Hexagenia rigida (Ephemeroptera) as a Biological Model in Aquatic Ecotoxicology: Experimental Studies on Mercury Transfers from Sediment

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ABSTRACT

The accumulation of two mercury compounds—$HgCl_2$ and CH_3HgCl—by Hexagenia rigida *(burrowing mayfly nymphs) from contaminated sediments was investigated experimentally. Three exposure periods were selected: 7, 14 and 28 days. Results reveal a high capacity of this species for Hg accumulation and considerable differences between the two chemical forms of the metal. Thus, the amount of total mercury accumulated after 28 days' exposure would be 60 times greater for the organic form if the two compounds were initially added to the sediment in the same concentrations. No significant growth inhibition appears for the different experimental conditions studied. Data treatment at the organism level showed a positive linear correlation between the fresh weight and Hg content in the nymphs; this was especially marked when the exposure time was relatively long and Hg was in the form of CH_3HgCl. The study of mercury distribution in the organs of* Hexagenia rigida *(gills and gut) and the examination of results obtained in similar experimental conditions after contamination of the nymphs via the water column showed the importance of the trophic route, via ingested sediment, for the bioaccumulation of the metal initially introduced into the sediment.*

INTRODUCTION

The research objectives are based on an experimental approach to mercury bioaccumulation and transfer within freshwater systems. All the experiments take into account the three fundamentals of ecotoxicology: abiotic,

biotic and contamination factors. This approach is based on devising and using multispecific ecotoxicological models. According to the criteria of complexity and representativity in relation to natural contaminated systems, these models represent, from a methodological point of view, an intermediate position between field studies, on the one hand, and monospecific tests or bioassays, on the other.

The first stage of the research program was based on a linear trophic transfer model—experimental trophic chain—made up of four links: *Chlorella vulgaris, Daphnia magna, Gambusia affinis* and *Salmo gairdneri* (Ribeyre *et al.*, 1979; Boudou & Ribeyre, 1983). Since 1982, multicompartmental models have been developed, based on a mixed biotope—water and natural sediment—and on different biological species, representing different trophic levels: producers, herbivores, detritivores (Ribeyre, 1985). Work carried out so far has consisted essentially of the quantification of the actions and interactions of different abiotic factors (pH, temperature, photoperiod, light intensity) and contamination factors (chemical forms of mercury, contamination levels and routes) on mercury bioaccumulation by various species of rooted macrophytes (Maury *et al.*, 1988; Ribeyre & Boudou, 1989, in press.

More recently, similar studies have been developed using a benthic species: a burrowing mayfly, *Hexagenia rigida*. The choice of this species is based on several biological and ecological properties:

—*Hexagenia rigida* nymphs are detritivores and live in burrows which they construct in the upper layers of freshwater lentic sediments (silty substrates). They make sporadic incursions into the water column, their duration and frequency increasing under stressful conditions;

—estimations of biomasses indicate that this species is present in large quantities in lakes and rivers (e.g. 187 000 t—fresh weight—in Lake Winnipeg, Canada; Flannagan and Cobb, 1984). Mayfly nymphs and adults represent a significant source of food for many predators: fish, amphibia, bats, birds;

—nymphs have a high tolerance for fluctuations in abiotic factors, such as temperature, pH or dissolved oxygen (Craven & Brown, 1969);

—the nymphal stage is the longest period in the life cycle, between 10 and 24 months in natural conditions, depending on the climate; under optimal conditions, especially temperature and food supply, this period can be reduced to 12 weeks (Fremling & Mauck, 1980);

—mass culture can be initiated in the laboratory from eggs collected in the field, during emergence periods, or obtained from virgin female imagines or subimagines originating from field collections or from continuous laboratory culture (parthenogenetic development) (Friesen, 1981).

Very few ecotoxicological studies have been carried out on this species (Fremling & Mauck, 1980; Friesen *et al.*, 1983; Henry *et al.*, 1986); the research program had therefore to go through successive steps, the first being acute toxicity tests, in order to estimate mortality rates after short-term exposure to the two mercury compounds. The results obtained reveal a high resistance to mercuric contamination, in comparison with other freshwater species: e.g. LC_{50} after 24 h direct exposure to mercury chloride was $550 \mu g \, Hg \, litre^{-1}$ (Saouter, 1985); for *Daphnia magna* the 48-hr LC_{50} was $5 \mu g \, Hg \, litre^{-1}$ (Biesinger & Christensen, 1972).

In this paper results are presented of an experimental study of the accumulation of two mercury compounds—$HgCl_2$ and CH_3HgCl—by *Hexagenia rigida* from artificially contaminated sediments. Three exposure periods were selected: 7, 14 and 28 days. Metal accumulation was estimated by determination of the total mercury at the organism level, expressed either as concentration and body burden, and at the organ level (gills and gut). During this experiment weight and length were measured, in order to assess nymph growth during the experiment and to look for relationships between these parameters and mercury bioaccumulation. The effect of the degree of aeration of the water column was also taken into account, in order to search for any possible impact of this variable on nymph growth and metal bioaccumulation.

MATERIALS AND METHODS

Culture technique for *Hexagenia rigida*

Mass culture of the nymphal stages 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 previous work done on this species by Friesen (1979, 1981). Hatching was carried out in dechlorinated tap water, saturated with oxygen, by raising the temperature from 4 to $24°C$ in $4°C$ stages, every 48 h. Under these conditions, the hatch percentage was over 95%. Newly hatched nymphs were gently transferred to glass tanks ($25 \times 25 \times 30 \, cm$), using a Pasteur pipette. Each tank contained a natural sediment layer (depth: 1 cm), identical to the sediment used for the experiments, and was filled with dechlorinated tap water to a depth of 20 cm. Nymph density was about 1–2 nymphs per cm^2. Food supplies—Tetra Min B— were added at the rate of 0.3–$0.5 \, g$ per tank, twice a month during the first two months. At this stage, about one-tenth of the original population was still alive. The nymphs—3 to 8 mm long—were then transferred to new tanks, with an average density of 1

nymph per 3 cm². During this period, it seemed preferable to remove the sediment periodically, by transferring the nymphs every three months, on average, to new tanks, rather than add artificial food, which could lead to excessive bacterial development. Moreover, sediment replacement made it possible to check the nymph density in each tank, a parameter which acted as an important limiting factor to nymph growth. Prior to emergence, the nymphs—now 25 to 30 mm long—swam to the surface, shed their exuvia and emerged in the terrestrial subimaginal stage. The number and frequency of instars for *Hexagenia rigida* has not been determined, but numerous molts occur during postembryonic development.

Nymphs used in the experiments were 15–20 mm long and 25–85 mg fresh weight. Growth heterogeneity was important inside each culture tank, and it was necessary to collect a large number of individuals in order to obtain, after a screening process, sufficient nymph batches for the experiments.

Experimental design

The basic structure used in the experiments was the experimental unit (EU): a glass tank (12 × 12 × 30 cm, lined with a plastic bag) containing sediment (depth: 5 cm), 2·9 litre of dechlorinated tap water (depth: 20 cm) and *Hexagenia rigida* nymphs.

Sediment was taken from the banks of the Garonne River, upstream from Bordeaux, France. Its geochemical and granulometric characteristics were determined by the Institut de Géologie du Bassin d'Aquitaine (Bordeaux I University). It was a very homogeneous silt, rich in clays (75–80%), with a low total organic carbon content (2% on average). The natural level of total mercury was $0·124 \pm 0·012$ mg Hg kg^{-1} (fresh weight), with a moisture content of 50%. A large quantity of sediment was homogenized by mechanical mixing, and three batches were made up, one for the control units and the others to be enriched with mercury by means of mixing sediment with aqueous solutions of CH_3HgCl or $HgCl_2$ (Merck) at 500 mg Hg litre^{-1}. After homogenization, ten sediment samples were taken from each batch to check contamination levels and metal distribution throughout the substratum. Mercury concentrations selected initially for the two compounds were 10 mg Hg kg^{-1} (fresh weight) for $HgCl_2$ and 1 mg Hg kg^{-1} for CH_3HgCl. These concentrations were essentially based on results from preliminary tests, showing very marked differences in bioaccumulation between experiments with the two mercury chemical forms added to the sediment (Saouter *et al.*, 1989). Sediments were introduced in the EUs using plastic containers (11·5 × 11·5 × 5 cm) which ensured identical sediment weight per EU and facilitated nymph recovery at the end of the exposure periods.

The physicochemical characteristics of tap water were relatively constant throughout the year and were checked periodically by the Laboratoire Municipal de Bordeaux. The main parameters were: $NO_3 = 0.14$ mg litre^{-1}; PO_4 = undetected (< 0.05 mg litre^{-1}); resistivity = 476 μSiemens cm^{-1}; organic matter = 0.64 mg litre^{-1}. Water column levels in the EUs were maintained constant by means of water additions throughout the experiment, to compensate for losses due to evaporation and sampling (mercury determinations). The pH in the water column was 7.5 ± 0.2 at the outset and changed little during the experiment (pH = 7.8 ± 0.3 after four weeks).

The temperature was fixed at $24 \pm 0.2°C$, which corresponds to the optimal value for nymph growth. EUs were placed in larger tanks ($140 \times 65 \times 30$ cm), which were themselves in enclosed containers. Each tank had thermoregulation equipment, which was very efficient, owing to the large volume of water constantly stirred by submerged pumps. In this way, contact between the regulation systems and the medium in the EUs was avoided. The photoperiod was 12 h/24 h; light was produced by two neon tubes (Sylvania F36W/GRO) positioned at 45 cm above the surface of the EUs and operated by a timer switch. Average light intensity at the surface was 2000 lux (Quantum Sensor, LI190SB). For half of the EUs, continuous aeration was provided by air pumps (RENA 301), the diffuser being placed in the upper layers of the water column, in order not to disturb the sediment–water interface too much. For the second experimental condition, no air supply was provided in the corresponding EUs during the experiment.

Four nymphs were introduced into each EU. This number was determined with reference to several elements and experimental constraints: nymph density usually observed in natural conditions, size and number of EUs, production capacity of the stock culture, statistical validity of results. In order to minimize weight heterogeneity between organisms, nymph selection was based on four weight classes (30–40, 40–50, 50–60 and 60–80 mg) and a similar biomass in each EU was then achieved by introducing one nymph of each class into each unit. This method led to an average total biomass/EU of 51.13 ± 1.86 mg (fresh weight) at the beginning of the experiment, with an inter-EU scattering of less than 4%. Before nymphs were introduced into the EUs, body length was measured from the head to the abdomen tip (without cerci). The nymphs were introduced 10 days after water and sediment had been added to the EUs. This delay allowed the physicochemical conditions of the water phase to stabilize. During this period, there was also a very rapid decrease of the mercury concentration in the water column, owing to metal transfers between sediment and water during the first hours of contact between these two compartments (Maury & Engrand, 1986; Ribeyre, 1988). No food supply

was introduced during the experiment. At the end of each exposure period, nymphs were collected by sifting the sediment; weight measurements—fresh weight and dry weight (70°C, 24 h)—were taken for each nymph and the biological samples were deep-frozen ($-20°C$). It is important to note that growth evolution during the experiment was not quantified for each larva; indeed, it was very difficult and subjective to distinguish individuals between the four nymphs inside each EU. Consequently, growth rate estimations were made from average weights per EU ($GR = W_t - W_0$).

The experiments were designed to test effects of six experimental conditions based on combinations of the three contamination modalities— natural sediment, sediment contaminated with $HgCl_2$ or CH_3HgCl—and differences in levels of aeration (water column with or without aeration). Three exposure periods were studied: 7, 14 and 28 days, time zero corresponding to the introduction of the nymphs into the EUs. Three replicates were set up for the contaminated units. Thus, the total number of EUs was 42.

Total mercury determination

For many benthic species, the determination of contaminants accumulated in the whole organism is often difficult because of the presence of sediment inside the digestive tract. For studies based on trophic transfers between *Hexagenia rigida* and predators, it is certainly better to measure total mercury content in the nymphs, including the contaminated sediment; for example, As, Cd, Cu, Zn in gut contents represented up to 22% of whole animal burdens in *Hexagenia limbata* (Hare *et al.*, 1989). On the other hand, in order to quantify the capacity for bioaccumulation via the sediment source, it is preferable to clear the intestine of its contents. In this experiment, the second solution was chosen. After the nymphs were recovered from the EUs, they were placed individually in small tanks with pure clay (kaolinite) and dechlorinated tap water, for 6 h. Visualization of the gut-loading was facilitated by the clear colour of this substrate. The time required for clearing was determined from preliminary experiments, in the course of which four clearing durations were studied (3, 6, 12 and 24 h, data not shown); this was in agreement with results published by Zimmerman & Wissing (1978).

Total mercury determination was carried out by flameless atomic absorption spectrometry (VARIAN AA475). Nymphs and sediment samples were first mineralized by nitric acid attack (concentrated HNO_3) in a pressurized medium (borosilicate glass tubes), at 95°C for 3 h. A bromine salts treatment was applied before the addition of stannous chloride (Farey *et al.*, 1978). The detection limit was 5 ng Hg (50 ml at 0·1 μg Hg litre^{-1}). The validity of the analytical method was checked periodically by means of

intercalibration exercises and reference standards (NBS, Washington; IEAE, Monaco; KFA, Jülich).

Total mercury accumulation in *Hexagenia rigida* (whole organism) was expressed by two criteria: concentration (ng Hg mg^{-1}, fresh or dry weight) and content or body burden (ng Hg). The natural level of mercury in the nymphs was 130 ± 12 ng Hg g^{-1} (fresh weight).

Data treatment

Data treatment was carried out in two stages. All data collected were first analysed using diagrams and basic statistical parameters (averages, standard deviation, etc.), in order to estimate intra- and inter-EU dispersion and also to detect any discrepancies in values (errors in measurement, mercury determination or result transcription; experimental problems; etc.). The second stage was based on multiple linear regression, in order to quantify actions and interactions of the different factors taken into account. Complete experimental designs and orthogonal polynomials simplified interpretation of the effects of each regressor because of the independence of the regression coefficients. The regressor coding was: exposure duration, time zero $= -7$, 7 days $= -3$, 14 days $= +1$, 28 days $= +9$ (corresponding square terms: $+7$, -4, -8, $+5$); sediment contamination, control $= -1$, $HgCl_2 = 0$, $CH_3HgCl = +1$ (corresponding square terms: $+1$, -2, $+1$); EU, with aeration $= -1$, without aeration $= +1$ (Snedecor & Cochran, 1971). 99% confidence limits were adopted to select the terms of the regression model. From previous analysis of relationships between log S/log M (standard deviation and average calculated for the three replicates) and of graphical representation of residues, it was decided to use the experimental variables without transformation.

RESULTS AND DISCUSSION

Characterisation of the exposure conditions of *Hexagenia rigida*

Total mercury determinations on sediment samples collected before the introduction of contaminated sediments in the experimental units (EUs) show good agreement between theoretical levels and those obtained after the enrichment of the substrate by the two mercury compounds: 10.6 ± 0.5 mg Hg kg^{-1} for $HgCl_2$ and 1.1 ± 0.3 mg Hg kg^{-1} for CH_3HgCl.

During the ten days preceding nymph introduction into the experimental units, total mercury determinations in the water column show that the highest concentrations, due to release processes, appear during the first 24 h after the EUs are set up. However, these concentrations are low—close to

the detection limit $(DL = 0.1\,\mu g\,Hg\,litre^{-1})$ for inorganic mercury and to $0.4\,\mu g\,Hg\,litre^{-1}$ for methylmercury—and they decrease very rapidly, with all the determinations made after 3–4 days being lower than the DL.

After nymph introduction into the EUs, total mercury concentrations in the water column increase rapidly, as does the quantity of suspended matter in this compartment, owing to bioturbation. Total mercury concentrations were measured after 7, 14 and 28 days. They are practically constant throughout the 28 days' exposure period, but differences appear between the two contamination conditions and also between EUs with or without aeration. For inorganic mercury, average values are close to $4\,\mu g\,Hg\,litre^{-1}$ in EUs with aeration and to $1\,\mu g\,Hg\,litre^{-1}$ in EUs without aeration; for methylmercury, these concentrations are respectively, $0.5\,\mu g\,Hg\,litre^{-1}$ and $0.2\,\mu g\,Hg\,litre^{-1}$.

Metal partitioning in the water column between dissolved and particulate phases based on sample filtration (Nalgene nylon filters—$0.45\,\mu m$), showed that the quasi-totality of the metal is linked to suspended matter; mercury determinations on filtrates (50 ml) were always lower than the detection limit.

Thus, mercury in the water column could represent a secondary contamination source for *Hexagenia rigida* nymphs, for example, from the deposition of suspended particles at the water–sediment interface or the water currents they create in their burrows.

However, mercury contamination levels measured in the aquatic phase (essentially in particulate form), are very low compared with concentrations of the two mercury compounds in the sediment. Moreover, in our experimental conditions, the burrows reached the maximum depth of the sediment (5 cm); thus, a very small amount of the ingested sediment came from the upper microlayers.

Growth analysis of *Hexagenia rigida*

From measurements taken at the beginning of the experiment, before the nymphs were introduced into the EUs, the relationship between dry weight (DW, mg) and body length (BL, mm) reveals a strong positive correlation: $DW = 0.009 \times BL^{2.51}$; $r = 0.92$. This regression model agrees substantially with the equation obtained by Zimmerman (1977) relating to nymphs of the *Hexagenia limbata* species collected from natural ponds ($DW = 0.019 \times BL^{2.26}$; $r = 0.96$); this species is taxonomically very close to *H. rigida*.

Nymph mortality during the experiment was 10% on average, without significant differences between control and contaminated conditions. This rate is similar to that observed in culture tanks.

Analysis of nymph growth during the three exposure periods is based on the average growth factors drawn up for each EU. For the two contamination conditions studied and for the control units, the growth rate (GR, mg) is close to linearity, the mean rate of increase being 4–5 mg (FW) per week. The corresponding regression model takes into account only the exposure duration (ED, days) variable: $GR = 7.79 + 1.13 \times ED$; contribution: 91·4%. The other two controlled factors—sediment contamination and aeration—do not act significantly on the growth of *Hexagenia rigida* during the 28 days' exposure.

From the data collected, the relationship between fresh and dry weights (FW and DW, mg) measured at the end of the three exposure periods reveals a very strong positive correlation between these two parameters: $FW = 5.25 \times DW$; $r = 0.89$. Nevertheless, when the fresh weight:dry weight ratios were analysed by multiple linear regression, taking into account the three controlled factors—exposure time, sediment contamination, and aeration—the model revealed little difference between the two chemical forms of mercury initially added to the sediment (95% confidence limit): for similar fresh weights, dry weights of the nymphs are slightly higher when the substrate is initially contaminated by inorganic mercury. This result can be linked with a low level of water overload in the organism, which could be attributed to the toxicological properties of the organic compound, particularly its effects on the structure and properties of biological membranes (Delnomdedieu *et al.*, in press).

Mercury bioaccumulation by *Hexagenia rigida*

Multiple regression models were established for the two accumulation parameters—total mercury concentration (ng Hg mg^{-1}) and burden (ng Hg) in the nymphs (average values/EU)—in relation to the three controlled factors—exposure duration, sediment contamination ($HgCl_2$ and CH_3HgCl) and aeration.

Hg concentration = 2·14 + 1·55 (sediment contamination) + 0·17 (exposure duration) + 0·12 (sediment contamination) × (exposure duration) − 0·11 (exposure duration)^2 − 0·08 (sediment contamination) × (exposure duration)^2. [*contribution: 99·8%*]

Hg burden = 124·63 + 89·5 (sediment contamination) + 11·5 (exposure duration) + 8·22 (sediment contamination) × (exposure duration) − 5·11 (exposure duration)^2 − 4·01 (sediment contamination) × (exposure duration)^2. [*contribution: 99·8%*]

The presence or absence of aeration in the water column does not significantly affect mercury accumulation by *Hexagenia rigida*. Similar results were previously obtained for nymphal growth. During the

experiment, measurements of dissolved oxygen concentration in the water column indicate only minor differences between aerated and non-aerated EUs: average values were respectively, 8.3 ± 0.2 and 7.8 ± 0.3 mg O$_2$ litre^{-1}.

Several factors could explain this result: a small biomass inside each EU, a low concentration of organic matter in the sediment, a large air–water exchange surface, a mixing of the medium due to nymph activity, and also an air flow limited to the upper layers of the aerated EUs. From a methodological point of view, the fact that the aeration had no significant effect on the growth of *Hexagenia rigida* or on mercury bioaccumulation via the contaminated sediment, suggests that EUs without aeration will be satisfactory for future experiments. This would have numerous practical advantages: indeed, the setting up and maintenance of similar aeration conditions in a large number of EUs is very difficult, if not impossible.

Total mercury concentrations in *Hexagenia rigida* after 7, 14 and 28 days' exposure for the two contamination conditions reveal considerable differences between the two chemical forms of the metal initially added to the sediment (Fig. 1). Thus, after four weeks, the ratio of mercury in nymphs from CH$_3$HgCl EUs to mercury in nymphs from HgCl$_2$ EUs is 6·3. In fact, this difference is even more marked, as the concentration of inorganic mercury in the sediment was ten times greater than that of methylmercury.

During the first two weeks, mercury accumulation in the nymphs is much more rapid for methylmercury. Between 14 and 28 days, the curves reflect a typical plateau for both contamination conditions.

For the second bioaccumulation parameter—mercury burden in the whole organism—comments on accumulation differences between the two contamination conditions are similar to those made for concentrations.
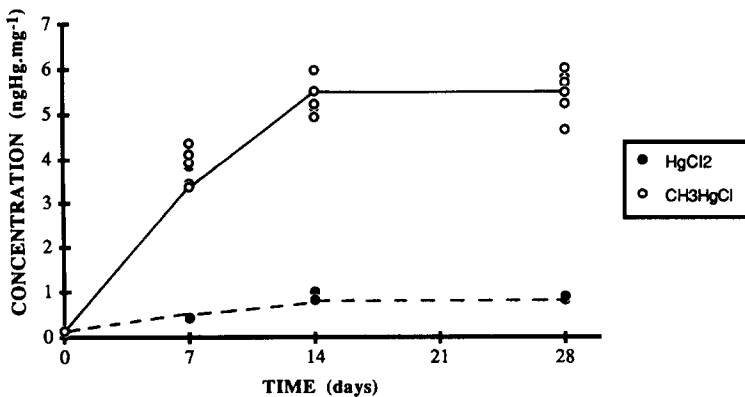

Fig. 1. Total mercury concentration in *Hexagenia rigida*, function of exposure duration and chemical form of the metal initially added to the sediment (HgCl$_2$, 10 mg Hg kg^{-1} and CH$_3$HgCl, 1 mg Hg kg^{-1}).

Indeed, ponderal growth was identical for the different experimental conditions studied. Nevertheless, the plateau observed for mercury concentrations after two weeks' exposure is associated with a slight increase in the corresponding burdens, owing to nymph growth during this period.

In conjunction with this analysis of results based on average values per experimental unit, our methodological choices enable us to take into account weight and accumulation measurements carried out at organism level. The aim of such an approach is to quantify the contribution of body weight with regard to mercury bioaccumulation by *Hexagenia rigida*, for each exposure period.

Data treatment based on individual values—fresh weight, mercury concentration or mercury burden—comes up against the problem of independence of the observations (four nymphs per EU). The first step is based on the setting-up of new multiple regression models taking into account data measured at the organism level, and the three controlled factors—exposure duration, sediment contamination and aeration. Two procedures can be applied: either direct use of all the weight measurements, or establishment of weight classes for nymphs collected after the three exposure durations. The first is easy to achieve, but requires orthogonality between regressors; the second is more difficult, owing to nymph growth during the experiment (weight dispersion increased) and mortality (missing values). For both procedures, it is necessary to centre the weight values with regard to the average values for each exposure period.

The first procedure was adopted and regression models were set up using centred individual weight values, the problem of orthogonality between regressors being minimized because of the preliminary distribution of nymphs into four weight classes.

For the mercury burden parameter, the new regression model confirms the effects of the controlled factors, observed after treatment of the average values, i.e. positive and important actions of exposure duration and sediment contamination modalities (Fig. 1). Nymph weight acts significantly on total mercury burden in *Hexagenia rigida*; its positive action is more evident as the exposure duration increases, and in experiments where the sediment was contaminated with the organic form of mercury (Fig. 2).

A similar study based on the concentration parameter shows an inverse relationship (negative correlation). This is more especially marked as the relationship between nymph weight and mercury burden moves away from linearity.

These results indicate that under our experimental conditions, weight differences have important repercussions on the total mercury burden in *Hexagenia rigida*. Consequently, when there exist weight differences between the different organisms inside the EUs, it will be important to consider
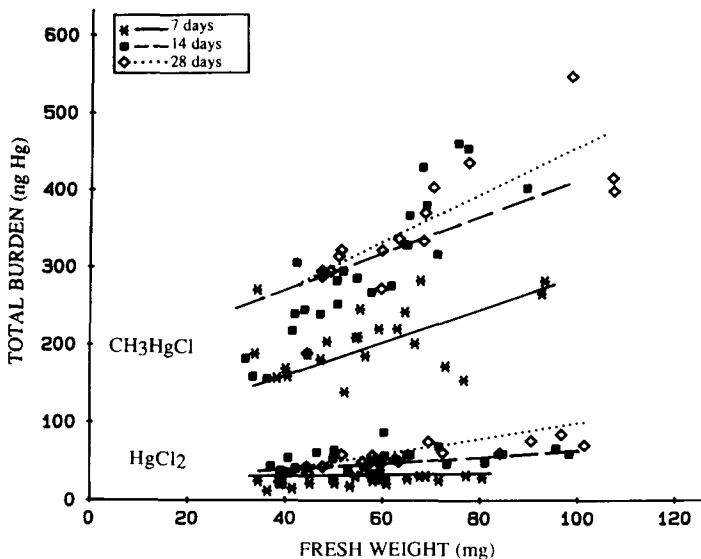
Fig. 2. Relationship between total mercury burden (ng) in the nymphs and fresh weight (mg), function of exposure duration and mercury chemical form initially added to the sediment. Regression model: Hg content = 125·45 + 11·22 (exposure duration) + 89·95 (sediment contamination) + 1·35 (nymph weight) + 8·19 (exposure duration) × (sediment contamination) − 5·37 (exposure duration)^2 − 4·31 (exposure duration)^2 × (sediment contamination) + 1·03 (sediment contamination) × (nymph weight) + 0·11 (exposure duration) × (nymph weight) [*contribution: 97%*].

individual weight measurements as an explanatory variable. This will help to improve the capacity to demonstrate the actions and interactions of controlled factors (increase of degrees of freedom, reduction of residual variance).

When mercury compounds are initially added to the sediment compartment, contamination of *Hexagenia rigida* nymphs may derive from a combination of two routes: first, the trophic route, by the intermediary of the metal associated with the ingested sediment—sediment particles and interstitial water—the biological barrier involved being the gut wall; second, the direct route, based on mercury transfers through the gill epithelium and the cutaneous coating, from dissolved mercury in the interstitial water and secondarily in the water column.

With this experimental approach it is not possible to quantify precisely these two contamination routes, mercury transfers from direct and trophic routes being superposed. Nevertheless, several conclusions derived from this or from complementary experiments give an indication of the contamination mechanisms in *Hexagenia rigida*.

In an experiment carried out in similar conditions, where the sediment was contaminated with inorganic mercury ($HgCl_2$: 10 mg Hg kg^{-1}) and methyl-
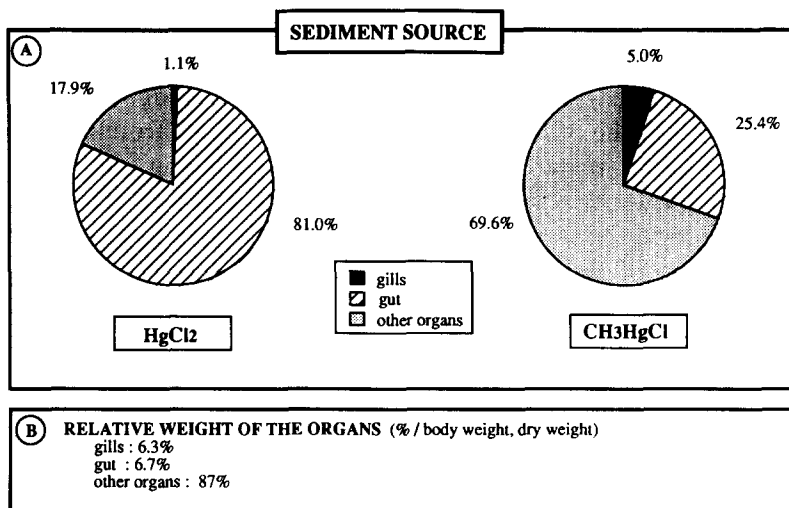
Fig. 3. (A) Mercury distribution in the gills, the gut and the rest of the body of *Hexagenia rigida*, expressed in relative burdens (%), after contamination via the sediment with $HgCl_2$ (10 mg Hg kg^{-1}) and CH_3HgCl (0·5 mg Hg kg^{-1}); (B) Weights of these organs, expressed as percentage of body dry weight.

mercury (CH_3HgCl:0·5 mg Hg kg^{-1}), metal distribution was measured after nymph dissection in the gills, the gut and the rest of the body. It was found that for the inorganic form of the metal, more than 80% of the Hg body burden was localized in the gut, the relative weight of this organ being only 6·7% of the average nymph weight, and only 1% was accumulated in the gills (6·3% of the average nymph weight). On the other hand, for organic mercury, these relative burdens are respectively, close to 25 and 5% (Fig. 3).

A similar distribution of the metal was observed in fish, *Salmo gairdneri*, after experimental trophic contamination with prey which had been exposed to mercuric chloride or methylmercury (Boudou & Ribeyre, 1985). In this case, the Hg burden in the lower intestine, beyond the pyloric coeca, was 35% of the body burden, while the relative fresh weight of this organ was 1·4%. For the organic compound, results showed a much lower accumulation capacity in the intestine of the fish, the relative burden in this organ being only 2·7%. However, large amounts of the metal were measured in the other organs, which gave rise to some wide differences between the average Hg burden in fish, after contamination by the two mercury compounds, with a ratio of about 3 in favour of methylmercury. Note that these differences are very much less than those observed in *Hexagenia rigida*, which were of the order of about 60 for similar contamination conditions of the sediment.

These results tend to show that for this burrowing mayfly species the trophic route, via ingested sediment, is predominant in our experimental

conditions. The gut of the nymphs, like the posterior intestine for *Salmo gairdneri*, would be a very efficient organ for inorganic mercury accumulation, metal transfer through this barrier being very low; for methylmercury, on the other hand, absorption capacity through this organ would be very great, metal accumulation in the gut being low.

Results obtained after complementary experiments based on contamination of the EUs via the water column, by means of twice-daily additions of identical amounts of $HgCl_2$ or CH_3HgCl (average mercury concentrations in water column samples, without filtration, are close to 6–$8\ \mu g$ Hg litre^{-1}, for the two compounds, after 10 days' exposure), could be used to support this hypothesis. In such experimental conditions, the direct contamination route must necessarily take priority. Mercury organotropism in the nymphs indicates strong differences compared to the metal distribution after contamination by the sediment source: Hg burdens in the gills and the gut represent respectively 50% and 8% of the body burden after exposure to inorganic mercury and 20% and 17% after exposure to methylmercury (unpublished results). Thus, when the direct contamination route has priority, the mercury burden in the gills is very high, jointly with their role in metal transfer between the surrounding medium and the internal tissues of *Hexagenia rigida* nymphs. The very low relative Hg burdens accumulated in this organ after contamination of the EUs via the sediment source leads us to suppose that mercury transfer from the water column as a secondary contamination source are very small, thus confirming the hypothesis that the trophic route predominates.

One last argument can be put forward to support this interpretation. When the EUs are contaminated via the sediment source, the differences observed between average mercury concentrations in the water column for the two conditions with and without aeration (4 and $1\ \mu g$ Hg litre^{-1} respectively for $HgCl_2$ and 0.5 and $0.2\ \mu g$ Hg litre^{-1} for CH_3HgCl) do not lead to any significant differences in the capacity of the larvae to accumulate inorganic mercury and methylmercury. If the mercury present in the water column played an important part in relation to nymph contamination, the differences observed between these two experimental conditions would be represented as differences in the amounts of metal accumulated by the organisms.

In the case of *Hexagenia rigida*, as for other species, the very great discrepancy between transfers of the two chemical forms of mercury introduced into the sediment can be attributed to two basic mechanisms:

—First, the different capacities of inorganic mercury and methylmercury to cross membrane barriers, especially the phospholipidic bilayer of the plasmic membrane, which represents an hydrophobic barrier. Methyl-

mercury is thus more liposoluble than mercuric chloride: the n-octanol/water partition coefficients are 2·54 for CH_3HgCl and 0·61 for $HgCl_2$ (Halbach, 1985). However, according to Lakowicz and Anderson (1980), the rapid diffusion of methylmercury across membranes, rather than lipoid affinity, is responsible for its transport across biological barriers;

—second, the different efficiency rates of decontamination processes within the organism, according to the chemical form of mercury absorbed. Several experimental approaches to different species of freshwater fish show that decontamination speed is slower for methylmercury than for inorganic mercury, this giving rise to wide differences in the corresponding half-lives (Ribeyre & Boudou, 1984).

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