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Kinetics and Body Distribution of Waterborne $^{65}Zn(II)$, $^{109}Cd(II)$, $^{203}Hg(II)$, and $CH_3{}^{203}Hg(II)$ in Phantom Midge Larvae (*Chaoborus americanus*) and Effects of Complexing Agents

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Uptake, elimination and body-distribution of waterborne $^{65}Zn(II)$, $^{109}Cd(II)$, $^{203}Hg(II)$, and $CH_3{}^{203}Hg(II)$ were studied in fourth instar larvae of phantom midge, *Chaoborus americanus*, using a two-compartment kinetic model and whole-body autoradiography (WBARG). The effects of complexation by sodium diethyldithiocarbamate (DDC) and humic material (HM) were also evaluated. Uptake of Hg(II) and $CH_3Hg(II)$ from water was 20−240 times higher after a 1 week exposure compared to Cd(II) and Zn(II). Unexpectedly, $CH_3Hg(II)$ uptake rate was 5 times slower than inorganic Hg(II). WBARG showed a strong $CH_3Hg(II)$ gradient between organs and haemolymph, indicating that its slower accumulation may be related to a slower rate of translocation within the body rather than to a difference in overall lipid solubility compared to Hg(II). DDC doubled the uptake rate of both Hg forms, probably as the result of lipophilic complexes formation, but its effect on Zn(II) and Cd(II) uptake was negligible. HM decreased uptake rate of Hg(II) by a factor 50, whereas it increased $CH_3Hg(II)$ uptake by 30%. These results cannot be explained solely from the complexation of dissolved Hg(II) and $CH_3Hg(II)$ by HM. They indicate that HM adsorbed on aquatic organisms could also directly affect the uptake process at the water−larvae interface.

Introduction

Insects represent one of the most common and diverse group of freshwater animals (*1*), and larval stages of aquatic insects readily accumulate metals from water (*1*−*3*). Uptake mechanisms of trace metals in insects have been investigated through the study of kinetics and body distribution, using one-compartment models and microdissection (*1*). However, microdissection techniques are limited to the largest body components, such as the gut. It is also well-known that the metal content of an animal is often not kinetically homogeneous, but partitioned between pools, or compartments, having different elimination rates (*4*). In such cases, a one-compartment model is a simplification that may be erroneous depending on the time scale considered. Thus, more detailed and precise data about fine-scale distribution and kinetics are needed to investigate more thoroughly the fate of trace metals in aquatic insects.

We compared the distribution and the kinetics of waterborne $^{65}Zn(II)$, $^{109}Cd(II)$, $^{203}Hg(II)$, and $CH_3{}^{203}Hg(II)$ in fourth instar larvae of phantom midge larvae (*Chaoborus americanus*), an important pelagic predator in lakes and ponds (*5*, *6*). We used whole-body autoradiography (WBARG) to examine the distribution of radiolabeled compounds in thin cryosections of whole larvae (*7*) and a two-compartment model to quantify uptake and elimination kinetics (*8*). As complexing agents can greatly affect the bioavailability of metal, we also studied the effects of humic material (HM) and sodium diethyldithiocarbamate (DDC) on the uptake of the above metals by larvae. Humic materials, responsible for the yellowish color of water in forest areas, are natural phenol-carboxylate polyelectrolytes able to complex metallic ions (*9*) which reduce metal uptake by aquatic organisms to various extents (*10*). Dithiocarbamates are complexing agents widely used as accelerators in the rubber and plastic industries and as pesticides (*11, 12*). DDC forms with most divalent metals neutral lipophilic complexes that passively cross biological membranes and increase uptake of waterborne metals by aquatic organisms (*13, 14*).

TABLE 1. Total Metal and Complexing Agent Concentrations and Metal-Complexing Agent Combinations Employed with *Chaoborus americanus*[a]

	metal alone (2 nmol L^{-1})[d]	metal (2 nmol L^{-1}) + DDC (50 nmol L^{-1})	metal (2 nmol L^{-1}) + HM (0.5 mg of C L^{-1})
Zn(II)[b]	X	X	
Cd(II)[b]	X	X	
Hg(II)[c]	X	X	X
$CH_3Hg(II)$[c]	X	X	X

[a] Experiments on Hg(II) and $CH_3Hg(II)$ uptake were performed in December 1994, while those on Zn(II) and Cd(II) uptake and excretion of the four metals took place in March 1995. [b] ^{65}Zn and ^{109}Cd = 7.4 kBq L^{-1}. [c] ^{203}Hg = 8.9 (December) or 27.4 (March) kBq L^{-1}. [d] Speciation of metal alone (46−47). Hg(II): 98% $Hg(OH)_2$, 2% HgOHCl. $CH_3Hg(II)$: 97% CH_3HgOH, 3% CH_3HgCl. Cd(II): 93% Cd^{2+}, 5% $CdHCO_3^+$, 1% $CdSO_4$. Zn(II): 96% Zn^{2+}, 1% each of $ZnOH^+$, $ZnSO_4$, $ZnHCO_3^+$, and $ZnCO_3$. See discussion for speciation in the presence of DDC and HM.

Experimental Section

Experiments. $^{203}HgCl_2$ (4.1 and 13.7 MBq $mmol^{-1}$), $^{65}ZnCl_2$ (3.2 GBq $mmol^{-1}$), $^{109}CdCl_2$ (15.4 MBq $mmol^{-1}$), and DDC were purchased from regular commercial sources. Radioactive $CH_3{}^{203}Hg(II)$ was synthesized in our laboratory (*15*). Allochtonous HM, which constitutes an important proportion of dissolved organic carbon in natural waters (*10*), was extracted from a soil sample collected in a fir forest with experimental water maintained at pH 7.4. This allowed the extraction of both humic and fulvic acid fractions, as operationally defined by Aiken et al. (*10*). HM concentration in the filtrate, measured as nonpurgeable organic carbon, was 104 mg of C L^{-1}.

Experimental schedule and conditions regarding metals and complexing agents concentrations as well as the metal-complexing agent combinations used are shown in Table 1. Exposure and elimination experiments were done in all-glass aquaria containing 100−250 larvae in 20 L of aerated freshwater (pCl = 4.2, pH 7.11 ± 0.14, T = 10.0 ± 0.5 °C). The larval densities were in the range of natural values found for

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other *Chaoborus* species (*16*). Larvae were acclimated to experimental conditions for 1 day prior to experiments. Metals and complexing agents were added to water 1 h before introduction of larvae. Exposure experiments lasted for 1 week. For elimination experiments, larvae were first exposed for 1 week to metals and then transferred to clean water for 8−20 days. Water in the aquaria was not renewed during exposure experiments, while it was renewed at least every day during elimination experiments to approximate an open system with no recycling of the metal. Larvae were not fed to avoid the trophic uptake of metal adsorbed on food. Although starvation may have resulted in some stress, mortality remained <10%, except for the group exposed to $CH_3Hg(II)$ + DDC.

Larvae were sampled with a Pasteur pipet, transferred to polypropylene counting tubes, and rapidly rinsed with clean water. Triplicate samples of two to three active, healthy-looking larvae were collected in the case of those exposed to Hg(II) or $CH_3Hg(II)$. Due to the low amount of radioactivity accumulated, a single sample of 15 larvae was collected for the Zn(II) and Cd(II) exposures. Water samples were also collected. The radioactivity of samples was determined by γ-spectrometry, and counts per minute (cpm) obtained were corrected for background, isotope decay, and sample geometry. After γ counting, larvae were homogenized in the tubes with 1 mL of 0.3 M NaOH containing 1% deoxycholic acid and the protein content of the resulting solution was determined (*17*). To convert protein measurement results to larvae dry weight, larger larvae samples (3−5 g) were freeze-dried and the protein content measured as above.

The metal content of larvae from exposure experiments (V_{ACC}) was expressed as an equivalent volume of exposure water per gram dry weight (dw) following

$$V_{ACC}\ (\text{mL of water g}^{-1}\text{dw}) = \frac{\text{cpm in sample} \times (\text{mg of protein/mg dw}) \times 1000}{\text{cpm mL}^{-1}\ \text{water at } t = 0 \times \text{mg of protein in sample}} \quad (1)$$

V_{ACC} is a volume of distribution that expresses the affinity or capacity of an aquatic animal for a particular metal in term of the equivalent volume of exposure water holding the same quantity of metal (*8*). For elimination experiments, metal content of larvae sampled at time t, Q_{EXC}, was expressed as a proportion of the metal content measured in larvae sampled at time 0. Larvae exposed to metals for 1 week were used for WBARG according to Ullberg et al. (*7*).

Kinetic modeling. Although various two-compartment models exist (*18*), they can be empirically described with the following biexponential equation:

$$V_{ACC} = W[BCF_{1SS}(1 - e^{-ct}) + BCF_{2SS}(1 - e^{-dt})] \quad (2)$$

where W equals 1 mL, BCF_{1SS} and BCF_{2SS} are steady-state bioconcentration factors (unit of gram^{-1} dry weight), whereas c and d (h^{-1}) are first-order apparent rate constants. Metal uptake in larvae must be negligible compared to quantity of metal in the water in the exposure vessel for this equation to be valid. The empirical biexponential expression for elimination in a two-compartment system is

$$Q_{EXC} = Q_1(0)e^{-ct} + Q_2(0)e^{-dt} \quad (3)$$

where $Q_1(0)$ and $Q_2(0)$ (unitless) represent the relative metal content at the beginning of the elimination period of the fast (Q_1) and slow (Q_2) eliminating metal pools. General eq 2 and 3 , or some modified forms that accounted for particular situations (see Results), were used to calculate values of BCF_{1SS}, BCF_{2SS}, $Q_1(0)$, $Q_2(0)$, c, and d from experimental data by nonlinear regression analysis (NLRA) using the software STATGRAPH.

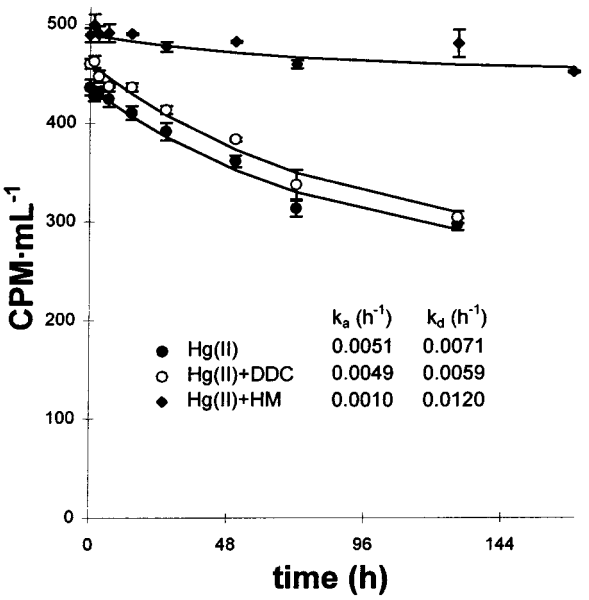

FIGURE 1. Radioactivity in water versus time during uptake experiments with inorganic Hg(II). Fitted curves were obtained by NLRA with eq 4.

Results

During exposure experiments, the radioactivity of water samples taken throughout the experiments was virtually constant for all metals (mean coefficient of variation 2.4%) except for inorganic Hg(II) (Figure 1). These losses of dissolved Hg, probably resulting from adsorption on glass, can be modeled as a function of time t with (*18*)

$$W = \frac{W_0}{(k_a+k_d)}(k_d + k_a e^{-(k_a+k_d)t}) \quad (4)$$

where W_0 is the quantity of metal in water at time 0. Rate constants k_a and k_d characterize adsorption and desorption processes, and their values were calculated by NLRA (Figure 1). Adsorption of Hg(II) was little affected by DDC, whereas HM strongly reduced it.

Exposure and Elimination Kinetics. Accumulation of Zn(II) and Cd(II) was low and rapidly reached a plateau (Figure 2). For both metals, only one kinetic compartment was detectable (Table 2). The value of BCF_{1SS} for Cd(II) was approximately three times higher than for Zn(II). The addition of DDC to water did not have an important effect on the accumulation of Cd(II) and Zn(II).

Accumulation of both Hg forms was much higher than for Zn(II) and Cd(II), V_{ACC} values at the end of exposure period being 20−240 times higher (Figure 2). Two modifications of eq 2 were introduced to quantify Hg(II) kinetics. Rate constant d was too small to be determined with the present set of data, so the term $BCF_{2SS}(1 - e^{-dt})$ was replaced by bt, in which b (units of gram^{-1} dry weight hour^{-1}) is the apparent uptake rate constant. W was substituted by its value expressed in eq 4 to account for the adsorption of Hg(II) on aquarium walls. This gives

$$V_{ACC} = \frac{W_0}{(k_a + k_d)}(k_d + k_a e^{-(k_a+k_d)t})[BCF_{1SS}(1 - e^{-ct}) + bt] \quad (5)$$

in which W_0 is also equal to 1 mL. Values of k_a and k_d used were those showed in Figure 1. Fitted curves calculated with eq 5 agreed well with experimental data from inorganic Hg(II)

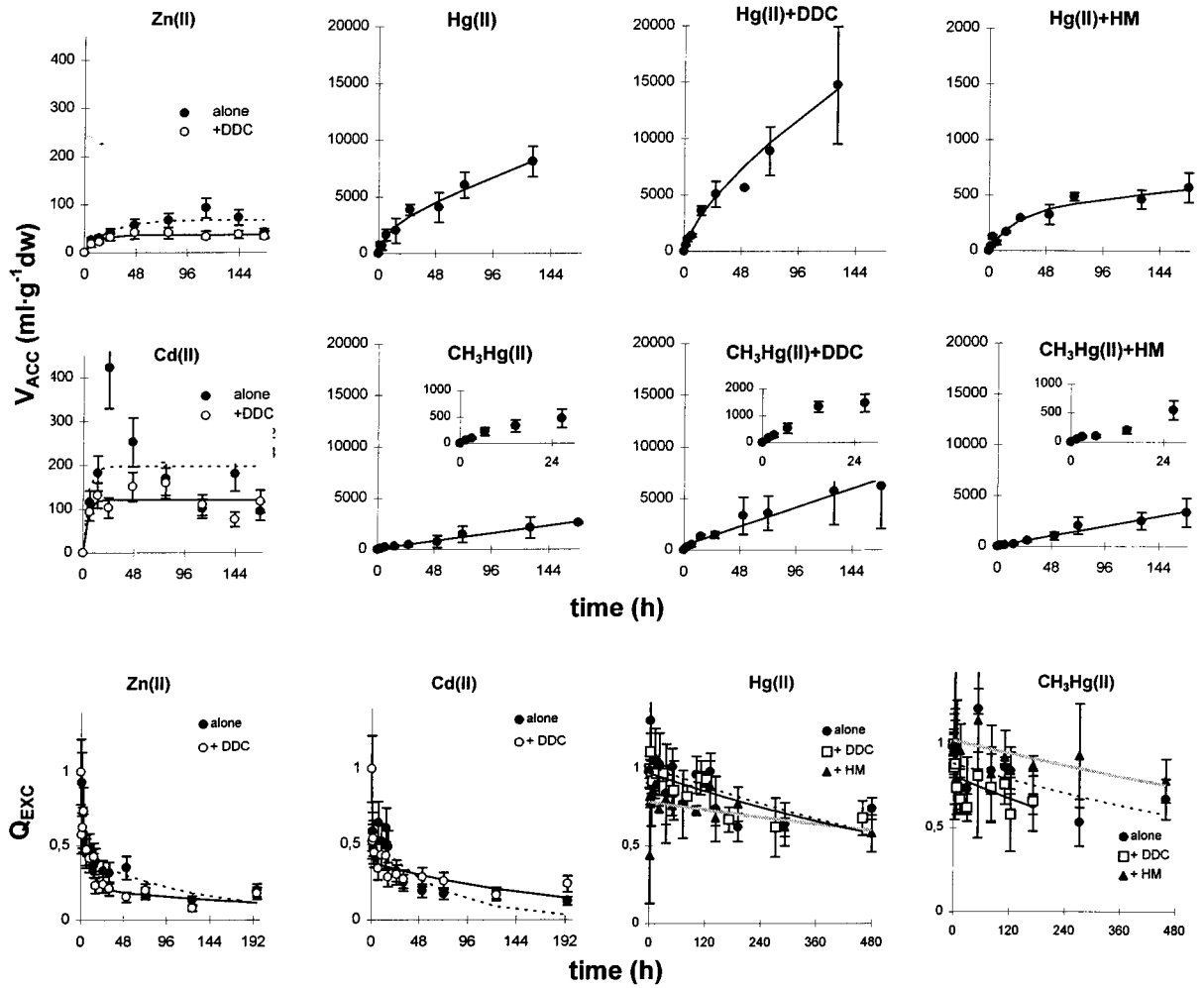

FIGURE 2. ^{65}Zn(II), ^{109}Cd(II), ^{203}Hg(II), and $CH_3{}^{203}$Hg(II) content of *Chaoborus americanus* larvae during uptake (V_{ACC}) and elimination (Q_{EXC}) experiments. Data points and bars are mean ±SD ($n = 3$) for Hg(II) and CH_3Hg(II). Data points for Cd(II) and Zn(II) are from a single sample and bars represent the sum of the mean coefficients of variation of protein measurement in experimental animals and dried larvae. Fitted curves for uptake were obtained by NLRA with eq 5 for Hg(II), by LRA for CH_3Hg(II), and by NLRA with eq 2 for Zn(II) and Cd(II) (dotted line for metal alone, solid line for metal + DDC). Fitted curves for elimination were obtained by NLRA with eq 3 for Zn(II) and Cd(II) and by LRA for Hg(II) and CH_3Hg(II) (dotted line for metal alone, solid line for metal + DDC, gray solid line for metal + HM).

TABLE 2. Values of r^2 for Fitted Curves in Figure 2 and Values (±SE) of Model Parameters for the Uptake and Elimination of Zn(II) and Cd(II) by *Chaoborus americanus* Larvae, Calculated by NLRA with Eqs 2 and 3, Respectively

uptake	r^2	BCF_{1SS} (g^{-1} dw)	BCF_{2SS} (g^{-1} dw)	c (h^{-1})	d (h^{-1})
Zn(II) alone	0.73	68 ± 7		0.05 ± 0.02	
Zn(II) + DDC	0.92	37 ± 2		0.09 ± 0.02	
Cd(II) alone	0.23	198 ± 42		0.22 ± 0.31	
Cd(II) + DDC	0.68	121 ± 11		0.26 ± 0.19	

elimination	r^2	$Q_1(0)$	$Q_2(0)$	c (h^{-1})	d (h^{-1})
Zn(II) alone	0.93	0.60 ± 0.07	0.43 ± 0.04	0.4 ± 0.1	0.007 ± 0.002
Zn(II) + DDC	0.85	0.62 ± 0.11	0.21 ± 0.10	0.12 ± 0.05	0.003 ± 0.005
Cd(II) alone	0.84	0.43 ± 0.11	0.57 ± 0.05	3.1 ± 5.8	0.015 ± 0.004
Cd(II) + DDC	0.90	0.61 ± 0.07	0.38 ± 0.03	1.0 ± 0.3	0.005 ± 0.002

experiments. The two-step process, i.e., nonlinear initial uptake followed by slower and almost linear uptake, was clearly evidenced. The apparent uptake rate constant b for the Hg(II) + DDC group doubled that observed for larvae exposed to Hg(II) alone, whereas values of BCF_{1SS} and rate constant c were similar (Table 3). The addition of HM to water resulted in a much slower accumulation of Hg(II) by larvae.

The accumulation of CH_3Hg(II) was lower than that of Hg(II) (Figure 2). It was not possible to use eqs 2 or 5 with data obtained from the CH_3Hg(II)-exposed groups as no convergence was found when performing NLRA. Linear regression analysis (LRA) was then used to determine the apparent uptake rate constant with $V_{ACC} = Wbt$. The value of b for larvae exposed to CH_3Hg(II) alone was 5.5 times lower compared to inorganic Hg(II) (Table 3). The presence of DDC increased uptake rate by a factor 2.5. HM resulted in a 30% increase of b.

Data from elimination experiments (Figure 2) show that for Zn(II) and Cd(II), with or without DDC, larval metal

TABLE 3. Values of r^2 for Fitted Curves in Figure 2 and Values (±SE) of BCF_{1SS}, c, and b Calculated for *Chaoborus americanus* Larvae Exposed to Hg(II) and $CH_3Hg(II)$[a]

	r^2	BCF_{1SS} (g^{-1}dw)	c (h^{-1})	b (g^{-1}dw h^{-1})
Hg(II) alone	0.98	1590 ± 550	0.16 ± 0.12	84 ± 10
Hg(II) + DDC	0.99	1290 ± 640	0.16 ± 0.18	159 ± 10
Hg(II) + HM	0.96	350 ± 100	0.05 ± 0.02	1.5 ± 1.0
$CH_3Hg(II)$ alone	0.99			15.4 ± 0.5
$CH_3Hg(II)$ + DDC	0.95			38.2 ± 3.0
$CH_3Hg(II)$ + HM	0.97			20.3 ± 1.0

[a] Data for Hg(II) were fitted by NLRA using eq 5. Data for $CH_3Hg(II)$ were fitted by LRA with V_{ACC} = Wbt ($p < 0.001$).

burdens were clearly not kinetically homogeneous. About half of the metal was eliminated within a few hours and elimination proceeded more slowly thereafter. On average, metals were distributed equally between $Q_1(0)$ and $Q_2(0)$, and values of c were 2 orders of magnitude higher than those of d (Table 2). The high variability of Q_{EXC} values obtained from larvae preexposed to Hg(II) and $CH_3Hg(II)$ prevented the resolution of elimination process into two steps (Figure 2). It can be seen however that elimination of Hg(II) and $CH_3Hg(II)$, with or without complexing agents, was much slower as 60−75% of the metal remained in the larvae at the end of elimination period. Rate constant d was estimated by LRA using the natural logarithm of Q_{EXC} data plotted against time [$\ln Q_{EXC} = \ln Q(0) - dt$]. Values found ranged (0.6−1.7) $\times 10^{-3}$ h^{-1} (r^2 range 0.14−0.66), an order of magnitude lower than for Zn(II) and Cd(II).

WBARG. Larvae exposed to Cd(II) alone (Figure 3A) had a high labeling of their gut whereas the exoskeleton showed a weak labeling. In the group exposed to Cd(II) + DDC (Figure 3C), the labeling of the exoskeleton was more important. The gut was the only structure labeled in larvae exposed to Zn(II), with or without DDC (not shown).

The distribution of inorganic Hg(II) in larvae was quite different (Figure 4A). All tissues were labeled to various extents. The exoskeleton was highly labeled, as well as the Malpighian tubules, gut, and haemolymph. Labeling of muscle was lower than that of haemolymph. In larvae exposed to $CH_3Hg(II)$ (Figure 4C), the exoskeleton was also labeled. The gut and Malpighian tubules contained high concentrations of radioactivity. The labeling of muscles was very high, much stronger than in larvae exposed to inorganic Hg, and that of the haemolymph was comparatively very low. The body distribution of Hg(II) and $CH_3Hg(II)$ in larvae was not affected by complexing agents (not shown).

Discussion

Zn(II) and Cd(II) Kinetics. Dissolved metals can reach the internal environment of insect larvae by diffusion through anal papillae or by swallowing of metal-containing water and subsequent diffusion through gut wall (*1, 19*). Though the relative contribution of each route cannot be assessed with the present set of data, the high concentrations of Zn(II) and Cd(II) observed in the gut at the end of the exposure experiment (Figure 3) indicate that water swallowing may have accounted for a significant proportion of the metal accumulated in this tissue. Elimination data indicated that an important fraction of the accumulated metal, Q_1, was eliminated within a few hours. This is similar to gut clearance time measured for other aquatic insect larvae (*20, 21*). Q_1 might then be related to the elimination of metal weakly bound to gut tissues once larvae were placed in uncontaminated water. Monoexponential uptake kinetics observed for Zn(II) and Cd(II) were not in accordance with the biexponential elimination kinetics. This may be related to interindividual variability, which would have masked the actual time trend of Zn and Cd accumulation.

Concentrations of Cd(II) and Zn(II) accumulated by *C. americanus* larvae in this work ($V_{ACC} \times [metal]_{water} = [metal]_{larvae}$) (*8*) were 3−4 orders of magnitude lower than values measured in *Chaoborus spp.* larvae collected in the field (*22−24*), even if our exposure conditions were in the range of natural ones (*23*). Cd(II) uptake via water in *Chaoborus punctipennis* was recently shown to be negligible compared with dietary uptake (*25*). Our results indicate that, for *C. americanus* as well, Cd(II) and Zn(II) uptake via water is probably not predominant, though long-term life cycle and ecosystems processes might also play a role in situ.

Inorganic Mercury and Methylmercury. Surface adsorption of metal can result in a fast initial uptake phase in insect larvae (*26*). Kinetic data clearly evidenced such an uptake pattern for inorganic Hg(II), and autoradiograms showed an important labeling of the exoskeleton. Uptake of $CH_3Hg(II)$ by larvae during the first hours tended to be faster (see insets in Figure 2), but it was not possible to resolve uptake kinetics in two pools. Though autoradiograms also showed a labeling of exoskeleton in $CH_3Hg(II)$-exposed larvae, the $CH_3Hg(II)$ quantity adsorbed may have been smaller, which combined to the rather high intersamples variability (mean coefficient of variation 23%) would have prevented the quantification of BCF_{1SS} with the NLRA procedure.

The high affinity of Hg(II) and $CH_3Hg(II)$ for biological ligands (*27*) as well as their passive diffusion through biological membranes as neutral species (*28*) likely explains their high accumulation in larvae compared to Zn(II) and Cd(II), of which the main dissolved species was the free ion (Table 1). Nevertheless, the slower uptake of $CH_3Hg(II)$ was surprising. The uptake of waterborne inorganic and organic mercury has been shown to be related to the value of the overall octanol/water partition coefficient, D_{ow}, which depends on pH and pCl (*29*). Under our experimental conditions, D_{ow} values were similar for both inorganic Hg and methylmercury (0.05−0.10) (*29*). Thus, a difference in lipid solubility can probably not account for the lower uptake rate of $CH_3Hg(II)$ by *C. americanus* larvae.

Following membrane crossing, the extent of metal accumulation within the body depends on the affinity for tissues and the transport toward them. The conventional opinion that $CH_3Hg(II)$ is distributed faster than inorganic Hg(II) in living organisms is based on the work of Rabenstein (*30*) demonstrating the lability of $CH_3Hg(II)$−sulfhydryl complexes. However, labile does not always mean fast. Actually, we have observed in other aquatic animals, upon exposure via water (*31, 32*) and via food (Rouleau, Gobeil, Ribeiro, Pelletier and Tjälve, unpublished data), internal translocation rates of $CH_3Hg(II)$ slower than those of inorganic Hg(II). In insect larvae, the haemolymph is the medium through which all the chemical exchanges between organs are effected (*19*). Its very low labeling compared to muscles and gut in $CH_3Hg(II)$-exposed *C. americanus* larvae reveals a strong gradient between tissues and the transport medium, much higher than what was observed for inorganic Hg(II). This may indicate a more difficult translocation of $CH_3Hg(II)$ toward internal accumulation sites, that would explain its slower accumulation rate.

The biological half-live (0.693/rate constant d) of both mercury forms is in the order of a month compared to a few days for Zn and Cd. This is likely to result in a much higher steady-state value of V_{ACC}, which can be roughly estimated from the ratio of uptake rate constant b over elimination rate constant d. Values of 15 400 and 84 000 mL g^{-1} dw are found for $CH_3Hg(II)$ and Hg(II), respectively. Considering that dissolved mercury concentrations in natural freshwater are typically less than 1 pM (*29*), the in situ steady-state $CH_3Hg(II)$

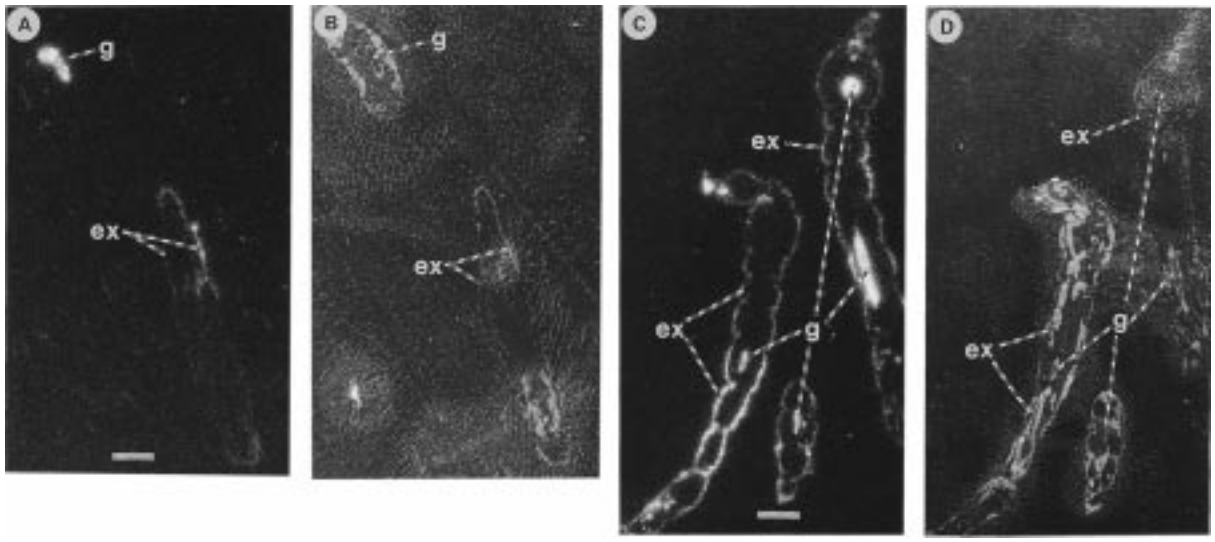

FIGURE 3. Whole-body autoradiograms of *Chaoborus americanus* larvae exposed for one week to $^{109}Cd(II)$ alone (A), or $^{109}Cd(II)$ + DDC (C). Panels B and D are tissue sections corresponding to panels A and C. The exposure time of the 20-μm-thick sections was 5 months. ab = air bladder, ex = exoskeleton, g = gut, h = haemolymph, m = muscle, Mt = Malpighian tubules. Bar is 1 mm.

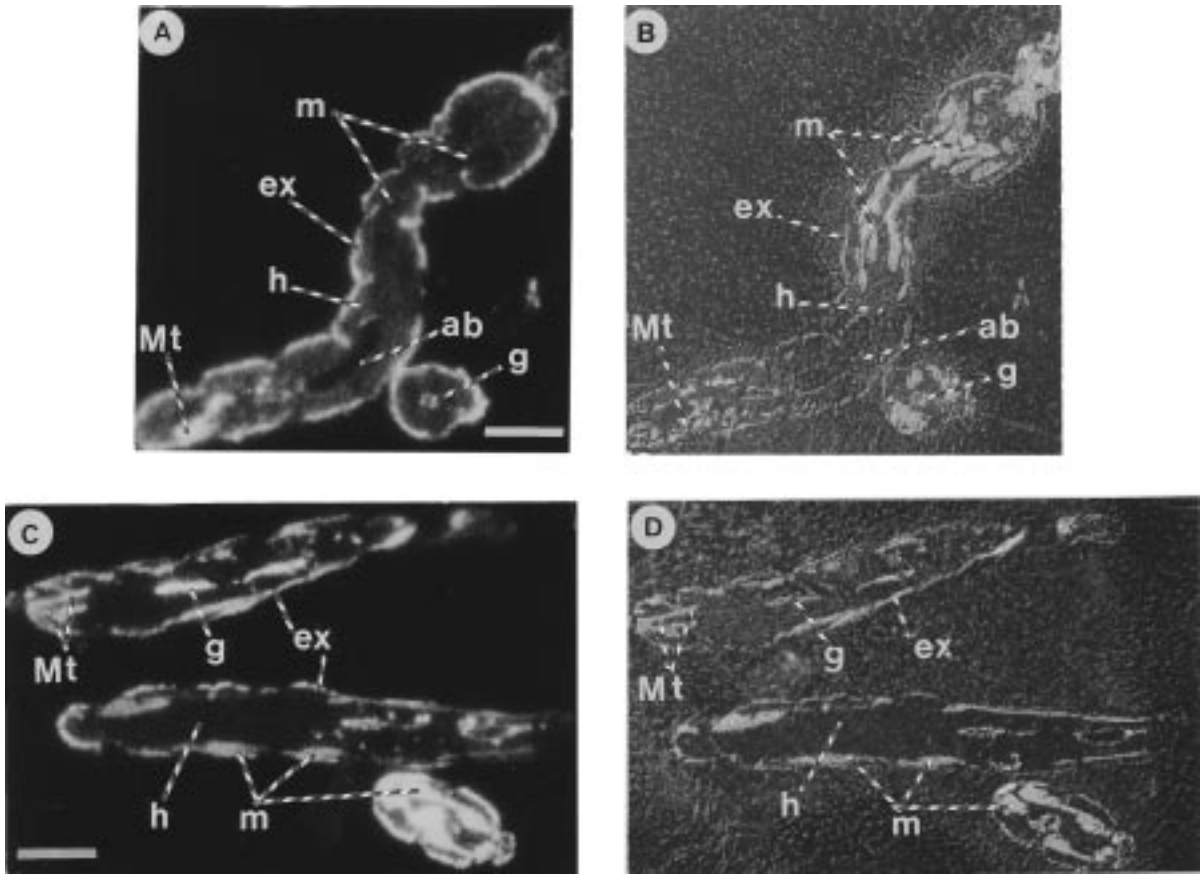

FIGURE 4. Whole-body autoradiograms of *Chaoborus americanus* larvae exposed for one week to $^{203}Hg(II)$ alone (A), or $CH_3{}^{203}Hg(II)$ alone (C). Panels B and D are tissue sections corresponding to panels A and C. The exposure time of the 20-μm-thick sections was 6 weeks. See Figure 3 for legend. Bar is 1 mm.

and Hg(II) concentrations of *Chaoborus* larvae would be <2 and <17 ng g^{-1}dw, respectively. This is much lower than natural values found for *Chaoborus spp.* and other dipteran larvae (*33, 34*) and indicates that uptake via water is likely not the predominant uptake pathway, though its contribution is more important than for Zn(II) and Cd(II). However, as we used an exposure concentration 3 orders of magnitude higher than in situ levels, it cannot be excluded that rate constant values measured were biased. Uptake from water at the pM level and trophic transfer kinetics need to be determined.

Effects of DDC. Calculation of metal speciation in the presence of DDC for this work, using log β_2 values published elsewhere (*11, 14, 35*), yield proportion of DDC-bound metal of 84% for Zn(II) and >99% for Cd(II), Hg(II), and $CH_3Hg(II)$. Thus, an increase of the accumulation of all four metals was expected. However, this was observed only for Hg(II) and $CH_3Hg(II)$. Actually, the formation and increased uptake of

lipophilic complexes of waterborne Cd(II) with DDC and xanthates has been shown to be significant only at a [complexing agent]/[metal] ratio above 100 (*36*, *37*). In our experiment, this ratio was 25. The lack of effect of DDC on Cd(II) and Zn(II) uptake might be related to their increased hardness compared to Hg(II) (*29*, *38*), resulting in an increased stability of the first hydration sheat of Cd^{2+} and Zn^{2+} ions of which H_2O dipoles could not be successfully displace by DDC at very low concentrations (*39*). The increased concentration of Cd on the exoskeleton may be the result of DDC adsorption that locally increased the [DDC]/[Cd(II)] ratio. This did not appear however to have an important effect on the overall amount of Cd accumulated by the larvae.

Effects of HA. The proportion of inorganic Hg(II) bound to HM in freshwater is usually important (*40*, *41*). The percentage of HM-bound Hg(II) in our experiment can be estimated from

$$100[1 - [(k_a/(k_a + k_d))_{Hg(II)+HM}/(k_a/(k_a + k_d))_{Hg(II)\ alone}]] = 82\% \quad (6)$$

Only one-fifth of Hg(II) in water was not HM-bound and likely bioavailable. As values of BCF_{1SS} and rate constant *b* were calculated from eq 5 with $W_0 = 1$ mL, i.e., 100% bioavailability, they should be 5 times lower than those found for Hg(II) alone. This was true for BCF_{1SS}, but rate constant *b* was more than 50 times lower, indicating some perturbation of the uptake process. Campbell et al. (*42*) showed that fulvic acid (FA) is adsorbed onto the surfaces of algal and animal cells. They calculated that FA concentration in the diffusion layer (1−30 μm outward the cell surface) would be 30−900 times greater than in the surrounding water. As a result, interactions of metal ions with FA adsorbed on cell surfaces would decrease their diffusion rate toward cell surface or their rate of reaction with cell surface, and hence decrease their bioavailability. It is likely that HM was adsorbed on *C. americanus*. The much lower uptake rate of Hg(II) than what would be expected from decreased unbound Hg(II) concentration alone is a clear experimental indication that HM affected the uptake process at the water−larvae interface itself.

At first insight, it could be expected from the similar values of stability constants of Hg(II)− and $CH_3Hg(II)$−HM complexes (*40*) that HM would have decreased uptake of both. HM has been observed previously to have no effect on the toxicity of nanomolar concentrations of $CH_3Hg(II)$ (*43*). This is likely related to the low binding capacity of HM for $CH_3Hg(II)$, which range from 0.2 to 0.7 nmol mg^{-1} (*44*). Thus, the HM concentration we used presumably bound a negligible quantity of $CH_3Hg(II)$ compared to the amount added to water. The 30% increase of $CH_3Hg(II)$ uptake rate by larvae observed in the present work is difficult to explain, though it might be related to direct interactions of HM with cellular membrane that can affect its permeability (*42*, *45*).

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Literature Cited

(1) Hare, L. *Crit. Rev. Toxicol.* **1992**, *22*, 327−369.
(2) Cain, D. J.; Luoma, S. N.; Carter, J. L.; Fend, S. V. *Can. J. Fish. Aquat. Sci.* **1992**, *49*, 2141−2154.
(3) Saiki, M. K.; Castleberry, D. T.; May, T. W.; Martin, B. A.; Bullard, F. N. *Arch. Environ. Contam. Toxicol.* **1995**, *29*, 484−491.
(4) Spacie, A.; Hamelink, J. L. In *Fundamentals of aquatic toxicology*, 1st ed.; Rand, G. M.; Petrocelli, S. R., Eds.; Hemisphere Publishing Corporation: New York, 1985; pp 495−525.
(5) Swüste, H. F. J.; Cremer, R.; Parma, S. *Verh. Int. Ver. Limnol.* **1973**, *18*, 1559−1563.
(6) Helgen, J. C.; Larson, N. J.; Anderson, R. L. *Arch. Environ. Contam. Toxicol.* **1988**, *17*, 459−471.
(7) Ullberg, S.; Larsson, B.; Tjälve, H. In *Biological applications of radiotracers*, 1st ed.; Gleen, H. J., Ed.; CRC Press: Boca Raton, 1982; pp 56−108.
(8) Barron, M. G.; Stehly, G. R.; Hayton, W. L. *Aquat. Toxicol.* **1990**, *18*, 61−86.
(9) Moore, J. W.; Ramamoorthy, S. *Heavy metals in natural waters: applied monitoring and impact assessment*, 1st ed.; Springer-Verlag: New York, 1984.
(10) Aiken, G. R.; McKnight, D. M.; Wershaw, R. L.; MacCarthy, P. *Humic substances in soil, sediment and water: geochemistry, isolation, and characterization*, 1st ed.; John Wiley & Sons: New York, 1985.
(11) Thorn, G. D.; Ludwig, R. A. *The dithiocarbamates and related compounds*, 1st ed.; Elsevier Publishing Company: New York, 1962.
(12) Hill, E. F. In *Handbook of ecotoxicology*, 1st ed.; Hoffman, D. J.; Rattner, B. A.; Burton, G. A., Jr.; Cairns, J., Jr., Eds.; Lewis Publishers: Boca Raton, 1995; pp 243−274.
(13) Tjälve, H.; Gottofrey, J. *Pharmacol. Toxicol.* **1991**, *69*, 430−439.
(14) Phinney, J. T.; Bruland K. W. *Environ. Sci. Technol.* **1994**, *28*, 1781−1790.
(15) Rouleau, C.; Block, M. *Appl. Organomet. Chem.* **1997**, *11*, 751−753.
(16) Tjossem, S. F. *Limnol. Oceanogr.* **1990**, *35*, 1456−1468.
(17) Lowry, O. H.; Rosenbrough, W. J.; Fari, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265−275.
(18) Whicker, F. W.; Schultz, V. *Radioecology: nuclear energy and the environment*, Vol. II, 1st ed.; CRC Press Inc.: Boca Raton, 1982.
(19) Wigglesworth, V. B. *The principles of insect physiology*, 7th ed.; Chapman and Hall: London, 1972.
(20) Avissar, Y. J.; Margalit, J.; Spielman, A. *J. Am. Mosq. Control Assoc.* **1994**, *10*, 45−50.
(21) Brooke, L. T.; Ankley, G. T.; Call, D. J.; Cook, P. M. *Environ. Toxicol. Chem.* **1996**, *15*, 223−228.
(22) Groulx, G. R.; Lasenby, D. C. *Arch. Environ. Contam. Toxicol.* **1992**, *23*, 370−374.
(23) Hare, L.; Tessier, A. *Nature* **1996**, *380*, 430−432.
(24) Hare, L.; Campbell, P. G. C. *Freshwater Biol.* **1992**, *27*, 13−27.
(25) Munger, C.; Hare, L. *Environ. Sci. Technol.* **1997**, *31*, 891−895.
(26) Krantzberg, G.; Stokes, P. M. *Environ. Toxicol. Chem.* **1988**, *7*, 653−670.
(27) Rabenstein, D. L.; Reid, R. S.; Isab, A. A. *J. Inorg. Biochem.* **1983**, *18*, 241−251.
(28) Bienvenue, E.; Boudou, A.; Desmazès, J. P.; Gavach, C.; Georgescauld, D.; Sandeaux, J.; Sandeaux, R.; Seta, P. *Chem.-Biol. Interact.* **1984**, *48*, 91−101.
(29) Mason, R. P.; Reinfelder, J. R.; Morel, F. M. M. *Environ. Sci. Technol.* **1996**, *30*, 1835−1845.
(30) Rabenstein, D. L.; Arnold, A. P.; Guy, R. D. *J. Inorg. Biochem.* **1986**, *28*, 279−287.
(31) Rouleau, C.; Pelletier, É.; Tjälve, H. *Aquat. Toxicol.* **1993**, *26*, 103−116.
(32) Rouleau, C.; Pelletier, É.; Tjälve, H. *Appl. Organomet. Chem.* **1995**, *9*, 327−334.
(33) Parkman, H.; Meili, M. *Can. J. Fish. Aquat. Sci.* **1993**, *50*, 521−534.
(34) Tremblay, A.; Lucotte, M. *Can. J. Fish. Aquat. Sci.* **1997**, *54*, 832−841.
(35) Labuda, J.; Skatulokova, M.; Nemeth, N.; Gergely, S. *Chem. Zvesti* **1984**, *38*, 597−605.
(36) Block, M. *Environ. Toxicol. Chem.* **1991**, *10*, 1267−1272.
(37) Block, M.; Wicklund Glynn, A. *Environ. Toxicol. Chem.* **1992**, *11*, 873−879.
(38) Sigg L., Stumm W., Behra P. *Chimie des milieux aquatiques*, 1st ed.; Masson: Paris, 1992.
(39) Stumm W., Morgan J. J. *Aquatic Chemistry*, 1st ed.; John Wiley & Sons: New York, 1981.
(40) Lövgren, L.; Sjöberg, S. *Water Res.* **1989**, *23*, 327−332.
(41) Mantoura, R. F. C.; Dickson, A.; Riley, J. P. *Estuarine Coastal Mar. Sci.* **1978**, *6*, 387−408.

(42) Campbell, P. G. C.; Twiss, M. R.; Wilkinson, K. J. *Can. J. Fish. Aquat. Sci*, **1997**, *54*, 2543−2554.
(43) Adare, K. I.; Welbourn, P. M.; Wright, D. A. In *Heavy Metals in the Environment*; Allan, R. J.; Nriagu, J. O., Eds.; CEP Consultants Ltd: Edinburgh, 1993; pp 112−115.
(44) Hintelmann, H.; Welbourn, P. M.; Evans, R. *Environ. Sci. Technol.* **1997**, *31*, 489−495.
(45) Parent, L.; Twiss, M. R.; Campbell, P. G. C. *Environ. Sci. Technol.* **1996**, *30*, 1713−1720.
(46) Schecher, W. D.; McAvoy, D. C. *Comput. Environ. Urban Systems* **1992**, *16*, 65−76.
(47) Shin, E.-B.; Krenkel, P. A. *J. Water Pollut. Control Fed.* **1976**, *48*, 473−501.

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