Trace Metals in the Environment 1

Heavy Metals in the Environment

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SYNTHESIS OF MERCURY CONTAMINATION MECHANISMS OF A BURROWING MAYFLY *(HEXAGENIA RIGIDA)*: METHODOLOGICAL BASES AND PRINCIPAL RESULTS

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SUMMARY

Experimental studies using a three compartment ecotoxicological model "water, natural sediment and *Hexagenia rigida* nymphs" revealed very high bioaccumulation capacities of this burrowing mayfly for two mercury compounds, $HgCl_2$ and CH_3HgCl. Contamination processes are strongly dependent on exposure conditions : contamination sources, Hg concentrations in the biotopes, length of exposure, etc.. By studying the metal organotropism between two principal target organs - gills and gut -, it was possible to link specific contamination routes (direct or trophic) with the chemical forms of mercury and the initial contamination sources (water column or sediment). Mercury partitioning in the water (dissolved and particulate phases) and in the sediment (interstitial water and geochemical phases) was studied, and transformations of the two Hg chemical forms were quantified. All these experimental approaches, taking into account the three fundamental poles of Ecotoxicology (abiotic, biotic and contamination factors) led to a better understanding of the range and kinetics of the contamination mechanisms for this intra-sedimentary species.

1. INTRODUCTION

Studies on the contamination of freshwater systems by trace metals clearly show the importance of sediments as reservoirs for storage and chemical transformations of these elements and also as a secondary contamination source, through release processes (1-3).

Metal fluxes to sediments are strongly dependent on inputs of anthropogenic origin. They result from gravitational and diffusive processes : the former generally predominate, owing to the strong affinity of metals to suspended matter ; the latter are the result of transfers from dissolved metal species in the aquatic phase (4-5). Metal partitioning in sediments results from actions and interactions of a great number of factors : physico-chemical properties of the metal considered, contamination modalities, relative importance of sediment sorbents, abiotic parameters, etc.. Many factors are able to induce, either directly or indirectly, metal transfers between the upper layers of sediments and the water column and/or the trophic networks. Among these factors are mechanical stirring (currents, dredging, navigation,...), bioturbations (burrowing species) and changes in the physico-chemical characteristics of biotopes (e.g. lake acidification) (6). In the same way, abundance and diversity of benthic and intra-sedimentary communities - bacteria, microalgae, rooted macrophytes, annelids, molluscs, insects,... - represent a potential trophic contamination source for

the aquatic networks and several terrestrial predators, such as piscivorous birds or man. In all cases, metal bioavailability is closely dependent on partitioning in the sediments and also on chemical transformations occuring in this compartment.

Our research objectives are based on an experimental approach to mercury bioaccumulation, transfers and chemical transformations 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 multispecies and interactive ecotoxicological models (water, natural sediments and species representative of different trophic levels) (7-8). 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 step of this new methodological approach has consisted essentially on the quantification of the actions and interactions of different abiotic factors (pH, temperature, photoperiod, light intensity) and contamination factors (chemical forms of mercury, contamination sources, ...) on mercury bioaccumulation by various species of rooted macrophytes (8-10).

More recently, similar studies have been developed using a benthic species : a burrowing mayfly, *Hexagenia rigida* (11-13). Several authors have described the biological and ecological characteristics of this species (14-17). The choice of this species is based on several biological and ecological properties. The nymphs are detritivores and live in burrows which they construct in the upper layers of freshwater lentic sediments (silty substrates). The nymphal stage is the longest period in the life cycle, between 10 and 24 months in natural conditions, depending on the climate, and has a high tolerance for fluctuations in abiotic factors, such as temperature, pH or dissolved oxygen (18-19).

The nymphal stages of *Hexagenia rigida* thus provide a very good means of studying at the laboratory level mechanisms of mercury bioaccumulation and transfer in relation to quantitative and qualitative modifications in several ecotoxicological factors. Contamination processes of this benthic species are fairly complex, however, being based on the transfer not only of mercury linked to sediment particles ingested by the nymphs - trophic route - but also of metal present in the water (interstitial water in the sediments and water column) - direct route -. These two routes are always more or less concomitant, but with one or other route predominating, according to whether contamination of the experimental systems is initially via water column or via the sediment compartments.

In this paper we shall present a synthesis of the main results from our experimental approach to the bioaccumulation, transfer and chemical transformations of two mercury compounds ($HgCl_2$ and CH_3HgCl). These results were obtained in the context of our research programme using a three compartment system "water, natural sediment and *Hexagenia rigida* nymphs", contamination of the experimental units (EU) being based either via the sediment or the water column (Fig. 1). Complementary studies of the metal partitioning in the biotopes were set up, in order to obtain a better understanding of the differences in distribution and bioavailability between the two compounds. Finally, chemical transformations of inorganic or organic mercury initially introduced in the water or in the sediment - methylation and demethylation reactions - were quantified.

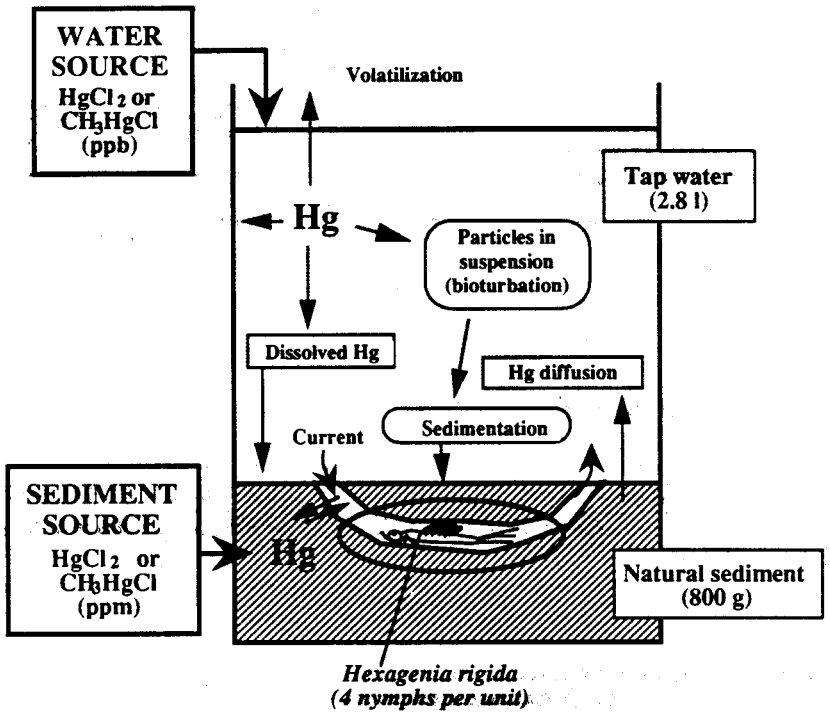

Fig. 1. Basic structure of the experimental model "water, natural sediment, *Hexagenia rigida* nymphs" : principal mercury exchanges between the different compartments, after initial contamination via the water or the sediment source.

2. MATERIAL AND METHODS

2.1 Culture technique for *Hexagenia rigida nymphs*

Mass culture of nymph stages was initiated in our laboratory from eggs collected each summer from Lake Winnipeg (Freshwater Institute, Winnipeg, Canada) and stored at +4°C. The culture technique (20-21) has been adapted in our laboratory (12-13). Hatching was carried out in dechlorinated tap water, saturated with oxygen and raising the temperature from 4°C to 24°C in 4°C stages, every 48 hours. In these conditions, percentage hatched was over 95%. Newly hatched nymphs were gently transferred to glass tanks (25x25x30 cm), using a Pasteur pipette. Each tank contained a natural sediment layer (depth : 5 cm), identical to sediment used for the experiments. Nymphs selected for the experiments were 15-25 mm long and 25-80 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.

2.2 Experimental design

The basic structure used in the experiments was the experimental unit (EU) : a glass tank (12x12x30 cm - lined with a plastic bag) containing sediment (depth : 5 cm), 2.8 l of dechlorinated tap water (depth : 20 cm) and *Hexagenia rigida* nymphs (4 nymphs/EU) (Fig. 1). Sediment was taken from the banks of the Garonne River, upstream from Bordeaux. It was a very homogeneous silt, rich in clays (75-80%), with a low organic carbon particle content (2% on average). The natural level of total mercury was 0.124 ± 0.012 mgHg.kg^{-1} (fresh weight), with a fresh weight/dry weight ratio of about 2.0. A large quantity of sediment was homogenized by mechanical mixing and two batches were made up, one for the control units and for the EUs contaminated by the water source, and the second for the sediment source, to be enriched with mercury by means of aqueous solutions of CH_3HgCl or $HgCl_2$ (Merck - 500 mgHg.l^{-1}). After a second homogenization, sediment samples were taken from each batch to check contamination levels and metal distribution in the substratum. The contamination by the water source was realized by twice daily additions of CH_3HgCl or $HgCl_2$ in the water column ; Hg concentrations in this compartment were monitored throughout the experimental period, 2 or 3 times a week.

Water column levels in the EUs were maintained constant by means of periodic additions during the experiment, to compensate for losses due to evaporation and sampling (mercury determinations). EUs were placed in larger tanks (140x65x30 cm), which were themselves in enclosed containers. Each tank had thermoregulation equipment, which was very efficient, due to the large volume of water constantly stirred by submerged pumps. Light was produced by two neon tubes (Sylvania F36W/GRO) positioned at 45 cm from the surface of the EUs and operated by a timer switch. Average light intensity at the surface was 2000 lux (Quantum Sensor, LI190SB). Discontinuous aeration in EU was produced 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.

Four nymphs were introduced into each EU. In order to minimize weight heterogeneity between organisms, nymph selection was based on four weight classes and a similar biomass was then achieved in each EU, by introducing one nymph of each class into each unit. The nymphs were added 10 days after water and sediment had been introduced in the EUs. This delay allowed the physico-chemical conditions of the water phase to stabilize (22-23). No food supply was added during the experiment.

All the experiments were developed using complete experimental designs, which permit to quantify the actions of the different factors studied and also their interactions, using multiple regression method (24-26). 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 giving by Snedecor and Cochran tables (27). 99% confidence limits were adopted to select the terms of the regression models. In this paper, most of the results presented will be based on theoretical models, representative of all the observations made during our research programme (12).

2.3 Total, organic and inorganic mercury determination

For many benthic species, the determination of contaminants accumulated in the whole organism is often difficult because of the sediment inside the digestive tract (28-29). To clear the intestine of its contents after the contamination phase, nymphs were collected from the EUs and introduced in a new sediment without mercury. The time required for clearing the gut - 6 hours at 20°C - was determined from preliminary experiments (12) ; this was in agreement with results published by Zimmerman and Wissing (30).

Total mercury determination was carried out by flameless atomic absorption spectrometry (VARIAN AA475). Nymphs and sediment samples were first mineralized by nitric acid attack (pure HNO_3) in a pressurized medium (borosilicate glass tubes), at 95°C for three hours. A bromine salts treatment was applied before the addition of stannous chloride (31). The detection limit was 5 ngHg. 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 determination in the gills and the gut of the nymphs was determined using radioactive isotope ($CH_3{}^{203}HgCl$ - Amersham, UK, and $^{203}HgCl_2$ - Dupont, USA). Radioactivity was measured in an "LKB Wallac 1282 Compugamma" gamma particle counter. Correction was made for radioactive decay and for background radiation. Measurements were carried out at the "Institut National de la Recherche Scientifique, INRS-Eau", University of Quebec, Ste Foy (Canada).

Organic and inorganic mercury determinations were carried out by flameless atomic absorption spectrometry, the metal being preconcentrated on gold wire and the organic and inorganic forms separated by anion exchange chromatography. All these measurements were achieved at the Institute of Applied Physical Chemistry, KFA, Jülich (Germany), according to Horvat's method (32-33).

Mercury accumulation in *Hexagenia rigida* (whole organism) was expressed by the concentration criterion ($\mu gHg.g^{-1}$, fresh weight) ; Hg bioaccumulation in the two organs selected - gills and gut - was expressed as relative burden (%). The natural level of total mercury in the nymphs was 130 ± 12 $ngHg.mg^{-1}$ (fresh weight).

3. RESULTS AND DISCUSSION

The bioaccumulation of the two mercury compounds - $HgCl_2$ and CH_3HgCl - in the nymphs is strongly influenced by the contamination source - water column or sediment. When the EUs were initially contaminated via the sediment source (identical initial concentrations of inorganic mercury and methylmercury), average Hg concentrations in the nymphs differed by between 40 and 60 fold, more in certain cases, in favour of sediments enriched with organic mercury (Fig. 2). Similar studies on the accumulation of organic and inorganic mercury by Shrimps (*Crangon crangon*) via the trophic route also showed very high contamination differences (a factor close to 40, in favour of methylmercury) (34).

When Hg is introduced in the water column, differences between total Hg concentrations in the nymphs tended to be very small, between 1.2 and 1.8, yet always in favour of EUs contaminated by methylmercury.

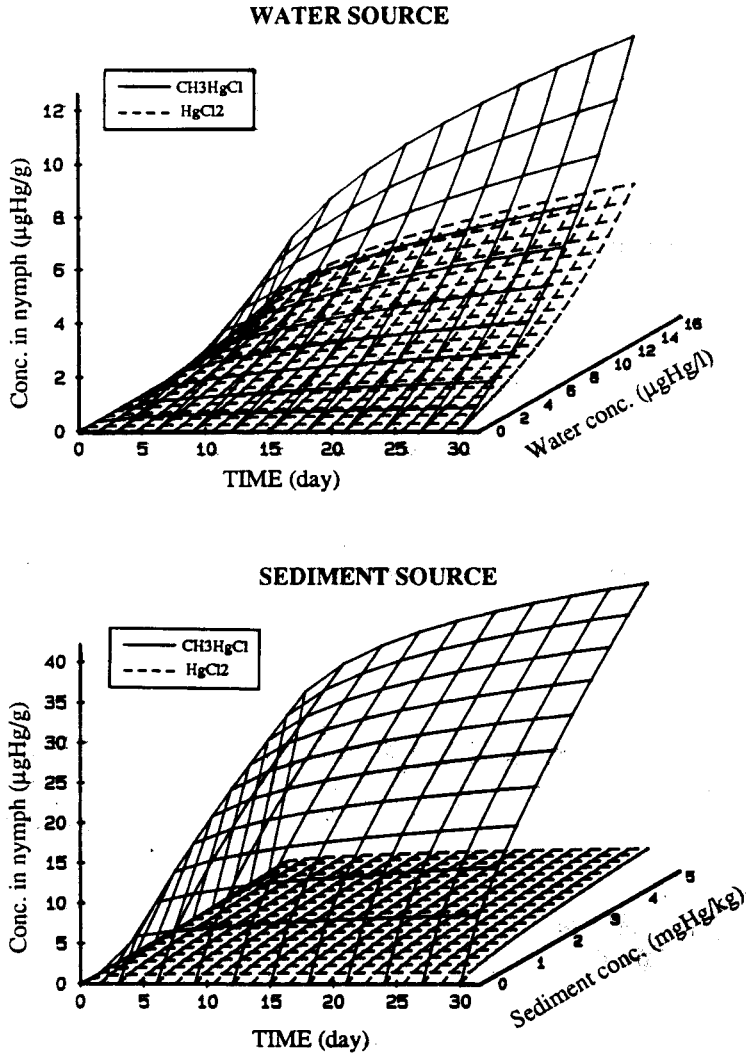

Fig. 2. Synthetic representation of total mercury concentrations accumulated in the nymphs ($\mu gHg.g^{-1}$, fresh weight), as a function of the contamination sources (water column or sediment), the chemical forms of mercury ($HgCl_2$ and CH_3HgCl), the metal concentration in the water column or in the sediment and the exposure length.

Although it is very difficult to achieve a strict comparison between the two contamination sources, it is clear that contamination via the water source does give rise to much greater transfers of

mercury into the organisms than contamination via the sediment source. Daily additions of mercury into the water column of the EUs produced concentrations in this compartment of about $\mu gHg.l^{-1}$, after 28 days' exposure, whereas the initial contamination levels in the sediment were about $mgHg.kg^{-1}$. Corresponding average Hg concentrations measured in the nymphs were in the same level order of magnitude, of about $mgHg.kg^{-1}$. In terms of bioconcentration factors (BCF = $C_{organism}/C_{biotope}$), it can be seen that accumulation capacities are greater from the water source by a factor of almost 1000. In fact, these differences could be linked to the degree of bioavailability of the metal in the water phase and in the sediment (2-3, 35).

The kinetics of accumulation also varied greatly depending on the contamination source. Via the sediment source, mercury bioaccumulation, asymptotic in nature, was very rapid, the maximum concentrations in the nymphs being observed after 10-15 days' exposure ; after this period, an "equilibrium" between the ad-absorption of the metal and its elimination gives rise to a well-defined plateau tendency. We should note that for exposures of over 30 days, we observed some fluctuations in this plateau tendency, with sharp decreases in the metal concentrations accumulated in the organisms, followed by a resumption of the bioaccumulation kinetics. These phenomena may be associated with the specific type of growth of *Hexagenia rigida* nymphs, which involves a series of moults. The rapid turn-over of the biological structures during the phase that precedes the moult may be a very efficient decontamination mechanism for the nymphs. We have no precise informations available on this phenomenon, but it is interesting to note that a chronological approach to the bioaccumulation of cadmium, zinc and lead by this mayfly species, under similar experimental conditions, gives rise to similar observations (36).

When the EUs were contaminated via the water source, mercury accumulation by the nymphs was also asymptotic, but the period of equilibrium between the metal's entering and leaving the organisms occurred very much later (Fig. 2). The differences between the two contamination sources could be associated with the specificities of the contamination modalities : when mercury compounds were added twice daily to the water column, the contamination pressure tended to remain constant throughout the experiment, and logically this exposure conditions contribute to maintain the metal transfers between the biotope and the organisms during the experiment. When the EUs were contaminated via the sediment source, however, the two mercury forms were added to the systems in a single addition, at the beginning of the experiment and the bioavailability of the metal may therefore decrease over time, in relation to the high complexation capacity of the particulate phase of the sediment.

Differences in levels of bioaccumulation between the two mercury forms tended also to vary according to the metal concentration in the biotopes :

- for the sediment source, these differences were not proportional to the amounts of mercury initially introduced into this compartment. They increased as mercury concentrations in the sediment increased, the relationships between bioaccumulation and sediment contamination level being close to linear for methylmercury, but highly asymptotic for the inorganic compound. We should note that whatever the metal concentrations initially introduced into the sediments, the

accumulation kinetics were always similar, with a plateau tendency appearing after several days' exposure ;

- for the water source, the relationships between Hg burdens in the nymphs and metal concentrations in the water column were close to linear or even slightly exponential, for the two mercury compounds.

Mercury distribution in the main organs of *Hexagenia rigida* nymphs revealed some considerable variations, depending on the chemical form of the metal and the contamination source considered (Fig. 3). By looking at the mercury organotropism, we should be able to estimate the respective importance of the direct and trophic contamination routes.

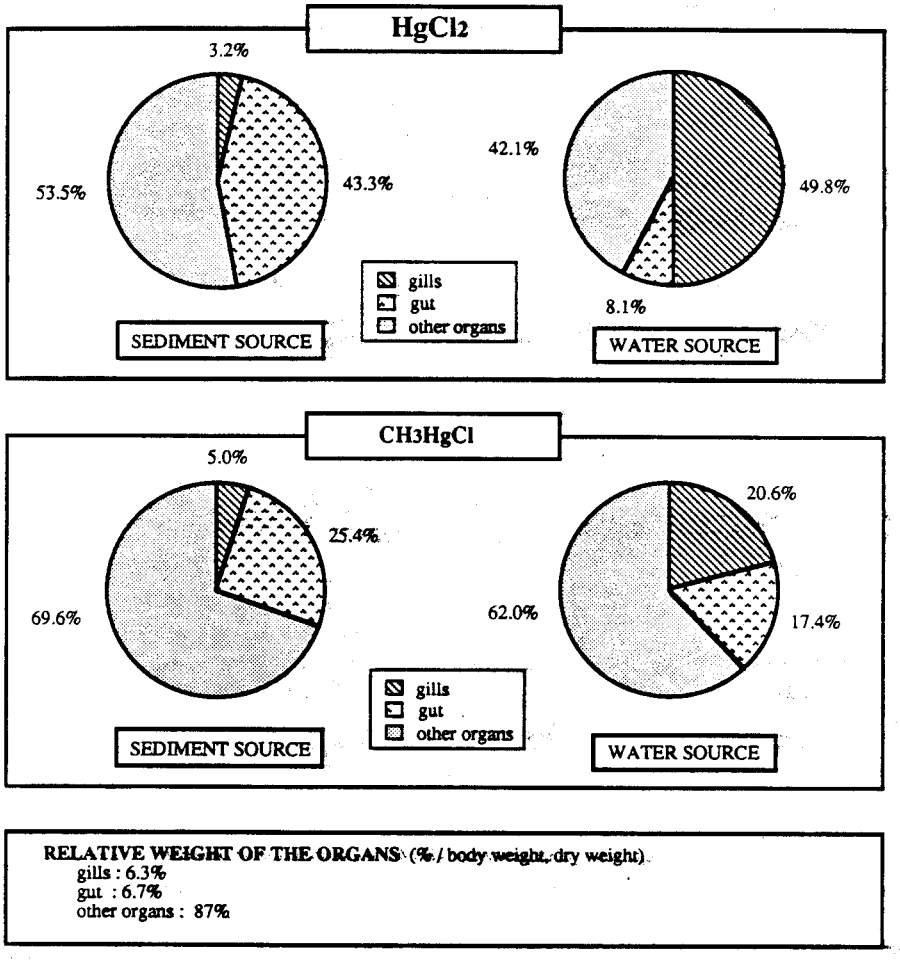

Fig. 3. Relative total mercury burdens (%) in the gills, the gut and the other organs of *Hexagenia rigida* nymphs, after contamination of the EUs via the sediment or the water source, by $HgCl_2$ or CH_3HgCl .

After contamination by inorganic mercury, via the sediment source, the relative mercury burdens were, on average, 43% in the gut and 3% in the gills. Via the water source, however, only 8% of the metal was accumulated in the gut, as opposed to approximately 50% in the gills. This mercury distribution would lead us to suppose that for the sediment source, the trophic contamination route, via the sediment ingested, was more important ; for the water source, the direct route, via the water column and/or the water in the burrows was more predominant. Nevertheless, in both instances, a high proportion of the metal was located in the rest of the body (53% and 42% respectively) ; this includes the cutaneous layer around the organism and all the organs and tissues not sampled (muscle mass, circulatory system, nervous system, haemolymph, etc.).

When the EUs were contaminated with methylmercury, via the sediment source, 25% of the metal was accumulated in the digestive tract, as opposed to 5% in the gills. As before, this distribution tends to suggest the predominance of the trophic route, by the intermediary of ingested sediment. With the organic form of mercury, the contrast between the metal burdens of these two organs was much less marked than when the inorganic mercury was used, and almost 70% of the accumulated metal was in the rest of the body. This distribution can be interpreted by attributing to the gut a very high capacity for the absorption of organic mercury, which is then stored in the different organs and tissues within the nymphs. In the case of the water source, mercury burdens in the gills and the gut were 20% and 17% respectively. The difference between these readings is not great: this may be due either to a contamination predominantly via the direct route, but with inter-organ transfers bringing large amounts of the metal into the gut by means of the haemolymph ; or it may be due to a mixed "direct + trophic" contamination. In both instances, more than 60% of the metal was nevertheless accumulated in the rest of the body ; so the same hypotheses apply as were put forward above in relation to the "sediment - CH_3HgCl" source, i.e. when there is a high rate of transfer across the biological barriers or a high level of fixation on the cutaneous interface.

Results obtained with other aquatic biological models (36-40) are in agreement with these observations. Metal burdens in the gut and the gills differ widely, with one or other being greater, depending on whether organisms are contaminated by the trophic or the direct route. Also, when contamination is via the trophic route, large quantities of inorganic mercury appear to bind on the gut wall, while absorption rates remain relatively low, thus explaining the low contamination levels measured in the whole organisms (Fig. 2). Methylmercury, on the other hand, crossed the digestive barrier of the nymphs much more easily, with bioaccumulation capacities, at the organism level, 40 to 60 times greater than those observed after contamination of the sediment by the inorganic compound.

In fact, mercury accumulated in the gills or the gut of *Hexagenia rigida* nymphs does not derive only from the water column or ingested sediment respectively. From the life style of the nymphs and the mercury exchanges established between water column and sediment compartments of the EUs, we can suppose that contamination is via the two routes, direct and trophic. The respiratory activity of the nymphs inside their burrows, with a current of water running through permanently (gill fluttering), does indeed contribute towards an increase in inter-compartment exchanges.

It is important to stress the role that the nymph's cutaneous coating may play in relation to mercury fixation and/or absorption. This barrier consists of a large surface area, in direct contact with the surrounding medium, and it is therefore able to participate in mercury accumulation by means of complexation reactions at the interface. With the micro-dissection procedure that we have adopted we are not able to estimate the quantities of mercury fixed on this barrier ; future experiments, using autoradiography (^{203}Hg), will permit to evaluate the role played by this barrier towards Hg fixation.

Lastly, it should also be noted that the relative Hg burdens in the gills and the gut sometimes represent very high concentrations, considering the low weight of these two organs relative to the rest of the body (6.3% and 6.7% respectively). Our estimates for the gut, for example, give concentrations of over 100 $\mu gHg.g^{-1}$ (fresh weight), for an initial concentration of 10 $mgHg.kg^{-1}$ ($HgCl_2$) in the sediment.

In this synthesis of results relating to the bioaccumulation of mercury compounds by *Hexagenia rigida*, at whole organism and main organ level, we are able to show the complexity of the phenomena involved and how closely dependent they are on initial contamination sources, chemical forms of mercury, contamination levels of the sources, length of exposure, ... For a better understanding of these processes, complementary studies were undertaken, based for example on the determination of mercury partitioning in the sediment, using chemical extractions (1,41-44) or on the quantification of the transformations of the two chemical forms of mercury initially introduced into the EUs.

When mercury is initially introduced into the sediments, its distribution between the dissolved and particulate phases differs, according to the chemical form considered - $HgCl_2$ or CH_3HgCl -. Our comparative study on the Garonne sediment in suspension in water shows that in the case of mercuric chloride, only 0.2% of the total mercury introduced was found in the dissolved phase ; for methylmercury, the percentages were between 2 and 5%. Moreover, with identical extraction conditions (1), the quantities of metal recovered from the oxic layers of the sediment after extraction with HCl (HCl 1N - iron and manganese oxide-bound Hg), ranged from 2 to 9% for the inorganic mercury and from 50 to 60% for the methylmercury. For mercury obtained after extraction with NaOH (NaOH 0.1N - humic acid fraction), the corresponding percentages are 13-17% and 15-20%. It was not possible to achieve satisfactorily experimental conditions for the determination of the metal extracted with H_2O_2 (H_2O_2 30% v/v - oxidisable organic matter) ; an important amount of mercury loss by volatilization was observed when samples were treated with peroxide (12,45).

Quantification of Hg dissolved and particulate forms in samples taken from the water column (filtration over nylon membrane - 0.45 µm - Nalgene 25 mm) in EUs contaminated by the sediment source shows that the metal concentrations are always greater when the sediment is first enriched with methylmercury. The presence of the metal in the aquatic phase is due to release processes from the sediments, strongly influenced by the bioturbation activity of the nymphs. The various results obtained demonstrate that the organic form of mercury has a smaller overall capacity to bind on the sediment particles. When mercury is introduced into the water column, average Hg concentrations in

this compartment remain similar for the two compounds, but the amount of dissolved metal is always greater when the EUs are contaminated with the organic form. Differences between inorganic and organic mercury are very closely dependent on the extent of the particle phase (turbidity of the water column). This phase is itself dependent on the degree of activity of the nymphs, the exposure period and the physico-chemical characteristics of the medium (e.g. temperature or pH). In any case, when mercury is initially added to the water column, the chemical form of the metal influences its distribution in this compartment much less than in the sediment source.

The fact that only small differences were observed between the two compounds in relation to mercury accumulation in the nymphs when contamination was via the water source could be linked with these results. In terms of bioavailability, there would seem to be little differences between $HgCl_2$ and CH_3HgCl as far as contamination conditions for the organisms in the water source were concerned.

Similarly, the very wide differences observed between the quantities of mercury accumulated in the nymphs, depending on the form of the metal introduced into the sediment - $C_{CH3HgCl}/C_{HgCl2} > 40$ - could be linked to the differences observed in metal concentrations in the interstitial water in the sediments or in the water column (release processes) : mercury is more abundant in these two compartments, and thus comparatively more bioavailable, when the sediment is initially enriched with methylmercury.

Based on these differences in the distribution of the two chemical forms of mercury throughout the biotopes, according to the contamination source, other arguments can be put forward to explain the differences observed between the amounts of Hg bioaccumulated in *Hexagenia rigida* nymphs.

First, let us review the physico-chemical properties of the two mercury compounds and how they could be linked with the transport processes across biological membranes. Many authors have established a close link between the high accumulation capacity of methylmercury and its liposolubility. In fact, the partition coefficient "n-octanol/water" (P) is 2.54 for methylmercury and 0.61 for $HgCl_2$ (46). These values are very low compared to highly liposoluble compounds, such as chlorinated hydrocarbons (47). Similar measurements on a "phospholipid (DPPC)/water" system gave similar P values for methylmercury (48). Studies on trans-membrane fluxes, using phospholipidic membrane models (planar bilayer membranes or BLM) show that methylmercury has a low affinity for the hydrophobic core of the bilayers, although its capacity for diffusion through the membrane is very great, and this explains how it is transported so efficiently (48-49). Moreover, the trans-membrane fluxes of inorganic mercury and methylmercury can be modified to a great extent, according to the physico-chemical conditions of the medium, especially pH and even more particularly the concentration in chloride ions (pCl). These processes are directly linked to the chemical speciation of dissolved mercury, where neutral species ($HgCl_2$ and CH_3HgCl for example) are better able to diffuse through the membranes than negatively charged ($HgCl_3^-$ or $HgCl_4^{2-}$)(50-51). Biochemical and biophysical studies of the binding of mercury compounds on membrane ligands have attributed a predominant role to the SH protein groups (thioloprive property). Recent

studies in our Laboratory, in collaboration with the Paul Pascal Research Centre (CNRS, Bordeaux) show that phosphatidylserine and phosphatidylethanolamine represent a new class of binding sites in cell membranes, the specific interaction between inorganic Hg and the primary amine groups on their polar heads giving rise to large-scale disturbances in the membrane micro-fluidity (52).

At the level of the biological barriers - gill epithelium and walls of the digestive tract -, structural and ultrastructural approach were realized in order to distinguish certain specific features of mercury accumulation and absorption. While the exchange surfaces of these two organs are very extensive - microvillosities of the middle gut and secondary lamella in the gills -, conditions for contact with the external environment and with the sediment ingested are quite different (12). Because of the presence of the peritrophic membrane which envelopes ingested sedimentary material, contamination via the trophic route requires that mercury pass through the dissolved phase in order to cross this first barrier and to reach after the apical face of the enterocytes. Thus, if the metal concentrations in the interstitial water are greater, as has been observed in the case of methylmercury, this may improve the metal accessibility to the interface zone between the lumen of the gut and the digestive tract wall. Mercury bound with the organic matter in the sediments can take advantage of different stages in the digestive process (proteolysis, for example, and liberation of small polypeptides or amino-acids) in order to be transported through the peritrophic membrane and then absorbed at the apical face of the enterocytes.

The ad- and absorption processes at the gut barrier level, in relation with the metal bioavailability, are minimized in the case of the gills, which are in constant contact with the surrounding environment. This obviously favours mercury transfers, especially of the dissolved chemical species. Moreover, our structural analysis of the gills reveals the presence of large muscle masses, especially at the base of the gill lamellae, which are in close contact with the haemolymph. This, in turn, represents an ideal site for mercury accumulation in this organ (12).

Many reactions of mercury complexation take place amongst particulate and dissolved ligands, which may contribute to strong ecotoxicological modifications within our experimental systems, especially in relation to mercury partitioning, physico-chemical properties and bioavailability. Running parallel with these processes, the inorganic and organic forms of the metal initially introduced into the water column or the sediment compartment may also be undergoing chemical transformations : methylation reactions, which produce from the inorganic mercury (HgII), methylmercury (CH_3HgX) or dimethylmercury ($(CH_3)_2Hg$, a volatile form) ; demethylation reactions, which transform the organo-mercurials into inorganic mercury (HgII) and in a second phase into elementary mercury (Hg^0, a volatile form)(3, 53, 54).

In our experimental conditions, we have recently begun to analyse these chemical transformation processes, by quantifying organic and inorganic forms of the metal accumulated in the sediment and in the *Hexagenia rigida* nymphs (whole organism level). The first results obtained refer to the mercury chemical form initially introduced into the water column or sediment compartments of the EUs ($HgCl_2$ or CH_3HgCl) and to the natural levels in the biotopes and in the organisms, in order to determine whether or not chemical transformations had taken place. Note that

with the determination method used, it was not possible to separate and quantify the different organic forms of the metal (methylmercury, dimethylmercury, etc.).

When mercuric chloride was used to contaminate the sediment compartment of the EUs, analysis of samples collected after 28 days'exposure shows that a significant amount of organic mercury was produced in the substrate. Concentrations were fairly low, between 2 and 11 $ngHg.g^{-1}$, which correspond to 0.09 and 0.8% respectively of the total mercury concentrations present in the sediment. These levels of methylation are in agreement with the results found in natural environments (1, 55-58).

When initial contamination of the EUs was with methylmercury, however, via the sediment source again, results show very high demethylation rates, which varied according to the depth of the substrate. Hence, depending on the abiotic conditions (temperature and pH), 4 to 33% of the organic compound initially introduced into the upper strates (oxic layer) was transformed into inorganic mercury ; in the lower layers (anoxic zone), these percentages were higher and more homogeneous at around 70% on average. We should mention that in several studies, rapid methylmercury decomposition has been observed, whether the compound was naturally occurring or artificially introduced into the sediments (56, 59-62).

For the water source, no determinations were made on samples collected from the water column ; on the other hand, we have some results for the upper layers of the sediment, at the water-sediment interface. When the EUs were contaminated with mercuric chloride, organic Hg was not detected in the surface layers of the sediment ; when contamination was with CH_3HgCl, however, very high levels of inorganic Hg were measured, corresponding to about 95% of the total mercury concentrations accumulated in this compartment ; these results clearly indicate very high rates of demethylation.

Inorganic and organic Hg determinations carried out on *Hexagenia rigida* nymphs produced some similar qualitative conclusions :

- when EUs were contaminated with mercuric chloride, organisms contained quantities of organic mercury at the end of the experiment (28 days for example) which were significantly greater than the natural background in the control nymphs, with relative burdens reaching a maximum of 3% in relation to the average total mercury accumulated in the nymphs ;

- when EUs were contaminated with methylmercury, a considerable proportion of the metal present in the nymphs after 28 days'exposure was in the inorganic form, with relative burdens between 25 and 55%, depending on the initial contamination source and the physico-chemical conditions of the environment.

At the stage we have currently reached in our experimental approach, it is not yet possible to establish any direct links between all the data collected, nor to define the mechanisms responsible for the chemical transformations. Nevertheless, several comments can be made :

- when the nymphs were contaminated via the sediment source, there were wide differences in mercury bioaccumulation, depending on the chemical form of the metal initially introduced. These differences could be as great as a factor of 40, in favour of methylmercury. Inorganic and organic Hg determinations in the sediment at the end of the exposure period show that

a high proportion of methylmercury had been demethylated, suggesting that the differences in the bioaccumulation capacities for the two compounds have, in fact, been underestimated when based on the initial concentrations of $HgCl_2$ and CH_3HgCl in the sediment ;

- in relation to the contamination of the EUs via the water source, we have no direct information on the extent of the chemical transformation reactions of the two compounds in the water column. Analysis of samples collected in the upper sediment layers, however, when methylmercury was added to the water column, shows that the metal present in this interface zone was mostly inorganic, as a result of direct water-sediment transfers or/and deposits of suspended particles. If this result is extrapolated throughout the whole experimental system, this phenomenon could provide an explanation for the very small differences observed between the accumulation capacities of the nymphs for the two experimental conditions "water source - $HgCl_2$" and "water source - CH_3HgCl" ;

- the presence of significant amounts of organic Hg in the nymphs after contamination of the experimental systems with mercuric chloride may be linked with the production of this organic form observed in the sediment ; it may also be due to methylation reactions within the organisms, especially in the digestive tract. Ultra-structural studies have indeed revealed the presence of a great number of bacteria in the gut lumen (12).

The presence of inorganic mercury in *Hexagenia rigida* nymphs after contamination of the EUs by methylmercury (water or sediment source) can also be correlated with the demethylation reactions observed in the biotopes. As has already been shown, inorganic mercury can accumulate in the nymphs when this compound is present in the surrounding environment, its physico-chemical properties and bioavailability being the reason for differences noted between processes of binding to and crossing the biological barriers. As well as methylation reactions, we can also suppose that demethylation reactions take place in the nymph's digestive tract, as a result of bacterial activity. Abiotic processes, such as UV radiations, could also participate to the production of Hg(II) and Hg^0 (52) ; experiments are actually under current work in order to compare the effects of different light intensities and photoperiods on these processes.

4. CONCLUSION

Our ecotoxicological approach to the processes of mercury bioaccumulation, transfers and chemical transformations within freshwater systems, based on a three compartment experimental model "water, natural sediment, *Hexagenia rigida* nymphs", provides a wide range of results. They reveal very strong effects of the different abiotic and contamination factors taken into account : temperature, pH, chemical forms of the metal, initial contamination sources (water column or sediment), length of exposure, ... The preliminary results on the quantification of the chemical transformations of inorganic and organic Hg, in the sediment and in the nymphs, after initial contamination of the EUs by the water column or the sediment, show the high potentialities of this

experimental model to reveal and quantify these essential processes, and to analyse the actions and interactions of many ecotoxicological factors.

Our research programme is actually directed towards a more detailed and mechanistic approach of these processes, in order to better understand the complex links occurring in natural conditions between mercury dispersion and partitioning in the biotopes, chemical transformations (methylation and demethylation reactions), biodisponibility, bioaccumulation and trophic transfers along the food chains and networks.

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REFERENCES

1 W.J. Langston, J. Mar. Biol. Ass. U.K., 62 (1982) 667-684.
2 W. Salomons and U. Forstner, Springer-Verlag, Berlin, 1984.
3 P.G.C Campbell, A.G. Lewis, P.M. Chapman, A.A Crowder, W.K. Fletcher, B. Imber, S.N. Luoma, P.M. Stokes and M. Winfrey, CNRC, Ottawa, Canada, K1A OR6, 1988.
4 A. Bourg, in: Boudou A. and Ribeyre F. (Eds), Aquatic Ecotoxicology : fundamental concepts and methodologies. CRC Press, Boca-Raton, USA, vol I, 1989, pp. 107-123.
5 P.G.C. Campbell and A. Tessier, in: A. Boudou and F. Ribeyre (Eds), Aquatic Ecotoxicology : fundamental concepts and methodologies. CRC Press, Boca Raton, USA, vol 1, 1989, pp. 125-148.
6 D.W. Schindler, Sci. 239 (1988) 149-157.
7 F.Ribeyre, Ecotox. Environ. Safety, 9 (1985) 346-363.
8 F.Ribeyre and A. Boudou, in: A. Boudou and F. Ribeyre (Eds), Aquatic Ecotoxicology : fundamental concepts and methodologies. CRC Press, Boca Raton, USA, vol 2, 1989, pp. 3-45
9 R. Maury, A. Boudou, F. Ribeyre and P. Engrand, Aquat.Toxicol. 12 (1988) 213-228.
10 F.Ribeyre and A. Boudou, in: W. Wang, J.W. Gorsuch and W.R. Lower (Eds), Use of plants for Toxicity Assessment. STP 1019, ASTM, Philadelphia, 1990, pp. 97-113
11 E. Saouter, F. Ribeyre, A. Boudou, in: J.P. Vernet (Ed.), Heavy metals in the environment. CEP Consultants, Edinburgh, 1989, pp.378-381.
12 E. Saouter, Bordeaux I University, Ph.D., n° 544, 1990.
13 E. Saouter, F. Ribeyre, A. Boudou and R. Maury-Brachet, Environ. Poll. 69 (1991) 51-67.
14 F. Neave, Contrib. Can. Fish. N.S., 7 (1932) 177-201.
15 C.R. Fremling, Trans. Amer. Fish. Soc., 97 (1968) 278-280.
16 W.P. McCafferty, Trans. Am. Entomol. Society., 101 (1975) 447-504
17 M.C. Zimmerman, Miami University, Ph.D., 1977.
18 R.E Craven, and B.E. Brown, Am. Midl. Nat., 82 (1969) 346-358.
19 R.H. Peterson, Can. Tech. Rep. Fish. Aquat. Sci., 1523 (1987) 1-7.
20 M.K. Friesen, J.F. Flannagan and J.F. Layrence, Can. Entomolo., 108 (1979) 665-673.
21 M.K. Friesen, in: S.G. Laurence (Ed.), Manual for the culture of selected freshwater invertebrates. Publ. Spec. Can. Sci. Halieut. Aquat.,1982.
22 R. Maury and P. Engrand, Bordeaux I University, Ph.D., n°667, 1986.
23 F.Ribeyre, Bordeaux University, Ph.D., n°957, 1988.
24 G.E.P. Box, W.G. Hunter and J.S. Hunter, Wiley, New-York, 1978.
25 R. Tomassone E. Lesquoye and C. Millier, I.N.R.A., Masson, Paris, 1983.
26 P. Dagnelie, Presses agronomiques de Gembloux, Belgique, 1984.
27 G.W. Snedecor and W.G. Cochran, Ass. Coordin. Techn. Agri, Paris, 1971.

28 C. Amiard-Triquet, C. Metayer and J.C. Amiard, Rev. Int. Oceanogr. Med., 73-74 (1984) 27-34.
29 L. Hare, P.G.C. Campbell, A. Tessier and N. Belzile, Can. Aquat. Fish. Sci., 46 (1989) 451-456
30 M.C. Zimmerman and T.E. Wissing, Freshwat. Biol., 8 (1978) 269-277.
31 B.J. Farey, L.A. Nelson and M.G. Rolph, Analyst, 103 (1978) 656-659.
32 M. Horvat, K. May, M. Stoeppler and A.R. Byrne, Appl. Organometallic Chem., 2 (1988) 515-524.
33 K. May, M. Stoeppler and K. Reisinger, Toxicol. Environ. Chem., 13 (1987) 153-159.
34 H.Y. Riisgard and P. Famme, Mar. Poll. Bull., 17 (1986) 255-257.
35 S.N. Luoma, Sci. Total Environ., 2 (1983) 73-82.
36 L. Hare, E. Saouter, P.G.C. Campbell, A. Tessier, F. Ribeyre, and A. Boudou, Can. J. Fish Aquat. Sci., 47 (1990) 39-47.
37 A. Boudou, and F. Ribeyre, W.A.S.P. 26,(1985) 137-148.
38 D.G. King, and I.M. Davies, Mar. Poll. Bull., 18 (1987) 40-47.
39 L. Albanus, L. Frankerberg, C. Grant, U. Haartman, A. Jernelov, G. Nornberg, M. Rudalv, A.Schutz, and S. Skerfuing, Environ. Res., 5 (1972) 425-442.
40 P.A. Cunningham and M.R. Tripp, Mar. Biol., 31 (1975) 321-334.
41 R.P. Gambrell, R.A Khalid, V.R. Collard, C.N. Reddy and W.H.Jr. Pattrick, in Dredging: Environmental Effects and Technology. Proceeding of Wodcom VII, World Dredging Conference, San Pedro, Calif., 1976, pp. 579-604.
42 A. Tessier, P.G.C. Campbell and M. Bisson, Anal. chem., 51 (1979) 844-851.
43 S.N. Luoma and G.W. Bryan, Sci. Total Environ., 17 (1981) 165-196.
44 S. Karlsson, K. Hakansson and B. Allard, J. Environ. Sci. Health., 6 (1987) 549-562.
45 P.G.C. Campbell and A. Tessier, in: J.P. Vernet (Ed), Heavy metals in the environment. CEP Cons., Edinburgh, UK, 1989, pp. 516-525.
46 S. Halbach, Arch. Toxicol., 57 (1985) 139-141.
47 D.R. Nimmo, in: G.M. Rand and S.R. Petrocelli (Eds), Fundamentals of aquatic toxicology. Hem. Publ. Corp., Washington, 1985, pp. 335-373.
48 J.R. Lakowicz and C.J. Anderson, Chem. Biol. Interact., 30 (1980) 309-323.
49 E. Bienvenue, A. Boudou, J.P. Desmazes, C. Gavach, D. Georgescauld, J. Sandeaux and P. Seta, Chem. Biol. Interact., 48 (1984) 91-101.
50 J. Gutknecht, J. Memb. Biol., 61 (1981) 61-66.
51 A. Boudou, D. Georgescauld and J.P. Desmazès, in : J.O. Nriagu (Ed), Aquatic Toxicology. Wiley, New York, 1983, pp. 117-136.
52 M. Delnomdedieu, A. Boudou, J.P. Desmazès and D. Georgescauld, Biochim. Biophys. Acta, 986 (1989) 191-199.
53 J.M. Wood, in: J.P. Vernet (Ed), Heavy metals in the environment. CEP Cons., Edinburgh, UK, vol 1, 1989, pp. 1-12.
54 T. Barkay, Appl. Environ. Microbiol., 53 (1987) 2725-2732.
55 P.D. Bartlett and P.J. Craig, Water Res., 15 (1981) 37-47.
56 A. Furutani and J.W.M. Rudd, Appl. Environ. Microbiol., 40 (1980) 770-776.
57 P.S. Ramlal, J.W.M. Rudd, A. Furutani and L. Xun, Can. J. Fish. Aquat. Sci., 42 (1985) 685-692.
58 S.M. Callister and M.R. Winfrey, W.A.S.P., 29 (1986) pp.453-465.
59 M.R. Winfrey and J.W. Rudd, Environ. Tox. Chem., 9 (1990) 853-869.
60 A. Kudo, D.R. Miller, H. Akagi, D.C. Mortimer, A.S. De Freitas, H. Nagase, D.R. Towsen, and R.G. Warnock, Prog. Water Technol., 10 (1978) 329-339.
61 P.S. Ramlal, J.W.M. Rudd and R.E. Hecky, Applied Environ. Microbiol., 51 (1986) 110-114.
62 F. Baldi, W.A.S.P., 38 (1987) 111-119.