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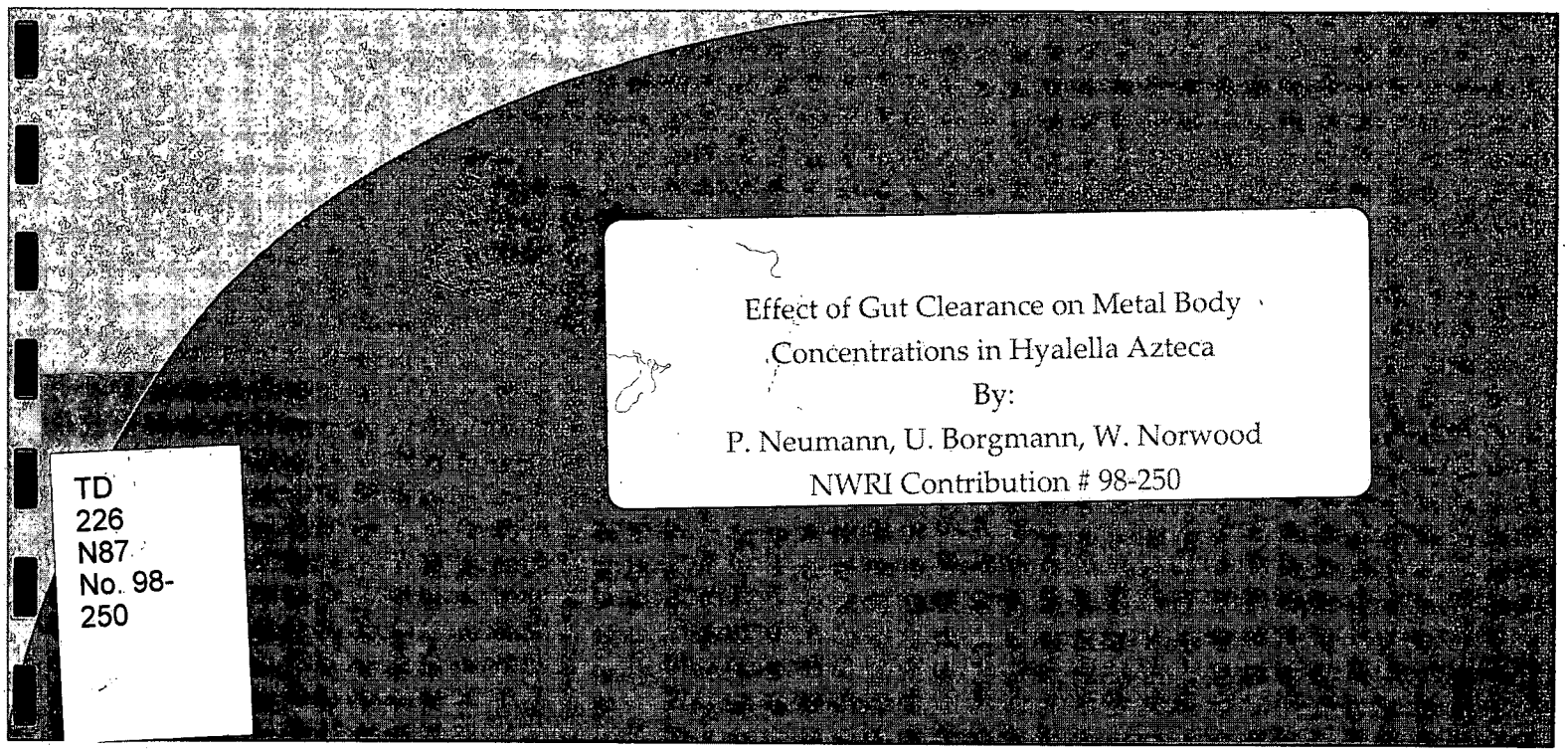

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NWRI Contribution # 98-250

98-250

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 18, No. 5, pp. 976–984, 1999

Printed in the USA
0730-7268/99 $9.00 + .00

EFFECT OF GUT CLEARANCE ON METAL BODY CONCENTRATIONS IN *HYALELLA AZTECA*

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(*Received* 27 *February* 1998; *Accepted* 13 *August* 1998)

Abstract—Gut content can contribute significantly to the metal body burdens in sediment-exposed *Hyalella azteca* even if it has no direct effect on toxicity. To determine the duration and the effect of gut clearance on total body concentrations, we exposed *H. azteca* for 1 week to a spiked sediment (lead, cadmium, zinc, and copper); a second set of amphipods was kept in cages above the sediment. Following transfer into clean water (25°C) for 96 h, lead and zinc concentrations showed a biphasic decline, with a stronger decrease in the first 4 to 6 h, when gut clearance contributed significantly to metal loss. After 6 h, metal loss was apparently due to excretion from the body. Without gut clearance, the "real" body concentrations of lead and zinc in sediment-exposed amphipods were overestimated by 438 and 44%, respectively. Gut clearance did not have a visible effect on cadmium and copper body burdens because the body and sediment concentrations were similar. After a depuration time of 6 h, direct excretion from the body resulted in a drop of less than 10% in the total body burdens of lead, cadmium, zinc, and copper compared to the gut-corrected time-zero body burdens. After 24 h, this loss increased up to 27%. Feeding during the depuration period did not have a significant influence on gut clearance. A model that allows estimation of the influence of gut content on the total body concentration of undepurated invertebrates from the bioconcentration factor is evaluated.

Keywords—Metal body burdens Gut clearance Depuration rates constants Aquatic invertebrates Model

INTRODUCTION

Measurements of metal body concentrations in aquatic invertebrates are an important tool for identifying the agents that are responsible for toxic effects of sediments [1]. In many cases, metal body burdens are a better indicator of bioavailable metal and its biological effects than the concentration of metals in the animal's surroundings [2]. The total body burden of metal in an animal is the sum of metal in its body and metal in the gut contents. Biological effects are usually ascribed only to metal in the body, requiring the need to differentiate between metal in the body and metal in the gut. Several authors have investigated the effect of gut content on metal body burdens in different aquatic invertebrates and found a significant contribution of the gut content to the total metal body burdens [3–6], but no data have been published for *Hyalella azteca*. A high metal content in the gut can lead to a significant overestimation of bioavailable metal in the environment if the metal is not absorbed by the body. Even if the main focus of an investigation is metal accumulation by higher trophic levels, the metal in the gut and the metal in the body of prey organisms could have different bioavailability for the predator. Therefore, reliable means of distinguishing between metal in the body and metal in the gut are required.

The purpose of this study was to determine the effect of gut content on total metal body concentrations of sediment-exposed *H. azteca*. Furthermore, we wanted to investigate how long *H. azteca* needs to clear its gut and whether feeding would have an influence on gut clearance. By comparing the decrease in total metal body concentrations in sediment-exposed amphipods to that in amphipods kept in cages above the sediment, we planned to separate the effect of gut clearance from the effect of metal depuration from the body. By measuring the change in body concentrations and applying a kinetic model to these data, we avoided the need to determine the change in body weight during gut clearance gravimetrically. Gravimetrical methods would be difficult to use because this would need to be done on live animals; the live weight of *H. azteca* is often below 5 mg and unstable because of evaporation when the animals are exposed to air while on the balance. A general model is presented to predict the influence of gut content on the total metal body concentration of aquatic macroinvertebrates from the bioconcentration factor and is applied to several studies in the literature.

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MATERIALS AND METHODS

Amphipods were cultured as described in Borgmann et al. [7] and were 6 to 7 weeks old at the beginning of the experiment. Burlington tap water (originating from Lake Ontario, hardness 130 mg/L, alkalinity 90 mg/L, pH 7.9–8.6), dechlorinated by bubbling with an aquarium charcoal filter, was used for culturing and experiments.

Sediment (density 1.22 g/ml, moisture content 71.5%, weight loss of 7.9% on ashing at 452°C for 48 h) was collected from site 1 in the west end of Hamilton Harbour and stored until use at 4°C. Sediments from this site consistently supported high survival in 4-week toxicity tests [8]. The sediment was spiked with the nitrate salts of copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn) by mixing equal volumes of spike solution (2 mM Cu, 0.1 mM Cd, 10 mM Pb, and 10 mM Zn in Milli-Q® deionized water, (Milli-Q, Bedford, MA, USA) and sediment in 1-L polypropylene bottles and rotating the mixture for 24 h at low speed. These concentrations were chosen to achieve metal concentrations in the sediment as high

as possible without resulting in a significant mortality after 1 week of exposure based on preliminary experiments. After mixing, the sediment was allowed to settle and the excess water decanted. This sediment caused 100% mortality of 5- to 6-week-old *H. azteca* after an exposure of 1 week and was therefore diluted fourfold with unspiked sediment and 10% dechlorinated tap water (to facilitate mixing) and rotated in polypropylene bottles for 5 d at 4 rpm. Again, the sediment was allowed to settle and the excess water decanted.

Amphipod exposures were conducted in two replicate 2-L polypropylene containers filled with 800 ml dechlorinated tap water and 200 ml spiked sediment carefully added to minimize mixing. After 3 d of aeration, 234 animals were added to each container and an additional 234 animals added to cages (volume 0.8 L) inside the containers. These cages were fixed to the wall of the containers 2 to 3 cm above the sediment. The bottom of the cages consisted of a 0.25-mm nylon screen that permitted water exchange but prevented access of the amphipods to the sediment. Experiments with varying densities (ratio of amphipods to sediment) showed no negative effect of these densities on growth or survival of the amphipods. An EDTA control was set up using 33 amphipods in a 2-L polypropylene container filled with 1 L of 50-μM EDTA in dechlorinated tap water and containing two pieces of cotton gauze (5 × 5 cm). The amphipods in all containers and cages were fed with TetraMin® (Ulrich Baensch, Melle, Germany) fish food. During the exposure period, all containers were gently aerated through Pasteur pipettes. The experiment was conducted at 25°C with a 16:8 h light:dark cycle.

After 1 week, the amphipods were removed from the cages and sieved from the sediment with a 500-μm nylon screen. To avoid gut clearance in the time-zero samples, the first 25 amphipods that were removed from each treatment and replicate were immediately rinsed three times with dechlorinated tap water containing 50 μM EDTA and once with Milli-Q water. Then 12 to 14 of them were randomly selected and dried at 60°C. The time between removing these amphipods from the sediment or the cage and the final rinse in Milli-Q water was shorter than 8 min. All amphipods in the EDTA control were treated in the same way. The remaining amphipods were separated from the sediment or taken from the cages and transferred three times into clean dechlorinated tap water with 50 μM EDTA. These were combined with the 11 to 13 remaining from the first 25 removed and then randomly distributed into 11 polypropylene containers for each treatment (cage, sediment) and each of the two replicates. These containers were filled with 200 ml of 50-μM EDTA solution and contained one piece of cotton gauze as clean substrate to cling to [7]. Seven of these 11 containers received 5 mg TetraMin fish food.

After 1, 2, 4, and 6 h all amphipods from two containers (one fed and one not fed) and after 24, 48, and 92 h all amphipods from one container (fed) of each treatment and each replicate were removed, rinsed in Milli-Q water, and dried at 60°C.

From each treatment (sediment fed, sediment not fed, cage fed, cage not fed) and each depuration time, six replicate subsamples of four amphipods (three replicate subsamples from each of the two replicate exposure containers) were digested and analyzed for their metal body concentrations. For each subsample, the total dry weight of four amphipods was measured. These were digested with 25 μl of 70% nitric acid at room temperature for 6 d, after which 20 μl of 30% hydrogen peroxide was added and samples were left for another 24 h at room temperature. Double-distilled water was added to a final volume of 250 μl. Dried samples (3–25 mg) of the spiked sediment were digested with the same method (250 μl of nitric acid, 200 μl peroxide, final volume 10 ml). Digested amphipods and sediment samples were analyzed for lead, zinc, cadmium, and copper on a Varian SpectraAA 400 graphite furnace atomic absorption spectrophotometer (Varian, Walnut Creek, CA, USA) with Zeeman background correction. After analyzing lead, we diluted all samples fourfold with double-distilled water before measuring the other three metals. If further dilution of individual samples was necessary, 0.7% nitric acid was used. Cadmium, lead, and zinc were analyzed using a platform and ammonium phosphate modifier. Copper was measured in a partition tube without modifier. Samples of National Research Council reference material (TORT-1, lobster hepatopancreas) were digested and analyzed using the same method. The average recovery (standard error) of Cd, Cu, Pb, and Zn in the TORT samples was 101% (±2.4), 95% (±1.9), 93% (±43), and 90% (±2.5) of the certified values, respectively (n = 9). The high standard error of the Pb readings was probably due to high variability of Pb in the TORT standard. The metal concentrations are certified for TORT samples larger than 500 mg, which is two to three orders of magnitude larger than the mass used in the digestion of the amphipods and the TORT samples in this experiment.

Statistical analyses were performed with Systat® 6.1 for Windows. Comparison between the different treatments and depuration times was done by analysis of variance (ANOVA). The pairwise comparison of all combinations was done with the Tukey method, and the comparison of all treatments with the EDTA control was done with Dunnett's test. Log transformation was necessary to establish homoscedasticity and normal distribution of the metal concentration data. Three data points of the Pb data and two data points of the Cd data were excluded as outliers from the data sets (n = 148) because the studentized residuals were larger than 3. The following equation was used to analyze the change in body concentrations during the depuration time:

$$C_{TB} = G_0 \cdot \exp(-k_g \cdot t) + C_{X0} \cdot \exp(-k_e \cdot t) + C_{Bk} \quad (1)$$

where

C_{TB} = whole-body concentration at time t (nmol/g dry mass),
C_{X0} = whole-body concentration due to metal in the body, excluding the gut content at the start of depuration (nmol/g dry mass),
G_0 = contribution of gut content to whole-body concentration at the start of depuration (nmol/g dry mass),
C_{Bk} = background body concentration (nmol/g dry mass),
k_e = depuration rate constant from the body (/h),
k_g = gut clearance rate constant (/h), and
t = time (h).

As a check on the reliability of the computer program for simultaneously fitting multiple constants using this equation, we also fit the data for $t \geq 6$ h (i.e., after completion of gut clearance) to Equation 1 with G_0 set equal to zero. In all cases, the estimates of k_e obtained in this way were not significantly different from those obtained using the full Equation 1. If not otherwise stated, all results shown are based on the amphipods that were fed during the depuration time. The data for the two replicates for each treatment (sediment, cage) were pooled for the final nonlinear regression. Statistically significant differences were seen between the body concentrations of Cd, Cu,

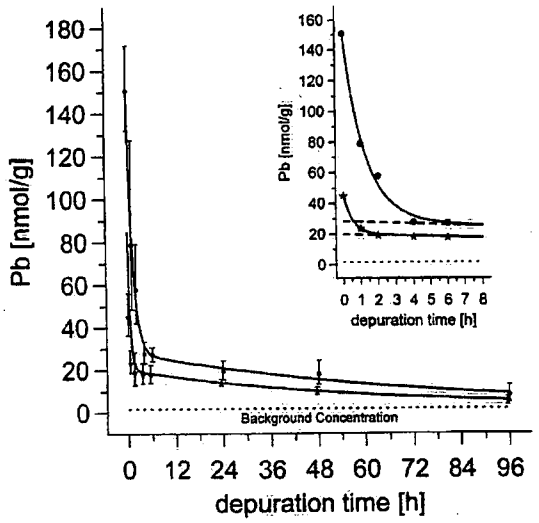

Fig. 1. Lead concentration in sediment-exposed (●) and caged (*) *H. azteca* during 96-h gut clearance in clean water. Geometric means with 95% confident limits; ——— = results of the linear regression of Equation 1; ····· = concentration of the EDTA-control amphipods (background concentration). The smaller graph within the graph shows the data of the first 8 h on an expanded time scale; – – – = real body concentration estimated with Equation 1.

and Pb between the two replicates (ANOVA, $p < 0.05$), but no significant differences were seen in the estimated gut clearance rate constant (k_g) and depuration rate constant (k_e) when they were estimated for each replicate separately.

RESULTS

The concentrations of Cu, Cd, Pb, and Zn in the spiked sediment were 2.8, 0.10, 7.8, and 22 μmol/g (dry wt.), respectively. Compared to sediment quality criteria, the Cd, Pb, and Zn concentrations were, respectively, 3, 18, and 5 times higher than the PELs (probable effect levels above which adverse biological effects are frequently observed). The Cu concentration in the sediment was slightly below the PEL [9]. The measured metal concentrations are in the same order of magnitude observed in contaminated freshwater sediments, such as those from Clark Fork River (MT, USA) [10]. The survival rates after 1 week in the EDTA control, the sediment exposure, and the cage exposure were 97%, 85%, and 97%, respectively. No additional mortality was seen during the 96-h depuration phase.

Body concentrations (95% confidence limits [CL]) in the EDTA control were 3.0 (2.5–3.5) nmol Cd/g, 999 (919–1,086) nmol Cu/g, 1.4 (1.0–1.9) nmol Pb/g, and 705 (595–835) nmol Zn/g. These data were used as the body background concentrations (C_{Bk}) in the nonlinear regression of Equation 1.

The sediment-exposed and the caged amphipods showed a significant decrease in the total body concentrations during the 96-h depuration for all four metals ($p < 0.01$). All these values were significantly higher than the values of the EDTA control ($p < 0.05$), with the exception of zinc in caged amphipods after a depuration time of 24, 48, and 96 h. The body metal concentrations of the caged amphipods were significantly lower than the body metal concentrations of the sediment-exposed amphipods (Figs. 1 to 4).

Lead

The total body lead concentrations of the sediment-exposed and the caged amphipods dropped quickly during the first 2 to 6 h of depuration and continued to decline at a slower rate thereafter (Fig. 1). The whole-body lead concentrations of the sediment-exposed amphipods were significantly higher than the whole-body lead concentrations of the caged amphipods during the whole depuration phase.

In both treatments (sediment, cage), the decrease in the body lead concentrations was described well by Equation 1 (Fig. 1). The depuration rate constants (k_e, Table 1) of the sediment-exposed amphipods (0.014/h) and of the caged amphipods (0.015/h) were not significantly different from each other. A depuration rate constant of 0.014/h is equivalent to a loss of 8% within 6 h and a loss of 27% within 24 h.

The gut clearance rate constant (k_g) of the sediment-exposed amphipods was 0.8/h (95% CL: 0.5–1.1/h). Therefore, the gut content is cleared by 99% in less than 6 h. The estimate for the gut clearance rate constant of the caged amphipods (1.9/h, 95% CL: 0.6–3.1/h) was higher but not significantly different from 0.8/h. The gut content of the sediment-exposed amphipods contributed much more to the total body metal concentration than that of the caged amphipods. Therefore, the k_g obtained from the sediment-exposed amphipods is more precise.

On the basis of the results of the nonlinear regression of Equation 1, the gut content (G_0) contributed 81% of the whole-body lead concentration of the sediment-exposed amphipods at the start of the depuration time (Table 1). The lead body concentration at this time (corrected for the gut content) was 28 nmol/g ($C_{X0} + C_{Bk}$). Lead in the sediment-exposed amphipods contributed a greater percentage of the whole-body metal concentration than any other metal. Therefore, this estimate of gut clearance (0.8/h) is considered to be the most reliable and is used in regressions in the following discussion

Table 1. Results of the nonlinear regression of Equation 1 for the caged and the sediment-exposed *H. azteca*. k_e = depuration rate constant, C_{TB0} = total body concentration at time zero, C_{X0} = above-background body concentration excluding the gut content at time zero, C_{B0} = body concentration excluding the gut content at time zero ($C_{X0} + C_{Bk}$), G_0 = contribution of gut content to the total body concentration at time zero.
For all data sets, K_g was fixed to 0.8/h, the value that was estimated for the Pb data of the sediment-exposed amphipods.

Type	Metal	k_e (h)	95% Confidence limits	C_{TB0} (nmol/g)	C_{X0} (nmol/g)	C_{B0} (nmol/g)	G_0 (nmol/g)
Sediment	Pb	0.0137	(0.0094–0.0181)	151	27	28	123
Cage	Pb	0.0153	(0.0117–0.0188)	45	18	20	26
Sediment	Zn	0.0108	(0.0040–0.0176)	1,646	438	1,143	503
Cage	Zn	0.0087	(−0.0003–0.0177)	1,190	228	933	257
Sediment	Cd	0.0069	(0.0043–0.0094)	27	21	24	3
Cage	Cd	0.0098	(0.0076–0.0120)	18	14	17	1
Sediment	Cu	0.0084	(0.0050–0.0119)	1,962	825	1,823	139
Cage	Cu	0.0069	(0.0038–0.0101)	1,615	512	1,510	105

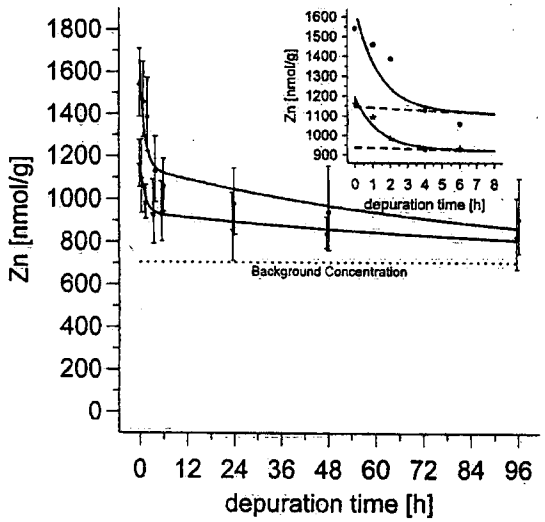

Fig. 2. Zinc concentration in sediment-exposed (●) and caged (*) *H. azteca* during 96-h gut clearance in clean water. Geometric means with 95% confident limits; ——— = results of the linear regression of Equation 1 (k_g fixed to 0.8/h, obtained for the Pb data); ····· = concentration of the EDTA-control amphipods (background concentration). The smaller graph within the graph shows the data of the first 8 h on an expanded time scale; – – – = the real body concentration estimated with Equation 1.

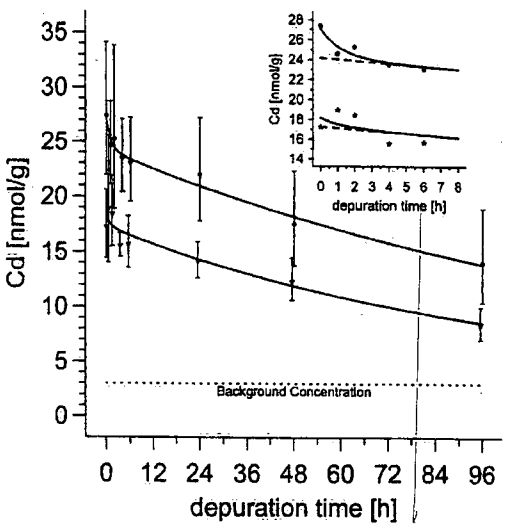

Fig. 3. Cadmium concentration in sediment-exposed (●) and caged (*) *H. azteca* during 96-h gut clearance in clean water. Geometric means with 95% confident limits; ——— = results of the linear regression of Equation 1 (k_g fixed to 0.8/h, obtained for the Pb data); ····· = concentration of the EDTA-control amphipods (background concentration). The smaller graph within the graph shows the data of the first 8 h on an expanded time scale; – – – = real body concentration estimated with Equation 1.

when reliable, independent estimates of k_g could not be obtained.

Zinc

The whole-body zinc concentrations of the caged and the sediment-exposed amphipods decreased quickly during the first 4 to 6 h of depuration (Fig. 2). Although the mean of the zinc concentrations decreased slowly during the remaining time (Fig. 2), the concentrations at 6, 24, 48, and 96 h were not significantly different from one another, and the body zinc concentrations of the caged amphipods at 24, 48, and 96 h were not significantly different from the EDTA-control amphipods ($p > 0.05$).

It was possible to fit Equation 1 to the observed body zinc concentration data to estimate the depuration rate constants (k_e) and gut clearance rate constants (k_g). This gives a k_g of 0.3/h (95% CL: 0.1–0.5) and a k_e of 0.0032/h (95% CL: −0.0058–0.0122). Although these estimates describe the observed zinc data well, they are not very reliable for two reasons. First, most of the body zinc concentrations were very close to the background body zinc concentration. Therefore, the estimate for the background concentration (EDTA control) has a strong influence on the estimates of the other parameters in Equation 1. Second, the estimated background concentration was low in comparison to previous investigations [11]. To get more reliable estimates for the depuration rate constants and to see whether the results for zinc are consistent with the results from the lead data, we fixed the gut clearance rate constant in the nonlinear regression to the value calculated from the lead data (0.8/h). The results of this nonlinear regression agree reasonably well with the measured zinc data (Table 1 and Fig. 2).

On the basis of the regression with a fixed k_g of 0.8/h, we estimated the gut content to constitute 31% of the whole-body zinc concentration at time zero. The depuration rate constant of 0.011/h implies that 6% (after 6 h) and 22% (after 24 h) of the previously mentioned background zinc concentration is excreted from the body.

Cadmium

The whole-body cadmium concentrations of the sediment-exposed amphipods showed a fast but very small drop within the first hour followed by a continuous, slower rate of decrease (Fig. 3). No clear trend was visible for the caged amphipods within the first few hours of depuration, but after 6 h the cadmium concentration decreased continuously at a rate similar to that of the sediment-exposed amphipods (Fig. 3).

For the caged amphipods, it was not possible to obtain estimates of k_g from Equation 1 with only the background concentration fixed for the nonlinear regression. To solve this problem, we set the gut clearance rate constant to 0.8/h and estimated the other parameter on the basis of this assumption. For comparability, we also did this for the sediment-exposed amphipods (Table 1). It was possible to estimate k_g for the sediment-exposed amphipods (1.1/h), but the confidence limits were very wide (−3.8–6.1) and overlapped the estimate of k_g obtained from the lead data.

The estimated cadmium depuration rate constant (k_e) for the sediment-exposed amphipods obtained using a fixed k_g of 0.8/h was 0.0069/h, a little lower than the depuration rate constant for the caged amphipods (0.0098/h, Table 1). A depuration rate of 0.007 is equivalent to a loss of 4% in 6 h and a loss of 13% in 24 h. On the basis of the results of the nonlinear regression of Equation 1 (Table 1), we estimated that the gut content contributes approx. 11% to the whole-body cadmium concentration at time zero.

Copper

The whole-body copper concentrations of the sediment-exposed and the caged amphipods decreased more or less continuously during the 96-h depuration (Fig. 4). It was not possible to fit Equation 1 to the data from the sediment-exposed

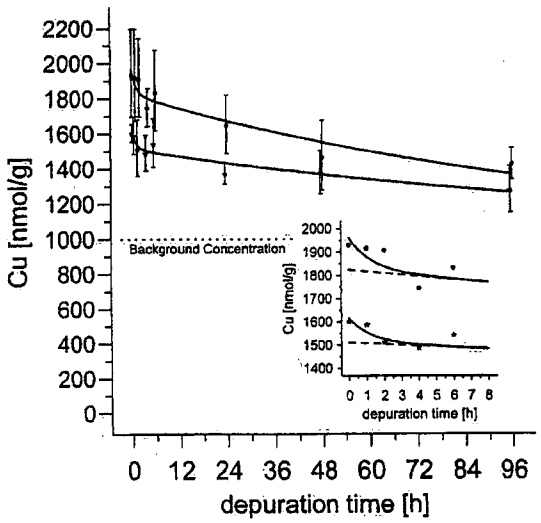

Fig. 4. Copper concentration in sediment-exposed (●) and caged (*) *H. azteca* during 96-h gut clearance in clean water. Geometric means with 95% confident limits; ——— = results of the linear regression of Equation 1 (k_g fixed to 0.8/h, obtained for the Pb data); ····· = concentration of the EDTA-control amphipods (background concentration). The smaller graph within the graph shows the data of the first 8 h on an expanded time scale; – – – = real body concentration estimated with Equation 1.

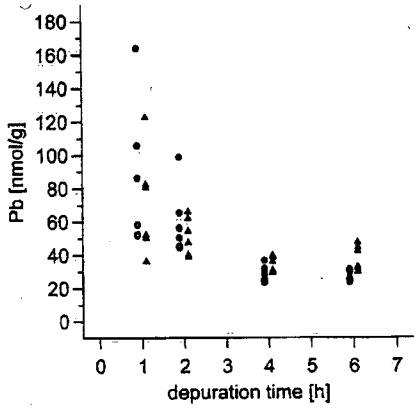

Fig. 5. Lead concentration in fed (●) and non-fed (▲) *H. azteca* during the first 6 h of gut clearance in clean water. All amphipods were exposed to sediment before the start of gut clearance.

amphipods, probably because of the small effect of gut clearance on the whole-body copper concentrations. To estimate the depuration rate constants (k_e), we set the gut clearance rate constant (k_g) to 0.8/h in Equation 1. The resulting estimates describe well the change in the body concentrations during the depuration time (Table 1 and Fig. 4). The depuration rate constant for the sediment-exposed amphipods was 0.0084/h, not significantly different from the depuration rate constant of the caged amphipods. On the basis of the results of the nonlinear regression of Equation 1 (Table 1), we estimated that the gut content contributes approx. 7% to the whole-body copper concentration at time zero.

Effects of feeding during gut clearance

No obvious effect of feeding was seen during the depuration time on the metal concentrations for any of four metals. We show the data only for Pb (Fig. 5) because the other metals were also similar for fed and nonfed amphipods. Although the ANOVA of the data from the sediment-exposed amphipods showed a significant effect of feeding for body lead concentrations, no significant difference was seen between the body lead concentrations of the fed and the nonfed sediment-exposed amphipods after 4 and 6 h.

DISCUSSION

The accurate estimation of gut clearance rate constants requires differentiation between gut clearance and the excretion of metals from the body because both parameters are estimated from the rate constant of decrease in whole-body metal concentrations. Comparison of sediment-exposed and caged animals and of excretion rate constant estimates from animals exposed to metals in spiked sediments and water-only experiments supports the conclusion that the decrease in metal concentrations in the first 6 h is, in fact, due primarily to gut clearance. Estimated gut contents of metals were much higher in the sediment-exposed animals than in the caged animals (Table 1), as expected if the sediment exposed animals ingested metal-contaminated sediments. In addition, the body depuration rate constants (k_e) for Pb, Zn, and Cu from the sediment-exposed animals were not significantly different from the body depuration rate constants of the caged animals. Only the Cd body depuration rate constants showed a significant difference ($p < 0.05$ for the effect of fixing k_e to 0.0098 for the sediment-exposed animals or to 0.0069 for the caged animals in nonlinear regression), but even here the 95% confidence limits overlapped. The rate constant of decrease in whole-body metal concentrations in the caged animals would be expected to be due primarily to excretion. Because this matches the rate constant of decrease in the second phase of the decrease in whole-body metal concentrations in the sediment-exposed animals, as estimated from Equation 1 with k_g set equal to 0.8/h, it is probable that 0.8/h is a reasonable estimate of the gut clearance rate constant. If the strong decrease in the lead and the zinc concentrations observed within the first few hours was due to a biphasic depuration from the body rather than gut clearance, this first phase of body depuration would have a rate constant an order of magnitude higher than reported for these metals in water-only exposures. MacLean et al. [12] estimated a Pb depuration rate constant of 0.022/h (95% CL: 0.018–0.025). Borgmann and Norwood [13] measured a Zn depuration rate constant of 0.028/h (95% CL: 0.022–0.035) and a Cu depuration rate constant of 0.0067/h (95% CL: 0.0046–0.0088). Stephenson and Turner [14] estimated a Cd depuration rate constant for *H. azteca* of 0.0038/h in a field experiment. All these rate constants are very close to the body depuration rate constants (k_e) that were estimated in this study (Table 1), indicating that the rapid decrease in total body metal in the first 6 h is probably due to gut clearance.

The estimated gut clearance rate constant of 0.8/h, equivalent to a gut clearance time of 4 to 6 h (94–99% gut clearance, 25°C), is similar to estimates reported in other studies with macroinvertebrates. Hargrave [15] observed gut passage times of 30 min for *H. azteca* feeding on sediment (15°C). The amphipod *Gammarus pseudolimnaeus* showed a gut clearance rate constant of 1.5/h at 18.5°C, equivalent to a gut clearance time of 3 to 4 h (95–99% gut clearance) [16]. *Gammarus pulex* has gut clearance times between 1 and 7 h at temperatures between 7 and 15°C [17–19]. The chironomid larvae *Kieffer-*

ulus barbitarsis has a gut clearance time between 2.7 and 7.7 h, depending on size [20]. The mayfly *Hexagenia limbata,* the midge *Chironomus tentans,* and the oligochaete *Lumbriculus variegatus* cleared, respectively, 75, 90, and 100% of their gut content within 12 h at 20°C [21].

Although no significant effect of feeding on the gut clearance rate constant was found in this study, we recommend feeding to reduce the risk of coprophagy during the gut clearance time. Furthermore, Brooke et al. [21] concluded that feeding could decrease gut passage time for *H. limbata,* and Hargrave [22] stated that the rate of fecal pellet production depends on the quality of sediment that *H. azteca* is feeding on. Therefore, feeding *H. azteca* during gut clearance time with an uncontaminated artificial food of constant quality should result in more consistent clearance rate constants.

Assuming that the concentration of metals in the sediment is a reasonable approximation of the metal concentration in the gut, it is possible to estimate the contribution of the gut contents to the dry weight of the whole animal. Using the sediment and body burden data for Pb, Zn, Cd, and Cu and the estimated contributions of the gut content to the body burden of these metals, we estimated the dry weight of the gut contents to be 2, 2, 4, and 15%, respectively. The estimate from the Cu data is not very reliable because the contribution of the gut content to the total body burden of each metal is an important factor in this estimation, and this contribution for Cu could not be estimated reliably from our kinetic data. The sediment concentration is only a crude approximation for the metal concentration in the gut because it has been shown that detritivore aquatic invertebrates feed selectively on fine detritus in the sediment [21,23–25]. Hare et al. [6] compared the As, Cd, Zn, and Cd concentration in the surrounding sediment and the gut content of *Hexagenia limbata* and found a ratio of 2.01, 0.23, 0.55, and 1.44, respectively. If similar ratios are representative for *H. azteca,* our estimates for the contribution of the gut content to the total body weight could be off by a factor of 0.5 to 4.

Amyot et al. [3] determined gravimetrically that the gut content contributes 3 to 11% (mean 6%) to the total body weight of *Gammarus fasciatus.* The inorganic gut content contributes between 9 and 10% to total body weight of the mayfly *H. limbata,* the midge *C. tentans,* and the oligochaete *L. variegatus* [21]. Chapman [5] found that the gut content contributes 14.9 and 16.8% to the weight of oligochaetes and chironomid larvae, respectively. The gut and gut content contributed between 5 and 19% to the body weight of the larvae of two stonefly and two caddisfly species [4]. These estimates are similar to our estimates of 2 to 15% for *H. azteca.*

Our results showed that although the gut content contributes only a small amount to the total body weight of *H. azteca,* it can be responsible for a significant overestimation of the real body metal concentration. If we express the total body concentration at time zero relative to the "real" body metal concentration at time zero, the Pb concentration is overestimated by 438% and the Zn concentration by 44%. The Cd and Cu body concentrations are overestimated by only 12 and 8%, respectively (Table 2). Amyot et al. [3] obtained similar results in experiments with *G. fasciatus.* The time-zero concentrations for Pb, Cd, Zn, and Cu in *G. fasciatus* were overestimated by 210, 20, 0, and 0%, respectively, relative to the total body concentration after 18-h gut clearance time, but they did not take depuration from the body into account. They also dissected several *G. fasciatus* and measured the metal concentrations in the different body parts. With this method, they concluded that the gut content contributed less than 20% to the total body concentration of Pb, Cd, Zn, and Cu. Chapman [5] estimated that the body concentration for Pb, Zn, and Cu in chironomid larvae without gut clearance is overestimated by 163, 41, and 20%, respectively, assuming that the metal concentration in the surrounding sediment is the same as in the gut.

Table 2. The bioaccumulation factor (BAF, C_{TB} at time zero divided by the sediment metal concentration) and the ratio of total metal body concentration (C_{TB}) at time t to the true body concentration at time zero (C_{B0})

		t		
Metal	BAF	0 h	6 h	24 h
Pb	0.02	538%	92%	73%
Zn	0.07	144%	98%	91%
Cd	0.27	112%	96%	87%
Cu	0.70	108%	98%	92%

Our results and the results from Chapman [5] and Amyot et al. [3] show that the effect of gut content on the total metal body burdens in aquatic invertebrates varies from metal to metal. Amyot et al. [3] obtained an empirical linear relationship between the logarithm of the quotient of the metal concentration of undepurated and depurated *G. fasciatus* and the logarithm of the metal concentration in the depurated amphipods divided by the metal concentration in the sediment. Amyot et al. [3] call the quotient the bioconcentration factor (BCF). The influence of the gut content increased with decreasing BCF. We refer to the quotient of the total body metal concentration (C_{TB}) of amphipods to the sediment concentration as the bioacumulation factor (BAF) because the term BCF is usually used in the literature to describe the quotient of the body concentration to the metal concentration in the surrounding water [26]. Like Amyot et al. [3], we also found an increasing influence of the gut content on the total body burden of *H. azteca* with decreasing BAF (Table 2). This correlation between BAF and the influence of gut content on the total body burdens is to be expected as long as the metal concentration in the gut content is related to the metal concentration in the surrounding sediment. If data on the metal concentration in undepurated amphipods and the metal concentration of the surrounding sediment are available, it is possible to predict the influence of the gut content on the total body concentration by a theoretical model for these amphipods. The model is based on the following equations and the assumption that the concentration of the metals in the gut is the same as in the surrounding sediment.

$$C_{TB} = (C_B \cdot m_B + C_S \cdot m_G)/(m_B + m_G)$$

$$= (C_B + C_S \cdot \beta)/(1 + \beta) \quad (2)$$

where

C_{TB} = whole-body concentration,
C_B = body concentration including the tissue background concentration but excluding the gut content,
C_S = concentration in the sediment,
m_B = body mass of the invertebrate without the gut content,
m_G = mass of the gut content, and
$\beta = m_G/m_B$.

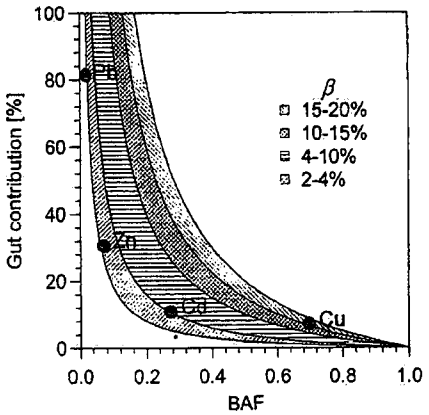

Fig. 6. Contribution of gut content to the total body concentration of invertebrates with different β predicted using Equation 5. A β of 2 to 4% was estimated for *H. azteca*. The bioaccumulation factors (BAF) were estimated for the undepurated *H. azteca*. The contribution of the gut content was estimated from the results of the nonlinear regression of Equation 1.

Equation 2 is a different arrangement of Equation 1 in Hare et al. [6]. Rearrangement of Equation 2 gives the following:

$$C_{TB} \cdot (1 + \beta) = (C_B + C_S \cdot \beta) \quad (3)$$

$$C_{TB} - C_B = \beta \cdot (C_S - C_{TB}) = \beta \cdot (C_{TB}/BAF - C_{TB}) \quad (4)$$

where $BAF = C_{TB}/C_S$ = bioaccumulation factor.

Dividing Equation 4 through by C_{TB} gives the fraction of metal in the gut:

$$((C_{TB} - C_B)/C_{TB}) \cdot 100 = ((\beta/BAF) - \beta) \cdot 100 \quad (5)$$

With this model it is possible to estimate the relative contribution of the gut content to the total body metal concentration for a given β and a given BAF. Figure 6 shows this model for different ranges of β.

Our estimates for the contribution of the gut content to the metal body concentrations of Pb, Zn, Cd, and Cu (Table 1) fit this model well (Fig. 6). Data of Amyot et al. [3] for *G. fasciatus* also fit the model well (β = 3–11%, BCFs estimated for undepurated *G. fasciatus*, Fig. 7). The model underestimates the contribution of the gut content to the total body concentration only for manganese. The data for Pb, Fe, Ni, Cr, and Zn are within the range predicted. Amyot et al. [3] estimated β gravimetrically and the contribution of gut content to the total body concentration by comparing undepurated amphipods with amphipods after a gut clearance time of 18 h. They did not take into account depuration from the body occurring during the 18-h gut clearance time; this can lead to an overestimation of the influence of the gut content.

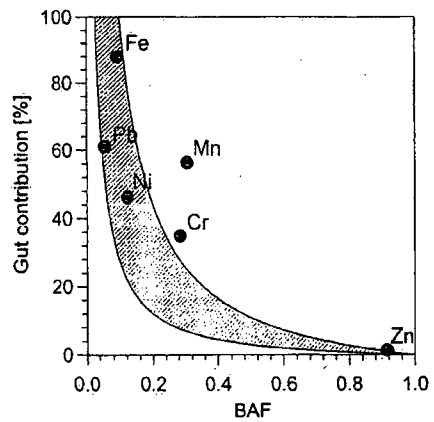

Fig. 7. Expected range of contribution of gut content to the total metal body concentration of *Gammarus fasciatus* as a function of the BAF based on Equation 5. The range of β (3–11%) and the value of the contribution of the gut content to the total metal body concentration are from Amyot et al. [3].

Table 3. Sediment concentration, total Cu body concentration, and bioaccumulation factors (BAF) of *H. azteca* exposed for 4 weeks to sediments from different locations (CF-01–CF-06) in the Clark Fork River [24–26]

Station	Sediment Cu (μg/g)	*H. azteca* Cu (μg/g)	BAF
CF-01	7,820	249	0.03
CF-02	583	87	0.15
CF-03	480	124	0.26
CF-04	478	127	0.27
CF-05	128	124	0.97
CF-06	16	84	5.25
Control	33	80	2.42

Application of Equation 5 to published data from Hare et al. [6] for *Hexagenia limbata* predicts that the gut content causes an overestimation of the metal concentrations of As, Cu, Cd, and Zn in the body by 22, 2, −9, and −1%, respectively. By dissecting the animals and measuring the concentrations separately in the gut content and in the body, Hare et al. estimated the real contribution of the gut content to be 22, 21, 13, and 23%, respectively. These differences between the predicted and the observed estimates of metals in the gut result from uncertainties in β and differences between the true metal concentrations in the gut and in the sediment. This demonstrates the limitations of the model. For this reason, we do not recommend the use of the model to correct data from undepurated invertebrates to estimate "real" body concentrations. However, the model is very useful to check existing data to determine whether the gut content could have a strong (e.g., >50%) influence on the measured body burdens.

It is important to recognize that the impact of the gut content on the body burdens can vary not only between different metals but also for the same metal in different sediments. To demonstrate this, we applied the model to Cu data for *H. azteca* published in three papers that deal with the toxicity of sediments from the Clark Fork River [10,27,28]. Table 3 shows the sediment concentrations of the different sampling sites along the river and the copper body concentrations in undepurated *H. azteca* after a 4-week exposure to these sediments. These data show that the BCF for the same metal can vary from 0.03 to 5.25. If we apply the model to these data (Fig. 8), we can conclude that the "real" (gut-corrected) Cu body concentration was significantly overestimated only at station CF-1. Toxicity tests (4-week, whole-sediment) showed a significant mortality (35%) relative to the control survival only for station CF-1. Borgmann and Norwood [29] observed a much higher mortality of 92% at a similar Cu body concentration (247 μg Cu/g). This supports the prediction of the model that the "real" Cu body concentrations from amphipods exposed to sediment from station CF-1 are overestimated because of the contribution of the gut contents. The model cannot be used to correct the data but can be used to explain them.

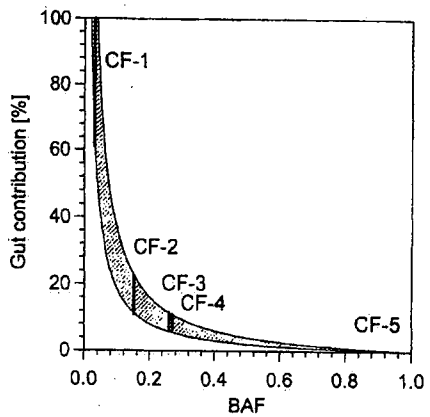

Fig. 8. Expected range of contribution of gut content to the total metal body concentration of *H. azteca* as a function of the BAF based on Equation 5; ▮ = expected range for *H. azteca*, exposed to sediments from different locations in the Clark Fork River (CF-1–CF-5); BAFs estimated from data in Brumbaugh et al. [10] and Ingersoll et al. [27].

Correction of metal body concentrations for gut contents has also proven problematic in other studies. Hare et al. [6] compared a model developed by Chapman et al. [30] to correct body burdens for the gut content. This model was the same as Equation 2 but rearranged to give C_B as a function of C_{TB}, C_S, $\dot{m}_G$ and $m_G + m_B$. For As and Cu, the model worked accurately, but for Cd and Zn the deviation of the corrected body burdens from the "real" body burdens was in the same order of magnitude as the deviation between the uncorrected body and the "real" body burdens. Amyot et al. [3] recommend empirical linear models to correct metal body burdens from *G. fasciatus* for gut content. This model could work reliably only if the bioavailability of a metal is more or less constant because with changing bioavailability the BCF will change, and this will result in a change in the relative contribution of gut content to the total metal body burdens.

Because of these limitations in the existing models, we recommend that the guts of invertebrates be cleared whenever possible, taking body depuration rate constants into account if necessary. At 25°C, 6 h is sufficient time to clear the gut of *H. azteca* and results in minor (<10%) loss of metal from the body. After a depuration time of 24 h, the loss of metal due to depuration from the body can already be as high as 27% (Table 2). If clearing of the guts is not possible for technical reasons, Equation 5 should be used to check whether the estimated total body concentrations are reliable estimates for the "real" body concentration.

Acknowledgement—The work by P.T.M. Neumann was supported by a grant from the Deutsche Forschungsgemeinschaft Ne 607/1-1.

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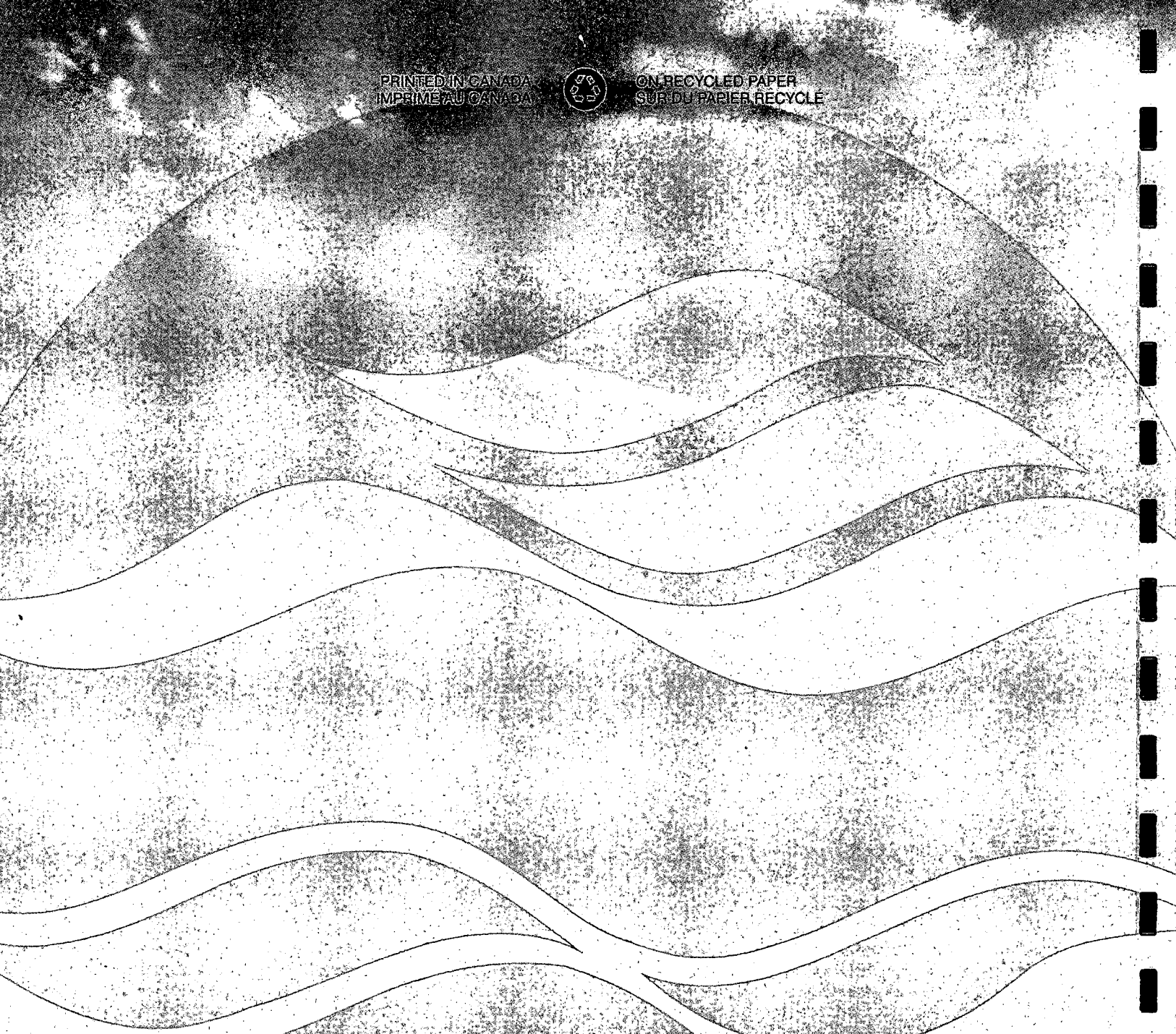

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