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Comparative Experimental Study of Cadmium and Methylmercury Trophic Transfers Between the Asiatic Clam *Corbicula fluminea* and the Crayfish *Astacus astacus*

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Abstract. Cadmium (Cd) and methylmercury (MeHg) trophic transfers were analyzed between the Asiatic clam *Corbicula fluminea* and the crayfish *Astacus astacus.* Metal bioaccumulation in crayfish was quantified after 5, 10, and 15 days of exposure via daily ingestion of soft bodies of *C. fluminea*, previously exposed during 7 days to Cd (20 $\mu g \cdot L^{-1}$) and MeHg (4 $\mu g \cdot L^{-1}$). Bioaccumulation kinetics in the predator were investigated at organ and tissue levels: hemolymph, tail muscle, hepatopancreas, gills, stomach/mesenteron, intestine, green gland, carapace. Trophic transfer rates were estimated at the whole organism level. Results showed marked differences (1) in assimilation efficiencies, mean transfer rates being 5% for Cd and 16% for MeHg; and (2) in the metal distribution within the different tissue compartments of the crayfish: for Cd, the trophic uptake leads to high concentrations in the hepatopancreas and small accumulation in the muscle tissue; for MeHg, the highest levels of bioaccumulation occur in the green gland and in the tail muscle. From an ecotoxicological point of view, these experimental data suggest a small risk of Cd transfer between the crayfish and predators, humans included; on the other hand, Hg distribution in the muscle and accumulation trends in this tissue represent an obvious risk of transfer.

Among the invasive species present in freshwater systems, bivalves occupy an important place in the colonization process of lotic and lentic environments. Like the zebra mussel (*Dreissena polymorpha*), which is today found abundantly in Canada (Great Lakes, St. Lawrence river, etc.) and northern Europe, the Asiatic clam *Corbicula fluminea* originated in China and was introduced into North America some time prior to 1930, where it developed invasive dynamics and has now become a major component of benthic communities south of latitude 40°N (Counts 1986). It first appeared in Europe around 1980 and is now present in very high densities in the majority of rivers, channels, and lakes in southwest France (Mouthon 1981; Dubois 1995). This filter-feeder has an average lifespan of 3 years, with shell sizes at the adult stage measuring from 1 to 5 cm. *C. fluminea* lives buried in the upper layers of sediments, filtering large volumes of water, about 10 L/clam/day on average, for respiratory and nutritional purposes (Way *et al.* 1990). This bivalve is thus able to accumulate very large quantities of metals and is used in many ecotoxicological studies both at field level, on endemic or transplanted populations, and in the laboratory in indoor microcosms or artificial streams (Graney *et al.* 1984; Belanger *et al.* 1986; Doherty *et al.* 1988; Inza *et al.* 1997; Baudrimont *et al.* 1997; Andres *et al.* 1999).

Given its bioaccumulation capacities, its invasive nature, and the very large biomasses found in the natural environment, this benthic species represents an important contamination source for predatory species over a very wide area. Some such species mentioned in the available literature are certain species of fish (bream, tench), crustaceans (crayfish), land mammals (muskrat, coypu), and some birds (Covich *et al.* 1981).

In this study, we have set up at laboratory level a comparative analysis of the trophic transfer of two metals, cadmium (Cd) and the monomethylated form of mercury (MeHg), between *C. fluminea* and the crayfish *Astacus astacus* L. This crustacea decapoda was once very plentiful in Europe but has been gradually ousted by two introduced species, the crayfish *A. leptodactylus* and, more recently, the red swamp crayfish (*Procambarus clarckii*) (Streit 1998). Whereas in the case of mercury, or more precisely MeHg, a large amount of data has indicated that the trophic route predominates in respect of the contamination of predatory species (Boudou and Ribeyre 1997; Jackson 1998), trophic transfer studies with Cd have shown considerable variation in the perceived importance of dietary exposure routes (Canli and Furness 1995; Devi *et al.* 1995; Kraal *et al.* 1995; Munger and Hare 1997). In our experimental conditions, metal transfers in crayfish were quantified after 5, 10, and 15 days of exposure via daily ingestion of soft bodies of *C. fluminea*, previously exposed to Cd or MeHg. Bioaccumulation kinetics were analyzed at organ and tissue levels (eight compartments); trophic transfer rates were estimated at the whole organism level.

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Materials and Methods

Collection of the Bivalve Prey C. fluminea *and Direct Exposure to Cd and MeHg*

About 1,000 adult *C. fluminea* were collected from a reference site on the banks of the Cazaux-Sanguinet freshwater lake (Aquitaine, France). This is an oligotrophic lake, with very little agricultural and industrial activity in its catchment basin. Average clam density was about 500 individuals $\cdot$ m^{-2}. An initial selection was made at the lake; the bivalves were then transported to the laboratory in cool boxes filled with lake water and constantly aerated. After a 3-day stabilization period, homogeneous batches were made up, with selection being based on the maximum anteroposterior length of the shell, between 1.5 and 2.0 cm.

Cd or MeHg exposure was carried out in two experimental units (EUs) of 30 $\times$ 50 $\times$ 40 cm, containing 45 L of dechlorinated tap water, permanently aerated by a diffuser; a sediment compartment, 5 cm deep, made of pure sand (98% silica; granulometry: 0.8–1.4 mm, Silaq, Gironde, France), and 120 *C. fluminea.* It is very important that there is a substratum in which the bivalves are able to burrow, as this notably increases filtration activity (Tran 1997). The EUs were placed in tanks with automatic monitoring of temperature (20 $\pm$ 0.3°C) and photoperiod (12 h light/day).

For each metal, EU contamination was based on an initial addition of adapted volumes of aqueous solutions of cadmium ($CdCl_2$, Merck, 10 mg $\cdot$ L^{-1}) or methylmercury (CH_3HgCl, Merck, 5 mg Hg $\cdot$ L^{-1}), in order to obtain nominal concentrations of 20 and 4 µg $\cdot$ L^{-1}, respectively. These conditions were selected based on previous experimental studies, so that after 7 days of exposure bioaccumulation levels in the soft bodies of the bivalves would be compatible with significant trophic transfer studies (Inza *et al.* 1997). Contamination pressure was kept constant throughout the exposure period by means of daily additions of aqueous Cd and MeHg solutions, adjusted according to the decrease in metal concentration, as determined from Cd and Hg concentrations measured on water samples collected at the end of each 24-h cycle.

At the end of the exposure duration, the bivalves were collected and stored at −20°C. Three animals from each EU were used to determine Cd and total Hg concentrations in the soft bodies.

Collection of the Crustacean Predator A. astacus *and Dietary Exposure to Cd and MeHg*

The animals used in this study were specimens of the freshwater crayfish *A. astacus* L., obtained from a breeding company in northeastern France (Frankhauser, Sarreguemines). They were all 1-year-old males. They were acclimatized in the laboratory for a period of 2 weeks; this also allowed them to become used to feeding on defrosted bivalves, which were put into their tanks daily with the shells opened. Preliminary studies have shown that crayfish predation is markedly inhibited if the soft bodies are introduced without their shells.

Before the beginning of the experiment, the animals were sorted to give a homogeneous batch of 18 animals: 10.4 $\pm$ 1.2 g (FW).

The comparative study of trophic transfer of the two metals was based on the crayfish being kept in individual glass containers (12 $\times$ 12 $\times$ 30 cm) containing 3 L of dechlorinated tap water and a sand compartment (5 cm deep). By isolating the organisms in this way, it was possible to monitor very closely the amounts of food consumed by each predator throughout the experiment. The water column in each EU was constantly aerated to ensure that the medium was saturated in oxygen, an important parameter for the ecophysiological requirements of this species. The EUs were placed in regulated tanks similar to those described above; only the temperature was different, in accordance with the thermal preference of this species: 16 $\pm$ 0.3°C.

The crayfish were fed Cd- or Hg-contaminated *C. fluminea,* with a ration of one bivalve per day. The soft body of the bivalve weighed on average 0.4 g (FW), or about 4% of the mean individual mass of the crustaceans. These conditions were similar to the food rations given under semi-intensive breeding conditions. Each bivalve introduced into the EUs was weighed (soft body + shell) after the shell was opened and the water in the palleal cavity eliminated; this enabled us to determine later the biomass ingested by each crayfish.

Each day, two-thirds of the water column in the EUs was renewed, and at the same time, the surface of the sand compartment was cleaned, in order to minimize variations in the abiotic conditions and reduce the risk of contaminating the crayfish via the direct route, possibly via metal transfers in the water column from urine and feces and/or the mastication of contaminated prey.

Three exposure durations were selected for the two metals: 5, 10, and 15 days. Three crayfish were used for each condition, with a total of 18 individuals. After each exposure period, the corresponding batches of crayfish were left in the EUs for 48 h without being fed, in order to ensure that the whole of the digestive tract was emptied and to prevent an overestimation of truly biologically incorporated metal (Langston and Spence 1996). Each crayfish was then sampled and weighed (total FW) after eliminating surface water with absorbent paper. Organs or tissue samples were taken from each individual in order to determine the organotropism of the two metals: hemolymph by aspiration using a 1-ml syringe, with the needle pushed into the articular membrane of the fifth leg; tail muscle (whole); hepatopancreas or digestive gland (whole); gills (all six pairs); stomach (all of the stomacal pouch and mesenteron, which in the crayfish is very small); intestine (whole); green gland (whole); and carapace (sample collected from the dorsal zone of the cephalothorax).

As well as collecting the crayfish, the shells of the bivalves left on the surface of the sand in the EUs were also sampled and weighed to quantify the biomass of the soft bodies consumed by each predator.

Cadmium and Mercury Analysis

The biological samples—whole soft bodies of the clams; organs or tissue samples of the crayfish—were first digested by nitric acid attack (3 ml of pure HNO_3, Merck) in a pressurized medium (borosilicate glass tubes) at 95°C for 3 h. After dilution of the digestates up to 20 ml with ultra-pure water (MilliQ plus), metal concentrations were measured by atomic absorption spectrophotometry. Cd determinations were made on a Varian AA 400 equipped with a model GTA 96 graphite tube atomizer and autosampler. Samples of 10 µl from the diluted digestates were taken for the determination and mixed before atomization with 4 µl of a ''50% Pd + 50% $Mg(NO_3)_2$'' mixture, to facilitate removal of the matrix. The detection limit in our analytical conditions was 0.1 µg $\cdot$ L^{-1}. Total Hg determinations were carried out by flameless atomic absorption spectrometry (Varian M 6000). A bromine salt treatment was applied to water samples and diluted digestates before the addition of stannous chloride (Farey *et al.* 1978). The detection limit was 10 ng Hg $\cdot$ L^{-1} within the analytical range 25–150 ng Hg $\cdot$ L^{-1}. The validity of the analytical methods was checked periodically by means of two standard biological reference materials (TORT-2, lobster hepatopancreas, and DOLT-2, dogfish liver from NRCC-CNRC, Ottawa, Canada). Values for Cd and Hg were consistently within the certified ranges for each element (data not shown).

Data Analysis

Means and standard errors of the mean (SEM) were calculated for all the parameters. Paired comparisons between Cd or Hg concentrations measured in the different control organs and contaminated crayfish (15 days) were made with the Student's *t* test at $p < 0.05$ or 0.1. The effect

of exposure duration on metal bioaccumulation in the organs was analyzed by multiple linear regression (STAT.ITCF software).

Results

Cd and Hg in Prey and Estimation of Dietary Input

After 7 days of exposure, the average metal concentrations measured in the whole soft bodies of *C. fluminea* were 4,850 ± 221 ng · g^{-1} (FW) for Cd and 3,370 ± 79 ng · g^{-1} (FW) for Hg (ratio FW/DW = 5.5). Background average levels were 198 ± 33 ng Cd · g^{-1} and 110 ± 30 ng Hg · g^{-1} (FW). No mortality was observed in the two batches of contaminated clams.

Twice-daily observations carried out on all the EUs containing the crayfish confirmed the efficiency of the predation, as each soft body introduced with its shell was consumed before the new batch was added. Based on the mean concentrations of the two metals measured in the bivalves and on the weight of the soft bodies ingested by each crayfish, the metal burdens brought in via the food could be determined for each of the three exposure durations studied (Table 1). Any differences between the burdens of the two metals added via the prey were fairly low and constant, with the ratio between mean values being about 1.3, in favor of Cd.

Cd and Hg Accumulation in the Crayfish

The distribution of the two metals among the different tissues of the control and contaminated crayfish after 15 days of trophic exposure are shown in Figures 1A (Cd) and 1B (MeHg). Bioaccumulation is expressed as average metal concentrations in the organs. In paired comparisons, Cd distribution in the control and the contaminated crayfish was significantly different in the gills, hepatopancreas, and stomach at $p < 0.05$; in the tail muscle and hemolymph at $p < 0.1$; but the green gland, intestine, and carapace showed no significant differences. The highest Cd concentrations were observed in the different organs of the digestive tract: 2,250 ng · g^{-1} (FW) in the hepatopancreas (×5.3/control), 1,125 ng · g^{-1} (FW) in the intestine (×6.6), and 615 ng · g^{-1} (FW) in the stomach (×7.0). Even though Cd bioaccumulation in the muscle was significantly higher than the background level (×3.8), concentrations measured in this tissue compartment after 15 days of feeding exposure remained very low, at about 20 ng · g^{-1} (FW). As can be seen in Figure 1B, mercury distribution in the principal organs of the crayfish was markedly different from that described earlier for Cd. Mean concentrations measured in the majority of tissues, apart from the hepatopancreas, were significantly greater than the reference values determined in the control animals. The highest concentration was observed in the green gland (2,510 ng · g^{-1}, FW), and then a relatively homogeneous bioaccumulation in the other tissue compartments—gills, tail muscle, hepatopancreas, stomach, intestine—with mean values being between 500 and 1,000 ng · g^{-1} (FW). The most marked differences in relation to the organotropism of Cd were in the green gland, and in particular the muscle (×92/control level). For both metals, concentrations measured in the hemolymph were very low (4 and 16 ng · g^{-1} FW for Cd and Hg, respectively).

Table 1. Quantities of cadmium (Cd, µg) and mercury (Hg, µg) ingested by the crayfish via the contaminated clams during the three exposure durations (mean ± SEM, n = 3)

	5 days	10 days	15 days
Cd burden (µg)	9.31 ± 0.62	17.39 ± 0.16	24.34 ± 1.1
Hg burden (µg)	6.93 ± 0.50	13.54 ± 0.96	19.11 ± 0.53

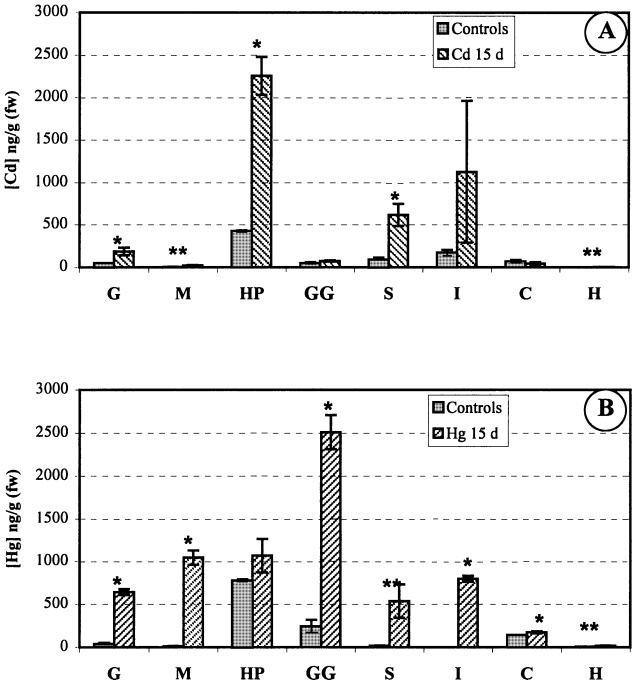

Fig. 1. Mean cadmium (A) and mercury (B) concentrations in the eight organs of the crayfish *Astacus astacus*, measured at time zero (background level/controls) and after 15 days of exposure via the trophic route. G: gills; M: tail muscle; HP: hepatopancreas; GG: green gland; S: stomach; I: intestine; C: carapace; H: hemolymph. Vertical bars: standard error of mean (SEM); *significant differences between controls and contaminated crayfish at $p < 0.05$; **significant differences at $p < 0.1$

The bioaccumulation kinetics of Cd and MeHg in the eight organs studied are shown in Figures 2 and 3, respectively. Note that for the hemolymph, the absence of regression models is due to the loss of samples collected at time 10 days; for the gills and hepatopancreas after Cd contamination (Figure 2), no significant models were obtained, except polynomial model of the third order, which were not representative of the biological processes studied. Once again, very marked differences emerged between the two metals. Observation of trends for Cd concentrations (Figure 2) reveals no significant effect of the variable ''exposure duration'' in the green gland and the carapace. In the case of the other organs, however, with the exception of the tail muscle (exponential trend), the evolution of concentrations shows a plateau tendency after 10 days. A closer analysis of the graphs, especially the comparison between the regression models and the broken lines joining mean concentrations, reveals that for the gills, tail muscle, hepatopancreas, hemolymph, and intestine, there is a very low increase, or even an absence of Cd bioaccumulation after 5 days' contamination of the crayfish via the trophic route.

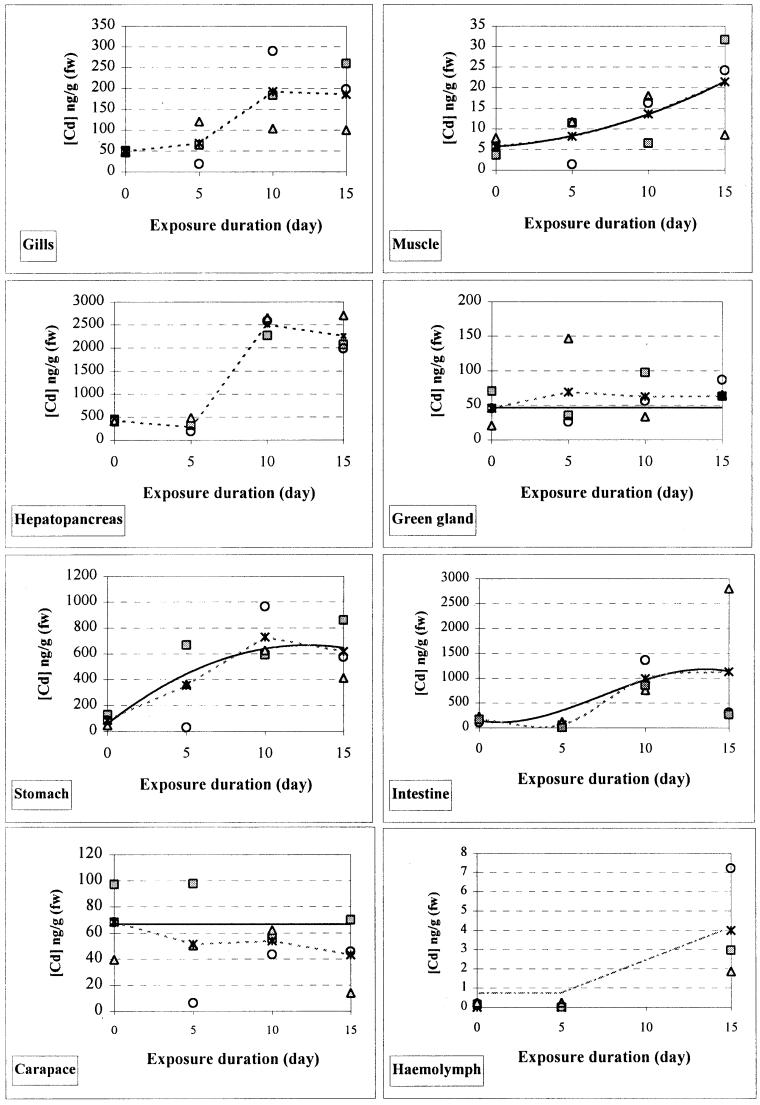

Fig. 2. Cadmium concentrations as a function of trophic exposure duration in the eight organs of the crayfish *A. astacus*. The bold curves represent the regression models ($p < 0.05$) and the broken lines the evolution of the mean measured values. Symbols correspond to the three replicates per exposure duration. $[Cd]_{Gills} = 48.97 - 22.31 * Exp.\ duration + 6.80 * Exp.duration^2 - 0.31 * Exp.duration^3$ ($R = 0.77$). $[Cd]_{Muscle} = 5.68 + 0.25 * Exp.duration + 0.054 * Exp.duration^2$ ($R = 0.71$). $[Cd]_{Hepatopancreas} = 424.08 - 589.87 * Exp.duration + 144.31 * Exp.duration^2 - 6.4567 * Exp.duration^3$ ($R = 0.82$). $[Cd]_{Stomach} = 58.413 - 95.99 * Exp.duration - 3.78 * Exp.duration^2$ ($R = 0.78$). $[Cd]_{Intestine} = 144.68 - 56.37 * Exp.duration + 25.1 * Exp.duration^2 - 1.13 * Exp.duration^3$ ($R = 0.75$). (Exp.duration = exposition duration)

In the case of mercury (Figure 3), concentrations in the majority of the organs (gills, intestine, tail muscle, green gland, stomach) show an increasing linear trend. In the hepatopancreas, bioaccumulation is very low, with the regression model showing no significant influence of the ''exposure duration'' factor. As for Cd, the regression model reveals no significant effect of the exposure duration in the carapace. It is important to stress that unlike Cd, there are no latency phenomena in the bioaccumulation processes, and all the mean concentrations of Hg measured after 5 days of contamination were significantly higher than the control values, with the exception of the carapace and the hepatopancreas.

In parallel with the study of bioaccumulation based on the concentration criterion, analysis of metal burdens in the different tissue compartments provided complementary data, taking into account the very wide disparity between the biomasses. When the organ samples were taken from the crayfish, several were collected whole, and the weight data enables us to calculate directly the Cd and Hg burdens, based on the measured concentrations. For the muscle tissue, the hemolymph, and the carapace, however, only fragments had been taken for metal dosage. In the literature consulted, there was no available data relating to the relative mass of the tissue compartments of the crayfish; we therefore used a batch of animals similar to those used during this experiment, to carry out a very careful dissection of all the muscle tissue—tail muscle, muscles in the five pairs of legs and in the cephalothorax—in order to determine the total biomass of this tissue. The weight of the entire carapace was also estimated (external carapace of the cephalothorax, legs and abdomen, ventral apodemes of the cephalothorax). For the hemolymph, mass was determined at the end of dissection from the difference between the initial weight of the animal and the total weight of all the organs and tissues sampled, taking into account also the quantity of haemolymph drawn off. Table 2 shows the relative mean masses of the different organs or tissues analyzed, based on fresh weight.

Cd and Hg relative burdens in the eight tissue compartments of the crayfish at time zero and after 15 days exposure via the trophic route are shown in Figure 4. It is important to remember that the values of the metal burdens correspond to estimates for the muscle, the hemolymph, and the carapace, which are based

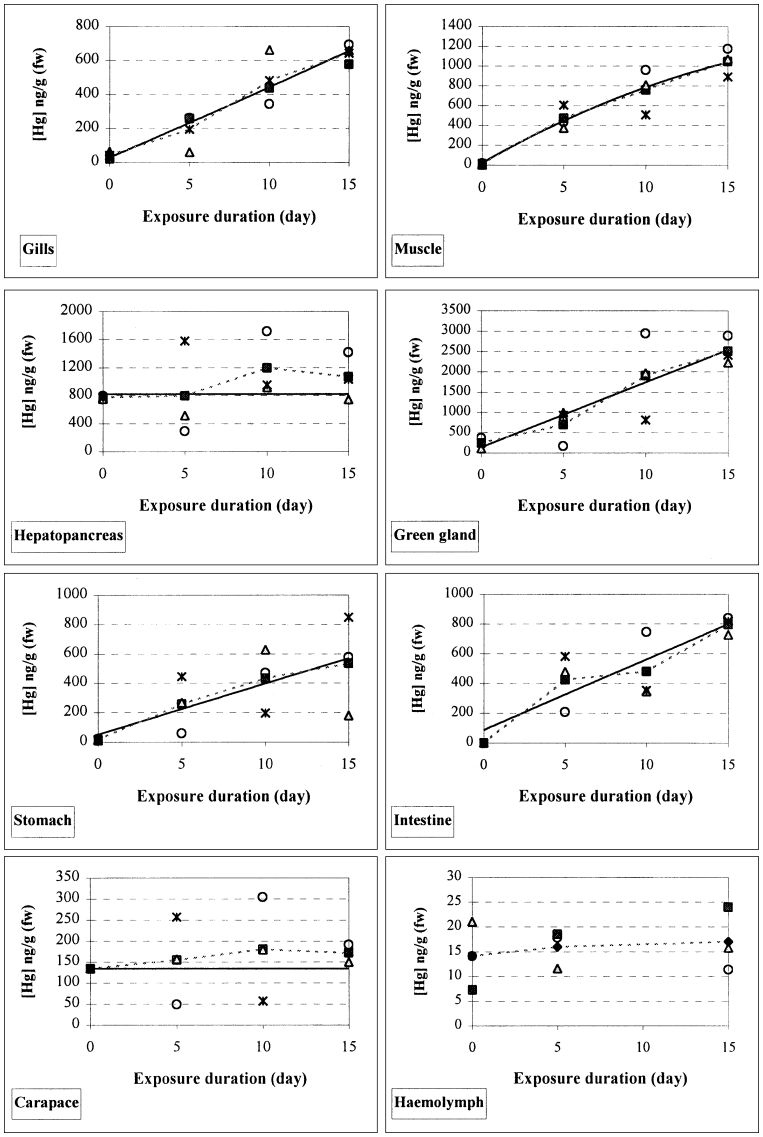

Fig. 3. Mercury concentrations as a function of the trophic exposure duration in the eight organs of the crayfish *A. astacus*. The bold curves represent the regression models ($p < 0.05$) and the broken lines the evolution of the mean measured values. Symbols correspond to the three replicates per exposure duration. $[Hg]_{Gills} = 25.61 + 41.87 *$ Exp.duration ($R = 0.93$). $[Hg]_{Muscle} = 84.5 + 67.791 *$ Exp.duration ($R = 0.94$). $[Hg]_{greengland} = 136.12 + 160.29 *$ Exp.duration ($R = 0.86$). $[Hg]_{Stomach} = 51.32 + 34.525 *$ Exp.duration ($R = 0.72$). $[Hg]_{Intestine} = 87.29 + 47.4 *$ Exp.duration ($R = 0.82$). (Exp.duration = exposition duration)

Table 2. Average relative fresh weight (%) of the different organs or tissue compartments of the crayfish *A. astacus*

Gills	Muscle	Hepatopancreas	Green Gland	Stomach	Intestine	Carapace	Hemolymph
1.9%	15.7%	4%	0.3%	2%	0.5%	47.6%	28%

on extrapolations from concentrations measured in samples taken during dissection and which presuppose a degree of homogeneity of distribution of the two metals throughout the respective tissue compartments.

At time zero, the cadmium is localized for the most part in the carapace, which represents more than 60% of the total burden, on the scale of the whole organism; 30% of the Cd is accumulated in the hepatopancreas. After 15 days of trophic exposure, the two methods of calculating relative burdens with or without deduction of background levels show considerable differences, mainly because of the quantities of metal in the carapace. In view of the relative mass of this compartment (47.6%) and the absence of Cd bioaccumulation at the end of the experiment, the carapace represents 9.5% of the total burden in contaminated organisms when the background level is not deducted and does not appear in the diagram corresponding to relative Cd burdens really bioaccumulated during the experiment. The hepatopancreas plays an important role: it represents almost 80% of the total burden of accumulated Cd in the organisms. The whole of the digestive tract (stomach + intestine) contains 16% of the Cd; the other organs have very low relative burdens, ranging from 2.7% (gills) to 0.08% (green gland).

In the control animals, Hg relative burdens in the carapace and the hepatopancreas predominated and were similar to those observed for Cd (62% and 30%, respectively). After 15 days of trophic exposure, the carapace represented only 26% of the total Hg burden in the organisms. We should recall that the regression model that was established based on data measured during

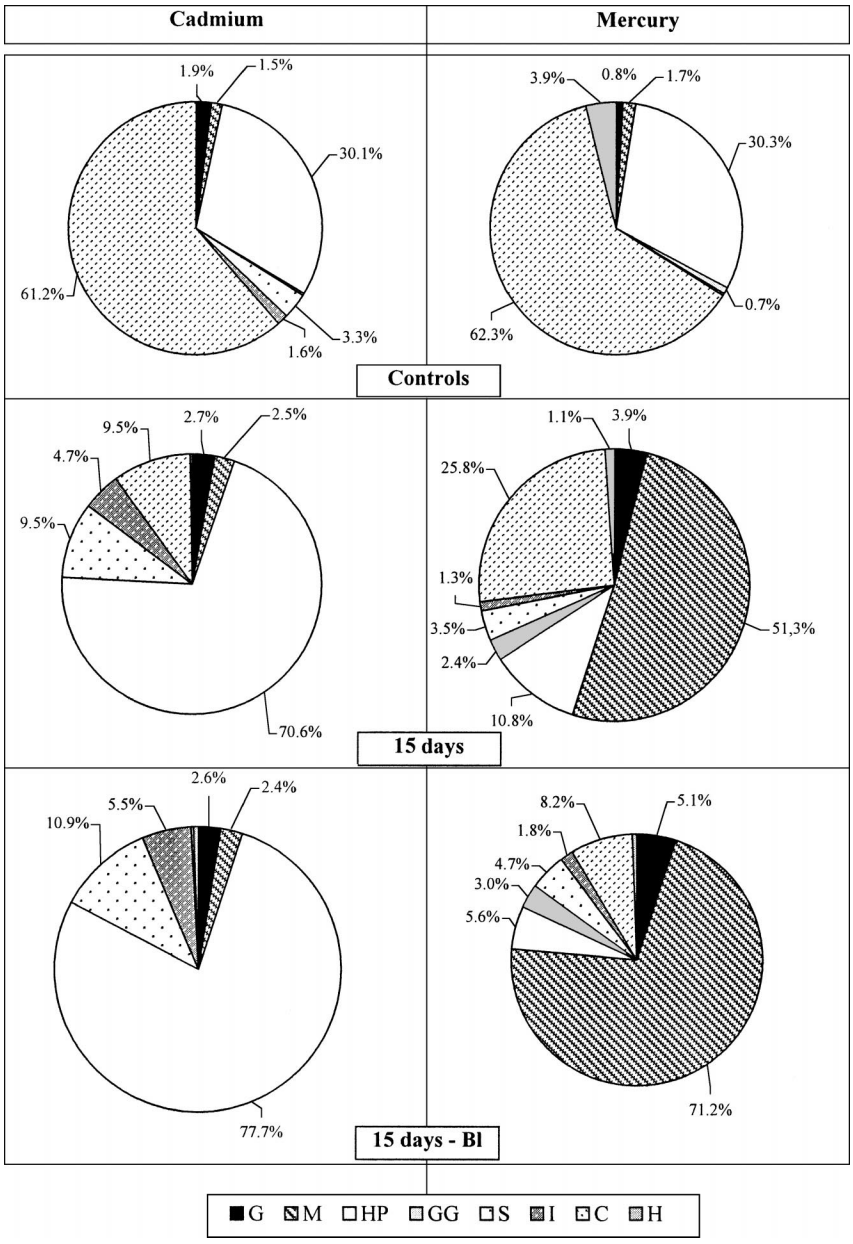

Fig. 4. Cadmium and mercury relative burdens (%) in the organs of the crayfish *A. astacus* at time zero (controls) and at the end of the trophic exposure: measured values (15 days) and measured values after deduction of the background levels (15 days - B1). The diagrams show only the relative burdens >0.7%

the experiment revealed no significant effect of the ''exposure duration'' factor on Hg concentrations in the carapace (Figure 3); in this instance, as was the case for Cd, the relative burden, after deduction of the background level, should be nil. In fact, the values measured indicate a slight increase in concentrations (Figure 1B), which is amplified when the burdens are calculated, due to the large biomass of this tissue; thus, the carapace represents 8% of Hg accumulated in the organisms at the end of the experiment. Unlike Cd, the predominant tissue compartment is the muscle, which contains more than 70% of the Hg total burden, after deduction of the background level, whereas the average relative burden in the hepatopancreas is less than 6%.

The marked differences in relative burdens between control and exposed crayfish could be linked to the exposure conditions during the rearing or farming period (indoor for the young crayfish and outdoor ponds for the adult stage). Thus, for Cd, uptake from water could be the predominant exposure route for the controls. For mercury, data suggest direct or/and trophic uptake of inorganic Hg.

*Estimation of Cd and Hg Transfer Rates Between Prey (*C. fluminea*) and Predators (*A. astacus*)*

The rates of trophic transfer of the two metals were determined from the total burdens accumulated in all the tissue compartments of the crayfish, background levels having been previously deducted, and burdens brought in via the soft bodies of the bivalves. As mentioned above, these calculations are based on extrapolations, especially for the carapace compartment, the hemolymph, and the muscle. For the first two of these, potential errors are negligible, given the very low level or the absence of bioaccumulation of the two metals in our experimental conditions; for the muscle, on the other hand, the quantities of Hg are very important.

After 10 and 15 days of contamination, the mean rates of Cd transfer between prey and predator were 6.0% and 4.4%, respectively. After 5 days, on the other hand, the mean rate estimated was very much lower, at less than 1%. For the MeHg, values after 5, 10, and 15 days were 14.5%, 18.5%, and 15.7%, respectively.

Discussion

Average transfer rates of cadmium between the soft bodies of *C. fluminea* and the crayfish, estimated after 10 and 15 days of contamination at the whole organism level and after a 48-h delay following the last food intake, are close to 5%. This value is low, but is nevertheless in agreement with the data available in the relevant literature, which show assimilation efficiencies of less than 10% (Shaikh and Smith 1980); thus, the maximum dose tolerable to man of 1 μg Cd · kg^{-1} · day^{-1}, as defined by the World Health Organization, is based on the postulate that 5% of ingested Cd is absorbed independently of the dose of ingested metal (WHO 1992). Note, however, that data giving a quantitative estimate of Cd transfer rates between contaminated prey and aquatic invertebrates are rare. In fact, the majority of published studies are based on measurements of concentrations of the metal in prey and predators, at whole organism and/or organ level, without determining Cd burdens, from which transfer rates could be calculated.

Thus, our results show that, despite the high bioaccumulation capacity of Cd in the soft body of *C. fluminea*, metal quantities accumulated in the crayfish are fairly limited overall and the risks of transfer in respect of predators are therefore low. In relation to humans, these risks are even smaller, because when crayfish is eaten, most of the ingested food consists of muscle tissue—tail muscle and muscles inside the claws at the end of the first pair of locomotive legs—where Cd concentrations are very low (20 ng · g^{-1} FW after 15 days). Nevertheless, Cd concentrations are exponentially increasing in the crayfish muscle tissue; the contamination period adopted in the present feeding experiment being quite low, it could be under much longer field exposure conditions a significant increase of the metal food chain transfer. However, the limits allowed for food consumption of Cd in molluscs, crabs, or other crustaceans are between 1 and 2 μg · g^{-1} FW; for the fish muscle, the norm is 0.1 μg · g^{-1} FW (Boisset 1996). At the end of the experiment, concentrations measured in the hepatopancreas were greater than 2,000 ng · g^{-1} (FW) or 10,600 ng · g^{-1} (DW), as this tissue compartment contains 80% of the total amount of metal accumulated in the whole organism. These results are in agreement with data published on other aquatic species: for example, crabs (*Carcinus maenas*) fed with Cd-rich *Artemia salina* (Jennings and Rainbow 1979); Norway lobsters (*Nephrops norvegicus*) fed with liver tissue from albatross (*Diomedea exulans*) (Canli and Furness 1995); lobsters (*Panulirus cygnus*) fed with Cd-enriched mussels (*Mytilus edulis*) (Francesconi *et al.* 1994); and red swamp crayfish (*Procambarus clarkii*) fed with contaminated duckweed (*Lemna gibba*) (Devi *et al.* 1995). In our experimental conditions, Cd concentrations measured in the hepatopancreas after 15 days can lead to toxicological effects; indeed, in *A. astacus*, Cd concentrations of almost 500 ng · g^{-1} (FW) in the hepatopancreas, after exposure via the water source for 10 weeks, can lead to structural impairments and the lowering of oxidative enzyme activities (Meyer *et al.* 1991). However, several studies have shown the high induction capacities of the biosynthesis of metallothioneins in crustaceans in response to an intracellular accumulation of Cd in the digestive gland (Roesijadi 1992); Cd complexes with these cytosolic proteins have been found in two genera of crayfish: *Procambarus clarkii* (Martinez *et al.* 1993) and *Austropotamobius pallipes* (Lyon 1984). Given their capacity for Cd sequestration and because the entry flow of the metal is compatible with the biosynthesis rates of metallothioneins, below the spillover situation, these cytosolic proteins may take on a protective role vis-à-vis other cell sites (enzymes, organite membranes, nucleic acids, etc.) (Roesijadi 1992).

The kinetic approach to the evolution of Cd concentrations in the different organs of the crayfish suggests the attainment of steady state in metal bioaccumulation in several organs after 10 days' exposure: gills, hepatopancreas, stomach, intestine. Given that the time of steady state is mostly governed by the rate of depuration, these results indicate a rapid depuration of Cd from the organisms. The kinetic approach also reveals a very low rate of bioaccumulation or even an absence during the first 5 days of exposure via the trophic route: only the stomach presented an overall increase in Cd concentrations (×7). It is difficult to account for this phenomenon, given that the contamination conditions for the crayfish are based for the first exposure duration on five successive additions of prey rich in Cd. At the present time, our knowledge of the structure of the digestive tract in decapod crustaceans and the mechanisms involved in digestion show that during the first stage (about 12 h in the crab *Carcinus maenas*) the food is ground up in the gastric mill, mixed with enzymes produced by the hepatopancreas, and sorted into two fractions: (1) the coarse fraction, containing all particles greater than 100 nm in diameter, is passed directly to the midgut; (2) the fine particles and liquids enter the hepatopancreas, where peritrophic membranes prevent particulate material from contacting the epithelium. During the second stage (12–48 h after feeding), numerous mature B cells from the hepatopancreas are extruded into the lumen, isolated from the epithelium by the production of a new peritrophic membrane, transferred into the midgut, and then voided. During the last stage, all the material derived from the hepatopancreas is passed to the hindgut and then voided (Barker and Gibson 1977; Hopkin and Nott 1980). Given these processes, the fact that we observe an accumulation of Cd solely in the stomach and the mesenteron (sampled together) after the first five feeds could derive from direct transfers of bioavailable Cd in the gastric cavity, as a result of acidic pH conditions and the enzymatic digestion within this organ; nevertheless, the majority of the ingested metal is not fixed in this part of the digestive tract, and the estimated transfer rate indicates that more than 90% of ingested metal is eliminated, probably via the feces. It is difficult to imagine that there is much Cd absorption through the digestive barrier during this phase, absorption that would then be counterbalanced by the almost total elimination via other excretory routes, without there being a significant degree of accumulation in the hepatopancreas. The digestive gland is involved in nutrient absorption, storage, transport to the hemolymph, and the synthesis of proteins/enzymes for secretion. This absence of Cd accumulation in the hepatopancreas after 5 days and, in contrast, the high concentrations observed after 10 days (×8) could be attributed to an evolution of the metal fixation sites in the digestive gland of the crayfish, where two types of sites are distinguished: during the first phase, the Cd

may be sequestrated on the structures at the interface with the lumen of the hepatopancreatic tubules, which are rapidly extruded after feeding (peritrophic membrane, B cells), and these may be responsible for the elimination of the Cd after the 48-h delay following the last feed; during the second phase, after the fifth feed in our experimental conditions, the Cd could reach more stable cell structures (with a longer lifespan) in the hepatopancreatic tissue, such as the R cells, with some of the metal crossing the digestive barrier and reaching the hemolymphatic compartment, with transport into other organs (gills, muscle) where metal concentrations increase significantly during the last 10 days of exposure. Histochemical observations of Cd accumulation in the crayfish *A. astacus*, based on the autometallographic procedure of Danscher (1984), have shown that Cd deposits in the digestive gland were found in the apical cytoplasm of the epithelial cells, mostly the R cells, after 10 weeks of exposure (Meyer *et al.* 1991).

The absence of any accumulation of Cd in the carapace during the 15 days of exposure indicates that contamination of the crayfish was predominantly via the trophic route. Indeed, when the crustaceans are exposed to Cd from the surrounding medium, concentrations measured at the level of the carapace are high (Meyer *et al.* 1991; Canli and Furness 1995). After contamination via the direct route in *A. astacus*, relative burdens of Cd in the gills were similar to those measured in the hepatopancreas (Meyer *et al.* 1991). In our exposure conditions, Cd concentrations measured in the gills after 15 days were about 10 times lower than those measured in the hepatopancreas, thus confirming the predominance of the trophic route. In these conditions, the Cd accumulated in the gills derives from an internal source, via the hemolymph. Metal transfers of this kind have been seen in the green crab (*Carcinus maenas*) after injection into the hemolymph or perfusion of isolated gills (Laporte 1997). Cd concentrations and burdens measured in the crayfish hemolymph after the three exposure durations studied in our experiment were very low: in fact, the hemolymph very quickly deals with the transfer of the metals between the uptake routes into the organism and the storage compartments; it has a negligible sequestration capacity compared with the metal burdens accumulated at whole organism level (Rtal and Truchot 1996; Laporte 1997).

Our results revealed no significant increase in Cd concentrations in the green or antennal glands. However, such glands play an important role in the excretory processes in crustaceans, the urinary fluid produced originating from filtration, resorption, or secretion of selected substances. The excretory organs usually accumulate large quantities of Cd, especially in fish and mammals: for example, uptake and tissue distribution of dietary Cd by carp (*Cyprinus carpio*) show that the kidneys have the second highest accumulation levels, less than the gut and a long way ahead of the liver (Kraal *et al.* 1995). Several hypotheses can be put forward in the case of the crayfish, in particular the excretion of Cd by other routes (nephrocytes in the gills, integument, digestive glands) or a delayed role on the part of the green gland, as the Cd does not accumulate in this excretory organ until after a longer exposure duration.

Our experimental approach to mercury trophic transfers between soft bodies of *C. fluminea* previously exposed via the direct route to the methylated form of the metal and crayfish shows a mean transfer rate of 16%. This transfer rate may be considered low compared with available data in the literature on this organomercurial, based on experimental approaches (Boudou and Ribeyre 1997). For example, after 30 days, rainbow trout alevins (*Onchorynchus mykiss*) fed with daphnia previously exposed to MeHg or HgII showed mercury transfer rates close to 90% and 40%, respectively (Boudou and Ribeyre 1997). Results from Mason *et al.* (1996) showed that MeHg in a coastal diatom was assimilated by zooplanktonic copepods four times more efficiently than was inorganic mercury; the trophic transfer rates were 62% and 15%, respectively. A possible demethylation of the MeHg during exposure of the bivalves (enzymatic transformation by bacteria or photochemical mechanisms) and/or during bioaccumulation in the different tissue compartments of the soft body, as has been described for several fish species (Jackson 1998), could be the reason for the transfer rates obtained in our experimental conditions. Nevertheless, the daily additions of MeHg into the tanks containing the clams, in order to compensate for the decrease in the metal concentrations in the water column, and the short exposure duration (7 days) tend to minimize the importance of the demethylation processes. Moreover, the organotropism of the metal in the crayfish, in particular the accumulation in the muscle compartment, which represents more than 70% of the total burden at whole organism level, follows a similar pattern. The structural and functional properties of the absorption zones on the digestive barrier of the crayfish may also play an important role in mercury assimilation efficiency. In contrast to the results from Cd, Hg burdens in the hepatopancreas, which in *A. astacus* represents the preferred assimilation route of the nutrients, are very low (6% after deducting background level). Several experimental approaches have shown, especially in fish, that the intestinal barrier is very permeable to the methylated form of Hg, whereas the rate of assimilation of HgII is very low, less than 10%. In this case, the MeHg would be rapidly transferred into the other tissue compartments, via the hemolymph, with a very low level of storage in the hepatopancreas after 15 days of trophic exposure. As was the case for the Cd, concentrations of Hg in the carapace did not increase significantly during the 15 days of contamination, thus indicating the minor role played by indirect contamination, via the presence of Hg in the water column. The background level of the gills, on the other hand, increased by a factor of almost 15; as was shown earlier, the hypothesis of metal transfer via the hemolymph may be put forward, given the abundant irrigation of the gill lamella.

Unlike the Cd, the linear increase for MeHg concentrations in the majority of the organs suggests a very slow depuration rate, which is consistent with the literature (Boudou and Ribeyre 1997). High accumulation of MeHg in the muscle of the crayfish may represent an important trophic transfer source for the predatory species and particularly for humans, who consume this tissue almost exclusively when eating these crustaceans. This transfer potential is all the greater when the evolution trend of the mercury concentrations in the tail muscle is almost linear, thus increased bioaccumulation can be expected when the exposure duration should increase.

Our results also revealed in the organs of *A. astacus* a significant increase in Hg concentrations from time 5 days, with the exception of the hepatopancreas and the carapace. The absence of a latency period for the methylmercury was also observed in shrimp *Crangon crangon* fed with contaminated

mussels: Hg accumulated immediately in numerous organs (Riisgard and Famme 1986).

Conclusion

The importance of food as a source of trace metal uptake in aquatic organisms depends on a combination of biological characteristics of the prey and predator species, as well as the chemical nature of the metals involved. Our experimental study of cadmium and methylmercury trophic transfers between the Asiatic clam *C. fluminea* and the crayfish *A. astacus* shows marked differences in assimilation efficiencies—5% for Cd and 16% for Hg—and also in the metal distribution within the different tissue compartments of the predator. From a toxicological point of view, the trophic intake of Cd leads to a high degree of accumulation in the digestive gland of the crayfish, which can give rise to structural and functional disturbances in this organ, depending on the efficiency of the sequestration processes of the metal, notably via the induction of metallothionein biosynthesis. On the other hand, the risks of transfer into the predators, humans included, are relatively small, as after 15 days of contamination the muscle tissue contains only 2.5% of the Cd burden in the whole organism. For the MeHg, on the other hand, the highest concentrations are observed in the green gland, the excretary organ in crustaceans, and, taking into account the background levels, the highest level of bioaccumulation occurs in the tail muscle ($\times 90$) with a tendency for concentrations to increase close to linearity; this organotropism represents an obvious risk of mercury transfer into the predators and in particular into man.

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