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MERCURY ACCUMULATION IN THE BURROWING MAYFLY *HEXAGENIA RIGIDA* (EPHEMEROPTERA) EXPOSED TO CH_3HgCl OR $HgCl_2$ IN WATER AND SEDIMENT

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Abstract—The accumulation of Hg and its distribution within nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) were investigated experimentally by exposure of nymphs to radiolabelled Hg in laboratory microcosms containing water and sediment. Mercury was introduced into the experimental units either in the overlying water (twice-daily additions) or in the sediment (single addition prior to introduction of the nymphs). At the whole animal level, Hg accumulation varied according to the chemical form of the Hg added and the contamination source. When added in an organic form (CH_3HgCl), Hg was accumulated to a greater extent than when added in an inorganic form ($HgCl_2$); the ratio of accumulation between the two compounds exceeded 60 (organic/inorganic exposure) when Hg was introduced via the sediment but was only about 2 when Hg was added to the overlying water. The distribution of Hg among the various body parts of the nymphs indicated that the Hg burdens in two major target organs—the gills and the gut—depended strongly on the initial contamination source. When Hg was added via the sediment, the contribution of the gut to the total body burden (43% for inorganic Hg, 18% for methyl-Hg) was much greater than that of the gills (3 and 5%, respectively). In contrast, when Hg was added to the overlying water, the contribution of the gills to the total body burden (49% for inorganic Hg, 20% for methyl-Hg) exceeded that of the gut (8 and 17%, for the two forms, respectively).

Key words—contamination, *Hexagenia rigida*, burrowing mayfly, mercuric chloride, methylmercury, microcosm, ^{203}Hg, bioaccumulation, gill and gut barriers

INTRODUCTION

Nymphs of the burrowing mayfly *Hexagenia rigida* have been used to study processes associated with the bioaccumulation and trophic transfer of Hg in freshwater benthic systems (e.g. Saouter, 1990; Saouter *et al.*, 1991b, c). These insects construct burrows through which they pump water from the sediment surface and in which they feed on bulk sediment (Zimmerman *et al.*, 1975). There are thus two potential routes by which nymphs can take up Hg; via the gut wall from fine-grained sediment consumed as food and via the gills from the oxygenated water used to irrigate their burrows. The proportion of accumulated Hg entering via these two routes appears to depend strongly on the contamination regime, i.e. the degree of Hg contamination in both the water and sediment and the chemical forms and species of Hg present (Saouter *et al.*, 1991b). Accumulation also depends on the structural and functional features of the biological barriers in direct contact with the external environment (Boudou *et al.*, 1991).

In this paper, we present the results of a study designed to measure the distribution of Hg in *H. rigida* nymphs after a 9-day exposure to radiolabelled Hg in laboratory microcosms containing both water and sediment. Mercury was introduced into the experimental units either in the overlying water (twice-daily additions) or in the sediment (single addition prior to introduction of the nymphs). This experimental design was used because *H. rigida* nymphs do not survive for appreciable periods of time in the absence of sediment, which serves as both cover and food for these insects. The concentration and distribution of Hg in the exposure media were monitored to define the contamination conditions to which the insects were subjected. Because there was some movement of the added Hg between the sediment and water compartments, the present study could not differentiate clearly between direct uptake (from water) and uptake via the trophic route (ingestion). The results do, however, provide an estimate of the relative importance of these two routes.

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MATERIALS AND METHODS

Preparation of experimental units

Each experimental unit consisted of a transparent cylindrical plastic container (20 cm high, 12 cm diameter) containing 5 cm of sediment, 1.5 l. of spring water (Ca 51.0 mg l^{-1}, Mg 2.2 mg l^{-1}, HCO_3^- 161 mg l^{-1}, pH 7.2) and eight *H. rigida* nymphs (background Hg concentration of 0.109 μg g^{-1}, wet weight). The sediment was from the Garonne river, upstream from Bordeaux, France. It was rich in clays (75–80%), had a low total organic carbon content (2%) and was 50% water by weight. The Hg concentration of the sediment was 0.12 ± 0.01 (SD) mg Hg kg^{-1}, wet weight. A large quantity of sediment was homogenized by mechanical mixing and divided into three batches. Mercury was added to two of the three batches to study uptake from sediment. No Hg was added to the third batch, which was used to study Hg uptake from water. The two batches of sediment were enriched with both non-radioactive Hg and radioactive ^{203}Hg as $HgCl_2$ (Dupont, Canada) or CH_3HgCl (Amersham, U.K.). The final Hg concentration in the sediment was 0.5 mg Hg kg^{-1} (wet weight) for each compound, with a specific activity of 24.6 mCi mg Hg^{-1} for CH_3HgCl and 49.5 mCi mg Hg^{-1} for $HgCl_2$. After homogenization, several sediment samples were taken from each batch to check contamination levels and Hg distribution throughout the substratum. For contamination by water, inorganic or organic Hg was added twice daily in the water column (2 ml of CH_3HgCl or $HgCl_2$ aqueous solutions which corresponds to 30 μg Hg day^{-1} with final specific activities of 24.6 mCi mg Hg^{-1} for CH_3HgCl and 4 mCi mg Hg^{-1} for $HgCl_2$). This contamination method ensured that experimental conditions could be well replicated from one experimental unit to another, but it caused a progressive increase in Hg concentrations in the water column during the experiment (Ribeyre, 1988).

The temperature was held at 22 ± 0.3°C (SD) and the photoperiod was 8 h/16 h (fluorescent light). The pH of the overlying water was not controlled, but varied little (pH 7.1 ± 0.2, SD). Two replicates were set up for each experimental condition, giving a total of eight experimental units in all. The duration of the experiment was 9 days. Controls without Hg were not run since previous experiments had shown that normal nymph growth was consistently achieved in the experimental system used (Saouter *et al.*, 1991b, c; Saouter 1990).

Rearing of nymphs and introduction into experimental units

Mass culture of *H. rigida* nymphs was initiated in the laboratory from eggs collected each summer from Lake Winnipeg (Freshwater Institute, Winnipeg, Canada) and stored at +4°C. The culture technique was based on a previously published method (Friesen, 1982) that was adapted for use in our laboratory (Saouter *et al.*, 1991c).

Nymphs (about 6 months old) were introduced 10 days after water and sediment had been added to the experimental units in order to provide sufficient time for conditions to stabilize. For units contaminated via the water column, additions of Hg were initiated several hours after introduction of the nymphs. No external food supply was introduced during the experiment since the sediment used contained sufficient organic matter to ensure the survival and growth of the nymphs for several months (Saouter, 1990). To reduce differences in nymph weights among experimental units, nymphs were grouped into several weight classes and one nymph from each class was introduced into each unit. The mean nymph biomass in individual units at the beginning of the experiment was 61.2 ± 8.5 (SD) mg, wet weight, with an inter-unit coefficient of variation of less than 3%.

Sample collection

After the 9-day exposure, nymphs were sieved from the sediment and individually weighted. Gut contents were replaced by clean sediment by placing nymphs in beakers containing untreated sediment for 6 h (Saouter, 1990).

After removal from the beakers, each organism was placed in a small amount of deionized water, and an incision was made between the thorax and head to sever the gut and immobilize the nymph (Hare *et al.*, 1989, 1991b). Gills, head and legs were removed, and the abdomen was opened ventrally to remove the mesenteron (midgut), proctodeum (hindgut), Malpighian tubules and the rest of the body (hereinafter referred to as "body"). In *H. rigida*, the stomodeal part (foregut) was very difficult to retrieve; it is only a few tenths of a millimetre long, in the foremost part of the gut just behind the buccal cavity (Saouter *et al.*, 1991a). The dissection liquid, including deionized water, haemolymph and organ fragments, was also collected. Nymph parts from two animals were combined to obtain sufficient quantities of tissue. After dissection, animal parts were placed into 10-ml polystyrene test tubes. Each sample was then digested in 5 ml of concentrated nitric acid (Aristar) for 24 h at room temperature, to ensure good homogenization prior to determination of its radioactivity in a gamma particle counter. The various biological samples were not weighed and results were thus expressed as Hg burdens (ng Hg) and relative burden (% of Hg found in an organ as compared with total Hg accumulated). The weights of the gills and gut, determined during earlier experiments (Hare *et al.*, 1991b; Saouter, 1990), were 6.3 and 6.7%, respectively, of the nymphs' fresh weight.

Throughout the experiment, water samples were collected from the overlying water in all units and were either counted whole or after filtration through Nylon membranes (0.45 μm, Naglene filters). On day 7 additional samples were collected to assess the spatial distribution of Hg in the water compartment and its rate of mixing. Discrete samples (5 ml) were taken from the surface, middle and bottom of the water column in two experimental units (one contaminated with $HgCl_2$, the other with CH_3HgCl; three samples for each level); samples were collected before and after the day-7 addition of Hg to the water column.

At the end of the experiment, but before the organisms were removed, three glass tubes (inner diameter 0.8 cm) were pushed gently into the sediments contaminated via the water source to assess the vertical distribution of Hg (as ^{203}Hg) in the sediment. The cores were stored at −20°C prior to counting. Mercury was determined on five strata from each core: the first four were each 5 mm thick, the uppermost stratum being in direct contact with the water; the average thickness of the fifth stratum was 3 cm. Each sub-sample was partially digested in 5 ml of concentrated nitric acid (24 h at room temperature) and then counted.

Statistical analyses were conducted by multivariate analysis of variance to determine the action and interaction of controlled factors (Systat, 1992).

Mercury analysis

Radioactive Hg was measured in a gamma particle counter [LKB Wallac 1282 Compugamma, NaI(Tl) well type]. Adjustment was made for radioactive decay (47 days for ^{203}Hg) and for background radiation. Counting times were fixed to obtain a relative counting error (standard deviation, SD) of less than 2%. Estimates of background radiation, as determined from counts of 20 blank samples, were deducted from sample counts. All samples exceeded background values and only net values exceeding the mean blank plus 2 SD ($\alpha = 0.05$) were retained. Counting efficiency (60% for Hg) was determined from standards of known radioactivity and used to convert counts per minute (cpm) values to disintegrations per minute (dpm), which were then converted to ng or mg of Hg per sample.

RESULTS

Characterization of the exposure conditions

Contamination via sediment. Mercury determinations in sediment subsamples collected before introduction of the sediments into the units showed a homogeneous distribution of the metal. Final concentrations in sediment were 0.55 ± 0.09 (SD) mg Hg kg^{-1} (wet weight) for $HgCl_2$ and 0.48 ± 0.07 (SD) mg Hg kg^{-1} (wet weight) for CH_3HgCl.

During the experiment, some of the Hg introduced into the sediments was transferred to the water column due to the burrowing activity of the nymphs (bioturbation, bioirrigation) and to diffusion of the metal from the interstitial water to the water column. Waterborne Hg concentrations were greater in units containing sediments treated with organic Hg than in those containing sediments treated with inorganic Hg (significant difference at $P = 0.001$) (Fig. 1). Total Hg concentrations in the overlying unfiltered water (i.e. dissolved + particulate) tended to increase during the experiment, though in the case of CH_3HgCl a certain stabilization was apparent after 4 days. Although Hg concentrations in filtered water were consistently below the detection limit ($0.02\ \mu g\ l^{-1}$), dissolved Hg could have constituted a secondary source of contamination for the organisms in these systems.

Contamination via water. In units to which Hg was added to the water column, waterborne concentrations of total Hg increased over the first few days ($P < 0.001$), as anticipated from the experimental design (twice-daily additions); concentrations then stabilized or decreased slightly (Fig. 2). After 2 days, total Hg concentrations were almost 1.5 times higher in the units contaminated with organic mercury, but towards the end of the experiment levels were similar for both compounds ($P = 0.001$). Differences between waterborne concentrations of the two forms of Hg were greater for dissolved Hg. After 2 days, 93% of the Hg in the water column was in dissolved form in the experimental units contaminated with organic mercury, compared with only 33% for units contaminated with inorganic Hg. At the end of the 9-day exposure period, the water was very turbid, due to the burrowing activity of the nymphs; the percentages of dissolved metal were then no more than 33 and 16% for organic and inorganic Hg, respectively. However, exposure to Hg still remained greater in the units contaminated with the organic form, whether total or dissolved mercury is considered.

The spatial distribution of Hg in the water columns was found to be homogeneous for the two sampling times studied in a single cycle (30 min and 17 h after Hg addition). Mercury rapidly dispersed throughout the water column after addition of the 2 ml solution ($HgCl_2$ or CH_3HgCl) and 30 min later no significant difference was evident among any of the nine sampling points. While this homogeneity in concentrations was observed for the two sampling times, concentrations had decreased by about 50–60% from their initial levels during the intervening 16.5 h.

Because some of the Hg added to the water column in dissolved form became associated with particles, a proportion settled out to become a part of the sediment compartment in the experimental units. Analyses of sediment strata showed that 95% of this sedimentary Hg was in the uppermost 1 cm of the sediment (Fig. 3). The average concentration of added Hg in the upper layer (0.5-cm) was 5.95 ± 1.2 (SD) mg Hg kg^{-1} (dry weight). Below this layer, the concentrations decreased markedly: 1.5 ± 0.23 (SD) mg Hg kg^{-1} in the second layer, 0.13 ± 0.01

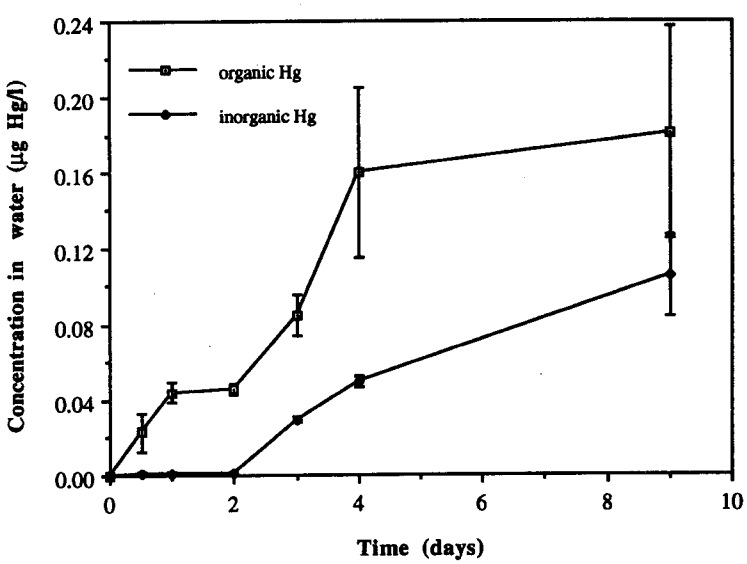

Fig. 1. Changes over time in the mean concentration (±SD) of total Hg in the water column of the experimental units used to assess contamination via sediment contaminated by $HgCl_2$ and CH_3HgCl.

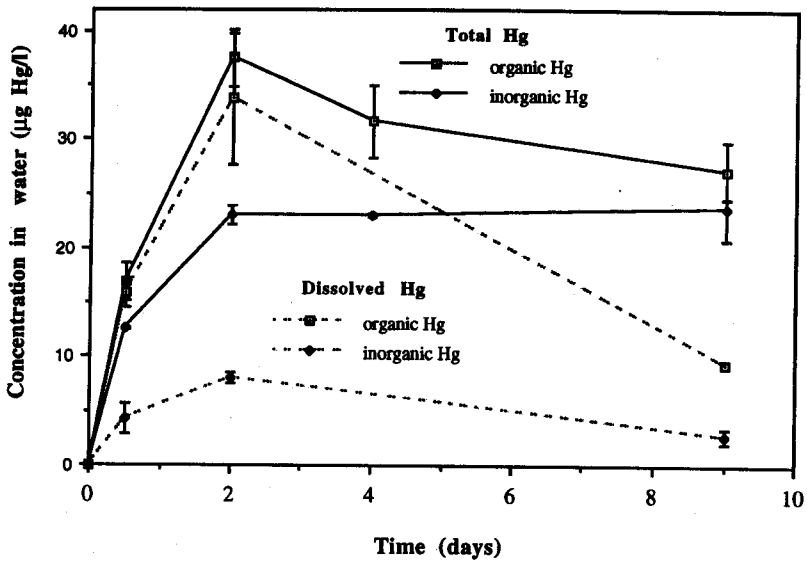

Fig. 2. Changes over time in the mean concentration (±SD) of total and dissolved Hg in the water column of the experimental units used to assess contamination via water by $HgCl_2$ and CH_3HgCl.

(SD) mg Hg kg^{-1} in the third, and for the two last strata, 0.035 ± 0.003 (SD) mg Hg kg^{-1}. As the upper sediment layers consisted mainly of redeposited material associated with bioturbation, we can assume that under our experimental conditions and for the period studied, there was little transfer of the added mercury from the overlying water column to the lower sedimentary layers. Consequently, although Hg in the surface sedimentary layers may have constituted a supplementary contamination source for *H. rigida* nymphs, this should have been minimal since the nymphs live mainly below the uppermost cm (up to 10 cm deep in a pond) and come into contact with the surface of the sediments only when they leave the sediments (once or twice every 48 h, visual observations, E. Saouter).

Mercury accumulation in Hexagenia rigida nymphs

The accumulation of mercury by *H. rigida* nymphs was influenced by the chemical form of Hg ($P = 0.01$) and the contamination source (sediment vs water) ($P = 0.002$). Nymphs exposed to treated sediment accumulated less Hg than those exposed to the treated water; in both water and sediment exposures,

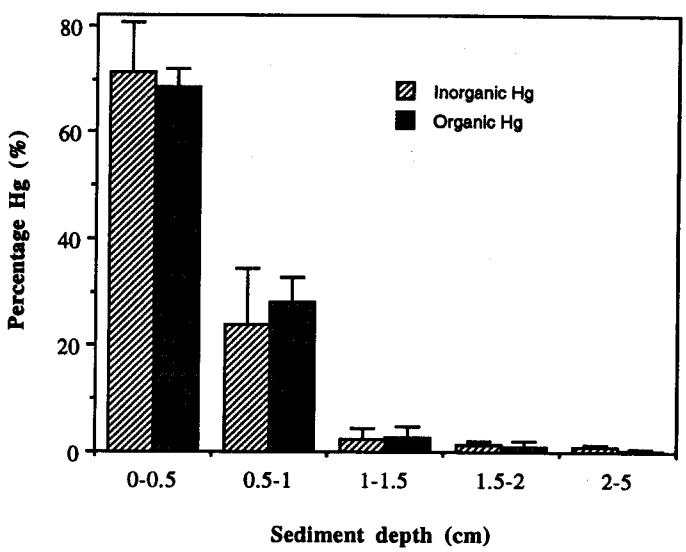

Fig. 3. Depth distribution of sediment-associated ^{203}Hg as a percentage (±SD) of total ^{203}Hg measured in all sediment strata (0 cm = sediment surface). Sediment cores were collected from experimental units where Hg had been added to the overlying water.

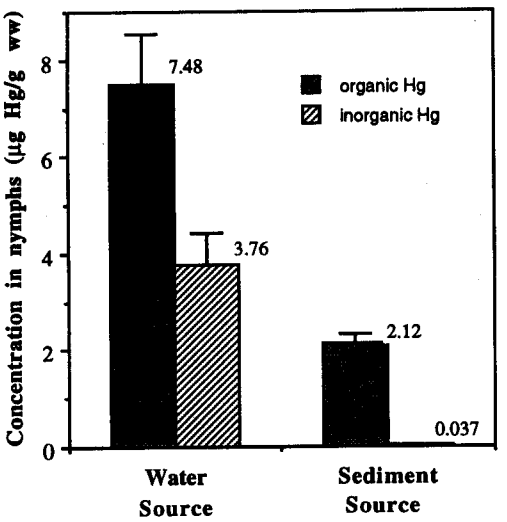

Fig. 4. Mean concentrations (±SD) of total Hg in nymphs of *Hexagenia rigida* (µg Hg g^{-1}; wet weight) in relation to the chemical form and source of Hg.

Hg was accumulated to a greater extent when added in an organic rather than an inorganic form (Fig. 4). The differences in accumulation between the two forms of Hg were especially marked for the sediment treatment. Concentration factors (CF) between Hg in nymphs and Hg in the environment suggests that Hg was assimilated more efficiently from the water (CFs of 180 for $HgCl_2$ and 380 for CH_3HgCl, based on average Hg concentrations in water) than from the sediment (CFs of 0.08 for $HgCl_2$ and 4.0 for CH_3HgCl, based on initial sediment concentrations). Calculated from previous experiments (Saouter, 1990) CF for control microcosms were close to 0.9 after several months in contact with untreated sediment.

Contamination of nymphs by Hg did not influence their growth; the average weight increase during the 9-day experiment was 5.8 ± 1.6 (SD) mg (wet weight), similar to that observed in previous studies in the absence of Hg (Saouter *et al.*, 1991c).

The distribution of Hg within the nymphs was strongly influenced by contamination conditions (Fig. 5). In the experiments with Hg-treated sediment, the gut (mesenteron and proctodeum) contained a much greater proportion of the total accumulated Hg, i.e. from 43 (inorganic Hg added) to 18% (organic Hg added), than did the gills, which contained from 3 to 5% of the accumulated Hg (inorganic and organic Hg, respectively). When exposure was via water, this pattern with the two groups of body parts was reversed: the gills contained 49% of the Hg after exposure to inorganic Hg, as against

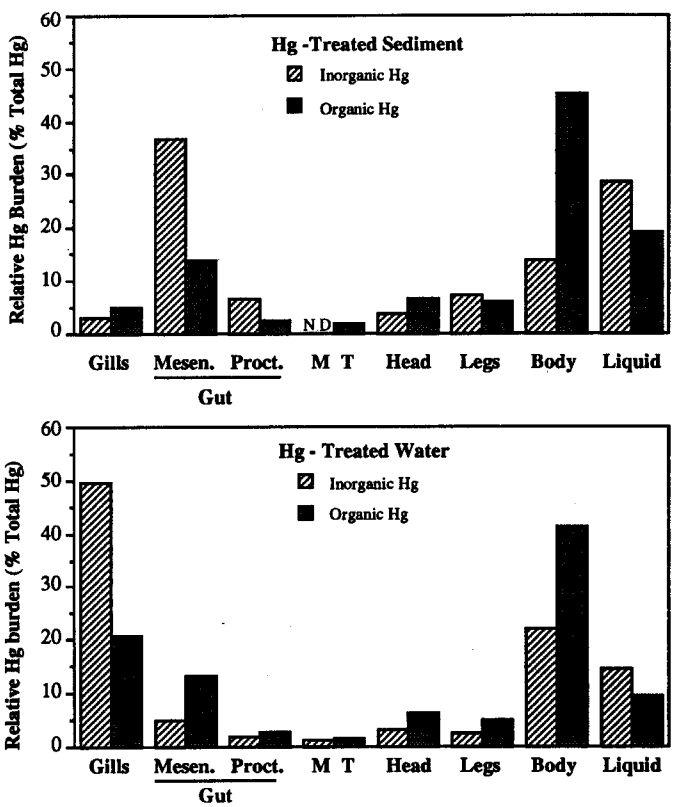

Fig. 5. Percentage of Hg in different parts of *Hexagenia rigida* nymphs in relation to the source of contamination and chemical form of Hg. Abbreviations: mesen. = mesenteron; proct. = proctodeum; MT = Malpighian tubules; ND = non-detectable.

Table 1. Mean Hg content (ng Hg ± SD, $n = 8$) in the various parts and in whole nymphs of *Hexagenia rigida* and the mean wet weight (ww) of nymphs in each of the EUs. The ratio, *R*, of the quantity of Hg in nymphs from EUs contaminated by organic Hg over that in nymphs from EUs contaminated by inorganic Hg is also given

	Hg-treated sediment			Hg-treated water		
	CH_3HgCl (ng Hg ± SD)	$HgCl_2$ (ng Hg ± SD)	*R*	CH_3HgCl (ng Hg ± SD)	$HgCl_2$ (ng Hg ± SD)	*R*
Parts of nymphs						
Gills	7.3 ± 1.8	0.07 ± 0.03	104	102.8 ± 45.9	133.8 ± 31.1	0.7
Mesenteron	19.8 ± 7.0	0.86 ± 0.97	23	65.8 ± 33.4	13.6 ± 7.2	4.8
Proctodeum	3.7 ± 1.3	0.15 ± 0.21	25	13.6 ± 5.0	4.6 ± 1.9	2.9
Malpighian tubules	2.5 ± 1.9	N.D.	—	7.4 ± 2.5	3.5 ± 1.9	2.1
Head	9.5 ± 1.8	0.09 ± 0.03	106	31.1 ± 11.7	8.3 ± 2.2	3.7
Legs	8.5 ± 2.7	0.17 ± 0.05	50	25.6 ± 7.6	6.8 ± 0.8	3.7
Rest of the body	65.3 ± 27.3	0.32 ± 0.04	204	205.1 ± 107.7	58.7 ± 8.5	3.4
Liquid	27.6 ± 16.8	0.67 ± 0.28	41	46.5 ± 18.1	39.2 ± 6.3	1.1
Whole nymphs						
Hg (ng)	144.4 ± 41.6	2.34 ± 0.85	$R = 61$	498.3 ± 74.7	268.8 ± 53.7	$R = 1.8$
Weight (mg)	68.1 ± 10.2	62.4 ± 6.14		66.5 ± 8.6	71.4 ± 13.7	

20% after exposure to organic Hg, whereas the gut contained 8 and 17%, respectively, of the total Hg.

Mercury accumulation in the compartment corresponding to the tissues remaining after dissection (skin, adipose tissue, nervous system, tracheal system, etc.), expressed as a relative burden, appeared to be independent of the mercury source (sediment vs water), but was affected by the chemical form of Hg (Fig. 5). This compartment contained 13–21% (for sediment and water sources, respectively) of the total Hg accumulated by the nymphs after exposure to inorganic Hg, as opposed to 41–45% after exposure to organic Hg.

When Hg accumulation in a given animal part was expressed as a burden (ng Hg per part), values were always higher for the organic than the inorganic form. These differences were especially great for nymphs from the Hg-treated sediments, e.g. amounts of metal in the gills and in the body were 100 and 200 times greater, respectively, after exposure to organic than to inorganic Hg (Table 1). When Hg exposure was via the water, differences in metal burdens were much smaller; the accumulation ratios between the organic and inorganic forms ranged from 0.7 (gills) to 4.8 (mesenteron).

Accumulation of Hg in the mesenteron was always greater than in the proctodeal part of the digestive tract (Table 1). However, since the mesenteron is up to 3 or 4 times heavier than the proctodeum, Hg concentrations in these two parts of the gut are probably similar, despite the different physiological structures and functions. The quantity of Hg present in the Malpighian tubules was comparable to that in the proctodeal part of the gut; given its small weight (<2% of total body wt; Hare *et al.*, 1991a), Hg concentrations in this organ must be very high.

Two compartments were influenced little by contamination conditions: the head and legs. They contained between 2 and 7% of the average Hg burdens accumulated in the whole organisms. Fairly large quantities of Hg were present in the dissection liquid, as much as 28% of the total Hg accumulated by the nymphs (Table 1). In an earlier study, in which only the gills and gut were sampled, the dissection liquid contained on average only 5% of the total Hg accumulated (Saouter, 1990). Since a larger number of biological compartments were sampled in the present study, the dissection times were longer (about 20 min) and thus more material may have been lost as tissue fragments.

DISCUSSION

Mercury in whole nymphs

At the whole animal level, metal accumulation in nymphs was always greater when Hg was introduced in the organic form. However, capacities for transfer between the exposure medium and the organisms were strongly influenced by the initial contamination source—water column or sediment. For the sediment source, differences in accumulation between the two compounds exceeded a factor of 60, in favour of experimental units contaminated with organic Hg, but these differences were only 1.8 for the water source. Similar results have been obtained in previous studies with the same experimental model (Saouter, 1990; Saouter *et al.*, 1991c).

The Hg forms initially introduced into the units were subject to various possible transformations (methylation, demethylation, reduction, volatilization), notably as a result of microbiological activity (Oremland *et al.*, 1991). Preliminary results from experiments based on the determination of inorganic and organic Hg in the sediment (Saouter *et al.*, in preparation) indicate that less than 1% of the added $HgCl_2$ was methylated. In contrast, appreciable demethylation may have occurred (up to 70% in the anoxic layer and in the units where the initial contamination was via the sediment). These results suggest that the differences in accumulation between inorganic and methyl-Hg by *H. rigida* were probably underestimated in the present experiments.

The preferential bioaccumulation of organic Hg, relative to inorganic Hg, has become a well-known

phenomenon, as shown in experimental studies with these two compounds and various aquatic taxa (crustacea, zooplankton, molluscs, fish, etc.) (Boudou and Ribeyre, 1985; Cunningham and Tripp, 1975; King and Davies, 1987). One factor that is often suggested to explain these differences in accumulation is the difference in liposolubility between the two chemical forms of the metal, which affects the metal's ability to cross biological membranes separating the organisms from their environment. Although this factor cannot be refuted, it should, however, be qualified. In fact, measurements of the *n*-octanol–water distribution coefficient (D) show that the difference between the two compounds is small: $D = 2.54$ for CH_3HgCl and 0.61 for $HgCl_2$ (Halbach, 1985). Solubility studies also indicate that the lipid–water partition coefficients for methylmercury are small (<2). In effect, it seems that enhanced diffusion across biological membranes, rather than liposolubility *per se*, is responsible for the preponderance of methyl-Hg bioaccumulation (Lakowicz and Anderson, 1980).

Estimates of excretion or decontamination kinetics also show major differences between the inorganic and organic forms of the metal. Biological half-lives are generally much shorter for the inorganic form than for methyl Hg. Because net bioaccumulation depends on both uptake and excretion kinetics, this more rapid elimination of inorganic Hg will also contribute to a preferential bioaccumulation of the organic form (Cunningham and Tripp, 1975).

Although these observations on the relative rates of Hg uptake and excretion are consistent with our results, they do not alone seem sufficient to explain the very large differences in bioaccumulation observed between organic and inorganic mercury when the metal was introduced into the sediment compartment (factor of >60 favouring methyl-Hg). Differences in the partitioning of organic and inorganic Hg between water and sediment would also seem to play a major contributing role. In effect, with comparable total mercury additions to the sediment, levels of dissolved Hg in the water column (and presumably in the interstitial water) were always greater when the experimental units were initially contaminated with organic Hg. Hence aqueous exposure was greater with CH_3HgCl than with $HgCl_2$, favouring Hg transfer to the organisms.

Mercury in nymph parts

The distribution of Hg among body parts was strongly affected by the mode of exposure (Fig. 5), the gills and the gut being the most sensitive organs. The marked differences in Hg distribution between the two exposure regimes suggest that appreciable internal transfer (e.g. from gills to the gut) did not occur during the 9 days exposure (a longer exposure could have changed the observed pattern). If appreciable inter-organ transfer of Hg had occurred, much smaller differences would presumably have been observed. When mercury was added to the overlying water, the gills were exposed to relatively high concentrations and under such conditions they accumulated appreciable quantities of Hg. On the other hand, for the experiments with contaminated sediments (a situation more closely resembling exposure conditions in natural systems), a consistently high proportion of Hg was in the gut tissues. This result suggests that accumulation via the trophic route (i.e. from ingested sediment) was more important than the direct route (i.e. from the interstitial water, through the gills or the cutaneous barrier).

The relative importance of the gut as a site for a metal accumulation in *H. rigida* exposed to contaminated sediments has also been established for Zn and Cd (Hare *et al.*, 1991b). When the different parts of the gut are considered, there seems to be a slightly higher accumulation of the Hg in the mesenteron, a site of intense physiological absorption activity. In *Hexagenia limbata*, the highest quantities of Zn and Cd are present in the anterior part of the midgut, whereas for Cu and Pb the levels are similar in the different gut regions (Hare *et al.*, 1991a). In chironomids, accumulation of Cd is generally greatest in the midgut region (Hare *et al.*, 1991a; Seidman *et al.*, 1986).

Appreciable quantities of Hg were present in the compartment corresponding to the remaining tissues not sampled during dissection (skin, nervous system, adipose tissue, tracheal network, muscle mass, etc.), most notably for specimens exposed to organic Hg. The cutaneous coating, which could not be isolated during dissection, was included in the "rest of the body" compartment. However, the relative insensitivity of this compartment to the mode of contamination of CH_3HgCl treated microcosms (relative Hg burden unaffected by the source of contamination, 45% for water source and 41% for sediment source, Fig. 5) suggests that the contribution from the cutaneous coating was small. In contrast, for the gills (also in direct contact with the surrounding environment) the relative burdens were small (5%) with the sediment source and 20% with the water source. This contrasting behaviour of the gills and the "rest of the body" compartment suggests that most of the Hg in the latter compartment, after contamination by methyl-Hg, was localized within the organism itself and that Hg fixed on the cutaneous coating of the nymph represented a relatively minor contribution. In the case of $HgCl_2$ contamination, the quantity of Hg in the "rest of the body" compartment seemed more sensitive to the mode of contamination (13% for sediment source, 21% for water source), suggesting a greater contribution from the cutaneous layer.

Differences in metal distribution among different body parts as a function of the source of contamination have been described for other benthic species. In the suspension-feeding mussel, *Mytilus edulis*, 37% of the Hg was localized in the gills when exposure occurred via water, compared to only 5% after

contamination via sediment (King and Davies, 1987). Comparable results were obtained for the American oyster, *Crassostrea virginica* (Cunningham and Tripp, 1975). Extrapolation of these trends to all benthic taxa would be risky, however, because their burrowing behaviour and feeding habits are likely to differ. For example, in field studies of the freshwater molluscs *Elliptio complanata* and *Anodonta grandis* (Tessier *et al.*, 1984, 1993) and of the estuarine gastropod *Littorina littorea* (Langston and Zhou, 1987), the gills appeared to be the primary route for Cd absorption, even though animals were living in, and ingesting, highly contaminated sediments. Indeed, bioaccumulation and the internal distribution of a given metal depend on several factors, including the chemical form of the contaminant and its bioavailability, the taxon considered (Hare *et al.*, 1991a), and the ambient water quality characteristics, such as pH and hardness. Note, too, that internal transfer of the metal may occur between different parts of the animal (Anderson and Baatrap, 1988).

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