organic and inorganic mercury concentration in perfusate solution during an exposure period of 2 hours is shown by the data in Table VI. The observed reduction in the mercury concentration of only 2 to 3% after 24 hours in the case of CH_3HgCl was in sharp contrast to the much greater concentration reduction of approximately 20% after 24 hours in the case of $HgCl_2$. This loss does not result from uptake by the amphipods. It can be observed in the absence of organisms as reported by Newton and Ellis (1974), who found that substantial amounts of $HgCl_2$ were lost at a concentration of 0.2 mg Hg l^{-1} and lower, and Dokiya et al. (1974), who also showed similar results. The results in Table VI also demonstrate that no significant methylation or demethylation of mercury in the exposure medium occurred during the exposure period.

The results in Table VII illustrate that the transfer coefficient (T_c) of about 12 h^{-1} for CH_3HgCl was independent of concentration over a 20-fold range. Studies

Table VII. Effect of concentration of CH_3HgCl_1 and $HgCl_2$ on uptake from dechlorinated water by *Hyalella azteca*.[1]

Chemical Form of Mercury	*Mercury Concentration in Exposure Medium* (*cpm ml-1*)	(*μg Hg ml-1*)	*Number of Amphipods*	*Uptake Rate* (*μg Hg/amphipod h-1*)	*Transfer Coefficient*[4] T_c (h^{-1})
CH_3HgCl[2]	2170	0.0008	10	0.88×10^{-4}	13.3±1.9
	17317	0.0060	10	4.26×10^{-4}	11.9±1.3
	40462	0.0140	9	15.99×10^{-4}	12.0±1.1
$HgCl_2$[3]	748	0.0005	8	0.42×10^{-4}	11.2±2.0
	7267	0.0050	6	3.45×10^{-4}	10.1±1.3
	76760	0.0500	7	26.00×10^{-4}	5.9±0.8

[1]Amphipods were exposed for 2 h at a water temperature of 19±1°C.
[2]Specific activity of $CH_3{}^{203}HgCl$ was 2.9×10^6 cpm μg^{-1} Hg.
[3]Specific activity of $^{203}HgCl_2$ was 1.5×10^6 cpm μg^{-1} Hg.
[4]Transfer coefficient values represent the weight of water, in grams, completely cleared of its mercury content by 1 g wet wt of amphipods in 1 h. Each value represents the mean± SE.

with fish of the same weight class also showed T_c to be indpendent of mercury concentration (de Freitas et al. 1974; de Freitas & Hart 1975). For 2-g fish (*Notemigonus crysoleucus*), T_c values were about 4 h^{-1} at methylmercury concentrations ranging from 1×10^{-5} μg Hg ml^{-1} to 1×10^{-3} μg Hg ml^{-1} (de Freitas et al. 1977). For $HgCl_2$, over a concentration range of 5×10^{-4} μg Hg ml^{-1} to 5×10^{-3} μg Hg ml^{-1}, T_c values for amphipods were similar at 11.2 and 10.1 h^{-1} respectively, but when the concentration of $HgCl_2$ was as high as 5×10^{-2} μg Hg ml^{-1} the value of the transfer coefficient dropped to 5.9 h^{-1}.

The concentration range, 1×10^{-4} to 1×10^{-3} μg Hg ml^{-1}, is realistic for many contaminated waters (Norstom et al. 1976). A concentration of 5×10^{-3} μg Hg ml^{-1} represents an extremely polluted enviornment (Gavis & Ferguson 1972). However, a $HgCl_2$ concentration of 5×10^{-2} μg Hg ml^{-1} is unrealistic for the natural environment and would likely be lethal to organisms exposed over a long period of time (Barnes & Stanburg 1948; Wisely & Blick 1967; Ray & Tripp 1976).

Direct uptake of chemicals from water involves a number of adsorption sites probably located over the amphipod's general integument, but uptake of mercury, particularly CH_3HgCl, into tissues may occur predominantly through the branchia, as was found with uptake of PCBs (Wildish & Zitko 1971). These authors also found uptake to be unaffected by the amphipod's stage of intermoult. Molts collected during the post-exposure clearance period did not contain significant amounts of ^{203}Hg, nor was there any detectable decrease in the body burden of ^{203}Hg as a result of molting. Mercury uptake due to drinking contaminated water should not be an important uptake vector as the drinking rate of *Gammarus duebeni*, for example, is less than 1 μl h^{-1} (Lockwood & Andrews 1969), and at a methylmercury concentration of 8×10^{-4} μg ml^{-1} the resulting uptake rate would only be 8×10^{-7} μg Hg/amphipod h^{-1} at a drinking rate of 1 μl h^{-1}, extremely small compared to the observed uptake rate of 4×10^{-5} μg Hg/amphipod h^{-1} as reported in Table VII.

Table VIII. Effect of temperature on uptake of CH_3HgCl from dechlorinated tap water by *Hyalella azteca*.[1]

CH_3HgCl[2] Concentration in Water (μg Hg ml^{-1})	Water Temperature (°C)	Number of Amphipods	Total Uptake Rate (μg Hg/amphipod h^{-1})	Transfer Coefficient[4] into Slow-clearing Compartment (h^{-1})
0.047	20	8	5.88×10^{-3}	10.8±1.0
0.053	10	8	3.31×10^{-3}	6.7±0.9
0.045	5	9	1.71×10^{-3}	3.5±0.6

[1]Amphipods were exposed for 2 h.
[2]Specific activity of $CH_3^{203}HgCl$ was 2.74×10^6 cpm μg^{-1} Hg.
[3]Acclimation of organisms was achieved by a 1°C da^{-1} drop in aquarium temperature, followed by a holding period of 1 wk prior to testing.
[4]Transfer coefficient values represent the weight of water, in grams, completely cleared of its mercury content by 1 g of amphipods in 1 h. Each value represents the mean±SE.

Our results on the effect of temperature on uptake from water presented in Table VIII, show that the transfer coefficient decreased from about 11 h^{-1} to 4 h^{-1} at 20°C and 5°C respectively. These results suggest that uptake is directly related to metabolic rate of the amphipod. Similar results on the effect of temperature have been reported for *Daphnia magna* (Huckabee et al. 1975; Trudel 1980), various species of fish (Murphy & Murphy 1971; MacLeod & Pessah 1973; Reinert et al. 1974; Ruohtula & Miettinen 1975; de Freitas et al. 1977) and mollusks (Pringle et al. 1968). However, Smith (Smith et al. 1975) concluded that temperature had no effect on direct uptake of mercury from water by clams. This conclusion may not be valid, as exposure of clams to a food-free medium results in a marked reduction in their filtration rate and a consequent reduction in uptake rate would be expected.

In the present study, 2 sources of water were used: dechlorinated tap water and unfiltered Ottawa River surface water. For both CH_3HgCl and $HgCl_2$, the transfer coefficient for mercury from water to the amphipod was lower in the unfiltered river water (Table IX). The importance of water quality on mercury bioaccumulation is well documented (Johnels et al. 1967; Nuorteva & Hasanen 1971; Karbe et al. 1975), but the type of field data reported in these studies cannot be used to distinguish between food and water vectors or clearance. Results from laboratory studies on fish (deFreitas 1977) show an enhancement of 2- to 3-fold in assimilation efficiency of methylmercury from Ottawa River water depending on season. Also, using filtered river water, the transfer coefficient from water to fish for methylmercury was increased by 40% compared to unfiltered water, similar to our present results with amphipods, as presented in Table IX. As applied to natural systems, our results suggest that transfer coefficient values can be assumed to fall in the range of 2.5 h^{-1} to 7 h^{-1} for CH_3HgCl and about 50% lower for $HgCl_2$ as shown in Table IX. Transfer coefficient values probably fluctuate to an even greater extent in natural environments due to the combined effects of seasonal changes in water quality parameters and temperature related changes in metabolic rate.

Table IX. Effect of water quality on direct uptake of CH_3HgCl and $HgCl_2$ from water by *Hyalella azteca*.[1]

Water Type	Chemical Form of Mercury	Mercury Concentration in Exposure Medium (μg Hg ml^{-1})	Number of Amphipods	Total Uptake Rate (μg Hg/amphipod h^{-1})	Transfer Coefficient[4] T_C (h^{-1})
Dechlorinated tap water	CH_3HgCl[2]	0.0003	8	7.0×10^{-5}	21.7 ± 1.6
Ottawa River water		0.0005	8	4.0×10^{-5}	6.8 ± 0.9
Dechlorinated tap water	CH_3HgCl	0.0580	10	1.4×10^{-3}	2.4 ± 0.5
Ottawa River water		0.0350	9	0.6×10^{-3}	1.5 ± 0.3
Dechlorinated tap water	$HgCl_2$[3]	0.0060	8	8.0×10^{-4}	11.1 ± 1.2
Ottawa River water		0.0070	10	3.0×10^{-4}	3.9 ± 0.5
Dechlorinated tap water	$HgCl_2$	0.3300	8	1.0×10^{-2}	2.8 ± 0.1
Ottawa River water		0.3700	8	0.6×10^{-2}	1.5 ± 0.4

[1] Amphipods were exposed for 2 h at a water temperature of 19±1°C.
[2] Specific activity of $CH_3{}^{203}HgCl$ was 2.16×10^6 cpm μg^{-1} Hg.
[3] Specific activity of $^{203}HgCl_2$ was 1.8×10^6 cpm μg^{-1} Hg.
[4] Transfer coefficient values represent the weight of water in grams, completely cleared of its mercury content by 1 g of amphipods in 1 h. Each value represents the mean ±SE.

It should be noted that our results in uptake from water and release of mercury by *Hyalella azteca* are in general agreement with corresponding values for *Daphnia magna* and various fish species, when differences in body weight are taken into account as shown for fish by deFreitas and Hart (1975). For example, transfer coefficient values, using CH_3HgCl, for *D. magna* (wet weight 0.1 mg) range from 100 to 300 h^{-1} (Huckabee et al. 1975; Trudel 1979). *Hyalella azteca* (wet weight 5.0 mg) has T_C values ranging from 8 to 25 h^{-1}. Studies on fish, *Notemigonus crysoleucus* and *Catostomus commensoni*, show T_C values of 4.4 h^{-1} for a 2-g fish, 3.0 h^{-1} for a 10-g fish and 1.7 h^{-1} for a 100-g fish (de Freitas 1977).

The efficiency of mercury uptake from water can be usefully compared to oxygen uptake by organisms (de Freitas 1974: Norstrom et al. 1976). Assuming oxygen uptake from water to be 100% efficient and an oxygen concentration of 9 μg ml^{-1} water, then *Hyalella* would remove the oxygen from 133 ml water/g amphipod h^{-1} (a T_C for 0_2 of 133 h^{-1}). With a transfer coefficient value for methylmercury of 20 h^{-1} (20 ml water/g wet weight h^{-1}), uptake of mercury from water would be 15% (20/133) as efficient as oxygen uptake. This value agrees with other studies as reviewed by Norstrom et al. (1976), and more recently by de Freitas (1977) and Phillips and Buhler (1978) who found mercury uptake by fish to be 15 to 30% as efficient as oxygen uptake.

Our results quantitatively define the bioaccumulation potential of both methylmercury and inorganic mercury in terms of three parameters: (1) efficiency of uptake from food, (2) efficiency of uptake from water and (3) elimination or fractional clearance from the whole body. The following discussion illustrates the use of these results in predicting the probable magnitude of mercury uptake via the food and water vectors in a natural environment like the Ottawa River.

The magnitude of the water vector and food vector can be calculated as follows: at a methylmercury concentration in water of 5 ng Hg l^{-1} (20% of total Hg concentration in water of 25 ng l^{-1}) and a T_c value of 20 h^{-1}, *Hyalella azteca* would accumulate 2.4 ng methylmercury/g amphipod da^{-1} by direct uptake from water. The concentration of total mercury in sediments from shallow areas of the Ottawa River in 1976 was 0.024 μg Hg g^{-1} (wet weight of sediment), of which 0.0024 μg Hg g^{-1} was proabably methylmercury (Miller et al. 1977). At an ingestion rate of 0.003 g wet weight of sediment/hour/amphipod (50% body wt/da) and an assimilation efficiency from the gastrointestinal tract of ~0.8, uptake of methylmercury via the food vector should be ~1.0 ng Hg/g amphipod da^{-1}. Under these conditions, the food vector would account for considerably less than 50% of the total uptake of methylmercury, ~3.4 ng Hg/amphipod da^{-1}. A continuous uptake of methylmercury at a constant rate of ~3.4 ng Hg/g amphipod da^{-1} (value for food and water uptake) will equal loss rate from the organism when its mercury body burden is ~230 ng Hg/g amphipod based on a fractional clearance rate of methylmercury from body tissues of 1.5% per day. This means that the concentration of methylmercury in *H. azteca* should approach an equilibrium value of about 0.23 μg Hg g^{-1} wet weight of tissue after about 4 months of exposure.

Corresponding calculations for uptake of inorganic mercury result in water and food vectors of about 5 ng Hg and 1 ng Hg/g organism/day respectively. Inorganic mercury is cleared from body tissues at about 4%/day, a much faster rate than methylmercury, and the concentration of inorganic mercury in *Hyalella azteca* should approach an equilibrium value of about 150 ng Hg g^{-1} amphipod after about 2 months of exposure.

These calculations do not take into account growth during this period. Mercury body burdens in amphipods would thus be somewhat lower than calculated above, owing to growth dilution. Table X shows actual field values for the mercury content in amphipods and other benthic invertebrates on a ppm dry-weight basis. The above predictions agree quite well with these field samples. It should be noted that the values for methylmercury in water and sediments used in the above example are close to levels observed in the Ottawa River and many other areas of Canada (Miller et al. 1977). It should also be stressed that this simple treatment of the uptake process can be used to distinguish between food and water uptake only when the concentration of mercury in the water or diet is known. The amphipod example used here demonstrates that the water vector is probably of equal or greater magnitude than the food vector, particularly in areas with low "background-type" contamination levels. One can speculate that the relative magnitude of the water vector will decrease during periods of decreasing mercury pollution and conversely, in areas of increasing pollution, the relative magnitude of the water vector will increase. If the water vector does in fact account for 50% or more of the mercury taken up by *Hyalella azteca*, then the hypothesis that biological magnification in aquatic environments is controlled by mass transfer of trace substances through the food web may have only limited application to the problem of high mercury levels in aquatic organisms. However, some degree of biomagnification in the benthic food web is virtually

Table X. Levels of total mercury in amphipods and other benthic invertebrates found in their natural environment.

Mercury Concentration (μg Hg g^{-1} dry wt.)	Location of Amphipod Collection	Date	Reference
2.16	Kettle Island Bay, Ottawa River	April 1977	Present study
2.75		July 1977	
0.29	Upstream, Upper Duck Island Ottawa River	June 1976	Miller et al. 1976
25.00	Downstream, Upper Duck Island	July 1976	
5.22	Lake Temiscaming, Quebec	May 1976	
0.05 - 0.07[1]	above mill, Sweden		Johnels et al. (1976)[1]
1.90 - 17.0	below mill, Sweden		

[1]This study does not specify the species of benthic invertebrates studied.

assured in the transfer of methylmercury from prey to predator, solely on the basis of its high assimilation efficiency of 80%, even in the case of a rapidly growing predator in which 10-20% of ingested calories are deposited as new tissue. In this context, it is obvious that a food conversion efficiency for growth of 10%, for example, precludes the food-chain biomagnification of any compound including inorganic mercury whose assimilation efficiency is $\leq$10%, regardless of how slowly it is eliminated from body tissues.

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