The Role of the Zebra Mussel, *Dreissena polymorpha*, In Contaminant Cycling: II. Zebra Mussel Contaminant Accumulation from Algae and Suspended Particles, and Transfer to the Benthic Invertebrate, *Gammarus fasciatus*

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ABSTRACT. *To determine the contribution of ingested material to zebra mussel contaminant accumulation, contaminant assimilation efficiencies (fraction of the total contaminant exposure that is accumulated into tissue, AE) from spiked algae and suspended sediment particles were measured for benzo(a)pyrene, the insecticide DDT, and selected polychlorinated biphenyl (PCB) congeners. Contaminant transfer from zebra mussel feces to the benthic invertebrate, Gammarus fasciatus, was determined by measuring AE from PCB contaminated mussel feces to gammarids. Further, mussel contaminant AE values coupled with physiological and environmental parameters were used in a steady-state model to examine the relative importance of the algal, suspended sediment, and water-borne exposure routes for a representative organochlorine compound, hexachlorobiphenyl (HCBP). The relative accumulation via the fecal and water exposure routes were modeled for gammarids. Mussel AE values for contaminant accumulation were greater from algae than from suspended sediments. Model estimates indicated that when contaminant concentrations in the water were near detection limits, dietary exposure was the primary route of contaminant accumulation (61.5% of the total contaminant concentration). Water was the most important route of contaminant exposure (89.5% of the total contaminant concentration) when contaminant concentrations in water were 10 times greater than the compound detection limit. Suspended sediment was the major dietary source of contaminants at all water concentrations. % AE for zebra mussel feces to gammarid transfer were high—79.0 and 89.4% for hexachlorobiphenyl and tetrachlorobiphenyl respectively—but not statistically different. Model estimates indicated that the dietary route of exposure was the primary source of PCB exposure for gammarids and indicated a potential for PCB biomagnification in the mussel-based detrital food chain. Results suggest that zebra mussels have the potential to change contaminant cycling in the Great Lakes by rerouting dissolved and particulate bound contaminants through zebra mussel food chains with possible biomagnification in upper trophic levels.*

INDEX WORDS: Assimilation efficiencies, Gammarus fasciatus, Dreissena polymorpha, bioconcentration, PCBs, benzo(a)pyrene, zebra mussels.

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INTRODUCTION

The zebra mussel, *Dreissena polymorpha*, was introduced into the Great Lakes ecosystem in 1986 (Hebert and Mackie 1989) and has successfully established extensive populations in littoral zones of four of the major Great Lakes, with highest numbers occurring in the western basin of Lake Erie (Hebert and Mackie 1989, Sea Grant 1991). These large populations of zebra mussel will be exposed to Lake Erie contaminants such as polychlorinated biphenyls (PCBs) through two major routes: 1) filtration of contaminated water; and 2) ingestion of contaminated algae or other suspended material. This exposure is expected to be particularly high in littoral areas that have large contaminant loads, producing a significantly large contaminant accumulation in zebra mussel tissue. Further, the biodeposition of contaminated material as both pseudofeces and feces will increase the potential for food chain transfer to benthic organisms associated with the zebra mussel colonies.

For filter-feeding bivalves such as the zebra mussel, freely dissolved, nonpolar, hydrophobic contaminants are readily available for uptake across the gill membrane (Zitko 1980; Spacie and Hamelink 1985; Fisher *et al.* 1993; Bruner *et al.* 1994). However, contaminants associated with particles (algae or suspended sediment) must desorb from the particles and then diffuse through the gut lining prior to assimilation into mussel tissue (Gobas *et al.* 1993, Klump *et al.* 1987). Therefore, the direct absorption of compounds from water is the fastest and the most efficient mode of contaminant accumulation for bivalves (Stegeman and Teal 1973, Pruell *et al.* 1986, Muncaster *et al.* 1990). Though contaminant uptake from water is significant for zebra mussels (Fisher *et al.* 1993), environmental concentrations of dissolved lipophilic contaminants are very low compared to contaminant concentrations in suspended solids and phytoplankton (Great Lakes Water Quality Board 1987, Swackhamer and Skoglund 1993). Thus, ingested material may represent a more biologically significant route of contaminant exposure for zebra mussels than contaminant uptake from water. This may be particularly true in waters with high particle loads (Langston 1978, Ekelund *et al.* 1987).

The contaminant route of exposure (freely dissolved vs particle-bound) may affect both steady-state concentration of contaminants in zebra mussels and the subsequent fate of those contaminants in the food chain. Exposure to dissolved contaminants will result in contaminated zebra mussel tissues (Fisher *et al.* 1993). The few predators which ingest zebra mus-

sels would then be exposed to zebra mussel-borne contaminants. In contrast, if zebra mussels are exposed to contaminated particles, this route of exposure could result in significant contamination of zebra mussel tissue and feces if not all of the contaminant is assimilated into the zebra mussel tissue during gut passage. Subsequent ingestion of contaminated feces by detritivores could represent an additional and pervasive way in which zebra mussels could introduce contaminants into specific portions of aquatic food chains (Pinkney *et al.* 1985).

Gammarus fasciatus, a benthic amphipod, is found in increasing numbers in zebra mussel colonies, purportedly in response to the structual complexity of the mussel colonies (Dermott and Barton 1991, Griffiths 1993). Gammarids, which feed on contaminated excrement and detritus generated by the zebra mussels may have increased contaminant concentrations compared to gammarids not living in zebra mussel colonies. Because gammarids are prey for most Lake Erie fish species at some point in their life cycle (Oster 1980, Weisberg and Janicki 1990), ingestion of contaminated gammarids could substantially increase the transfer of contaminants to gammarid predators.

Hence, this study determined the contribution of ingested material to zebra mussel contaminant accumulation by measuring mussel contaminant assimilation efficiencies (fraction of total contaminant exposure that is assimilated into tissue, AE) from spiked algae and suspended sediment particles. AEs of benzo(a)pyrene (BaP), the insecticide DDT, and selected PCB congeners were compared between the two dietary components. In addition, AE values were coupled with other physiological and environmental factors to parameterize a steady-state model. The model was used to calculate the relative importance of the algal, suspended sediment, and waterborne routes of exposure to zebra mussel steady-state contaminant concentrations for a representative organochlorine compound, hexachlorobiphenyl (HCBP). Finally, the potential for the zebra mussel to foster food chain contamination by routing unassimilated contaminants into feces and subsequent ingestion by gammarids was determined.

MATERIALS AND METHODS

Chemicals

^{14}C-labeled compounds were obtained from Sigma Chemical Company (St. Louis, Mo.). The specific activity for each chemical was: 2,2′,4,4′,5,5′-hexa-

chlorobiphenyl (HCBP) 20 mCi/mmol; 2,2',4,4'-tetrachlorobiphenyl (TCBP) 13.8 mCi/mmol; benzo(a)-pyrene (BaP) 52 mCi/mmol; and 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT) 12.2 mCi/mmol. All chemicals were greater than 97% pure as determined by thin layer chromatography and radiometric analysis (Leversee *et al.* 1982). All work with PAHs was performed under gold light to minimize photodegradation.

Organisms

Zebra mussels

Methods for collection and culturing of adult zebra mussels are described in Bruner *et al.* 1994. Zebra mussels used to determine contaminant assimilation efficiency were 21 ± 1 mm in length. Adult mussels (25–30 mm) were used for production of spiked feces.

Gammarids

Gammarids were collected from littoral zones of Lake Erie by kick-seining, off-shore of Gilbraltar Island, Put-In-Bay, Ohio. The gammarids were transported in iced lake water to holding facilities at The Ohio State University, Columbus, Ohio. Stocks were maintained in 55 L tanks filled with hard standard reference water (SRW) (USEPA 1975) at $17 \pm 2°C$ with a 12:12 light:dark photoperiod. Gammarids were fed Lake Erie detritus. Water temperature, dissolved oxygen and ammonia were checked daily and the water was replaced three times per week. All waste water was treated with chlorine bleach (50 ppm) before disposal to prevent introducing mussels or veligers into inland waters. Gammarids were acclimated to experimental conditions at least 4 days prior to use.

Algae

Initial populations of *Chlamydomonas rheinhardii* were obtained from stock cultures maintained by the Department of Plant Biology, The Ohio State University, Columbus, Ohio. Monocultures of algae were raised in an amended Bold's Basal Medium (Nichols and Bold 1965) under fluorescent lights (16:8 light:dark) at 20°C.

Media

Soft or hard SRW as described in USEPA (1975) and Bruner *et al.* (1994) was used in either zebra mussel (soft water) or gammmarid (hard water) exposures. Olentangy River sediment (total organic content = 0.9%) was the source of the suspended sediment particles. The dried sediment was sieved and then separated by suspension to yield particle sizes of ≤ 20 μm. PCB and PAH contaminant levels in the sediment were below detection limits (REA-Lab, Wooster, Ohio). The compound detection limits (μg/kg) are as follows: HCBP = 0.01; TCPB = 0.01; BaP = 0.05; DDT = 0.01). Method detection limits (MDL) were determined by relating the smallest detectable quantity to the standard deviation of sample analyte at or near zero analytical concentrations (MDL = $^t.(n-1, 1-a = 0.99)^{xSD}$; where SD = Standard deviation and t = Student's t-value appropriate to 99% confidence level and SD estimates with n–1 degrees of freedom, n = 7).

Media Preparation

Zebra Mussel Pulse-Chase Experiments

Algae (3.0×10^6 cells/mL) suspended in 90 mL SRW were spiked with a radiolabeled chemical dissolved in acetone (4-8 μL) and gently agitated for 3 h. Algal cell and suspended sediment particle numbers were determined using a Coulter Counter Model Z_{BI} (Hialeah, Florida). The algae were centrifuged, the supernatant removed, and the algal pellet rinsed with clean SRW to remove surface attached chemical. This process was repeated three times and then the algae were resuspended in 80 mL of SRW. Equal volumes (20 mL) of the final algae solution were added to each of four 4-L aquaria [algae contaminant concentrations (ng/g wet wt.): HCBP = 91 ng/g; DDT = 346 ng/g; BaP = 33 ng/g; TCBP= 210 ng/g].

SRW also was added to a volume of sediment slurry having sediment particles of diameter ≤ 20 μm so that the final volume was 90 mL SRW and particle concentration was approximately 2.0×10^6 particles/mL. This slurry was spiked with the appropriate radiolabeled chemical (4–10 μL) and gently agitated overnight. The slurry was then centrifuged for 0.5 h, the supernatant was removed, and the particles rinsed as described above for the algae. The rinsed particles were then resuspended in 80 mL of clean SRW and equal volumes (20 mL) were placed into each of four 4-L aquaria [sediment contaminant concentrations (ng/g wet wt.): HCBP = 22.4 ng/g; DDT = 45.1 ng/g; BaP = 8.6 ng/g; TCBP = 44.5 ng/g].

Gammarid Pulse-Chase Experiments

Three to four days before an experiment, dosed algae were prepared as described above. The algal pellet was resuspended in 90 mL of clean SRW and equal volumes (30 mL) of the algae solution were added to each of three 4-L glass aquaria filled with 30 zebra mussels (25–30 mm) so that algae concentrations were approximately 3.0×10^4 cells/mL. No pseudofeces were produced at this low algal cell concentration. Zebra mussel feces were removed by pipette from the aquaria two to three times a day after which the water was changed and new spiked algae were added to each aquarium. The feces were separated from the water by centrifugation then rinsed with clean SRW, centrifuged, and the supernatant discarded. Feces were refrigerated in a foil-covered 15-mL glass centrifuge tube until the day of the experiment.

Contaminant Assimilation Efficiencies

Zebra Mussels

Contaminant AEs were determined using a pulse-chase protocol developed from methods of Luoma *et al.* (1992). For 2–3 days prior to an experiment, 8–10 zebra mussels attached to each of three 100-mm glass petri dishes were held in aquaria filled with SRW and fed low concentrations of noncontaminated suspended sediments or algae (approximately 3.0×10^4 particles/mL). The aquaria were aerated and held in a darkened environmental chamber at 20°C.

On the day of the experiment, zebra mussels (8 mussels/aquarium) were exposed in three replicate 4-L aquaria filled with low concentrations (1.6 (0.03) $\times 10^4$ particles/mL, mean ± SE, n = 28) of spiked algae or sediment particles suspended in 2 L of SRW adjusted to pH 8. Previous experiments had shown that the low particle concentration prevented the formation of pseudofeces (D. Berg, pers. comm., Miami University, Oxford, Ohio, 1994). A fourth aquarium filled with spiked media (algae or sediment particles) but lacking mussels was used as a control. After a 2 h exposure, four mussels from each aquarium were processed for liquid scintillation counting (LSC) (Bruner *et al.* 1994) to determine the contaminant concentration in the animal (tissue and gut), C_0. The contaminant concentration of the particles and the particle number and mass in each aquarium were determined at the beginning and end of the exposure period. Feces produced during the experiment were pipetted from the expo-

sure aquaria, dried, and the amount (µg) of contaminant in the feces was determined by LSC analysis.

The remaining mussels were rinsed with clean SRW, transferred to uncontaminated water, and fed low concentrations of unlabeled algae or sediment particles (3.0×10^4 cells or particles/mL) in 2–3 pulses per day for 48 h to "chase" the unassimilated material through the gut. Feces were collected 2–3 times per day during this portion of the experiment to monitor gut passage of the contaminated media. Contaminant concentration in the mussels with guts purged of contaminated food (C_{48}) was determined after a 48 h purge and corrected for elimination of assimilated contaminant by the equation;

$$\ln C_{48} = \ln C_E - k_d t. \tag{1}$$

where C_{48} = contaminant concentration in the mussels at T_{48} (µg/kg wet wt)
   C_E = contaminant concentration assimilated by mussels (µg/kg wet wt)
   k_d = contaminant elimination rate constant (h^{-1})
   t = duration of the "pulse" exposure (h).

Zebra mussel contaminant elimination rate constants (k_d) were previously determined (Bruner *et al.* 1994, Fisher *et al.* 1993).

The amount of water-borne contaminant accumulated during exposure to spiked media was determined by centrifuging water from the control aquarium to remove the spiked media. Clean mussels were then placed in 250 mL of the supernatant for 1 h after which contaminant levels in the mussels' tissue were determined. The tissue concentration in these mussels, multiplied by the length of the pulse-chase exposure, was used to correct for uptake of desorbed, water-borne compound (C_w).

Percent assimilation efficiencies for each compound were determined using the following equation: Contaminant Assimilation Efficiency (AE) =

$$[(C_E - C_w)/(C_0 - C_w)] \tag{2}$$

where C_E = contaminant concentration assimilated into the mussel tissue (µg/kg wet wt)
   C_0 = total contaminant concentration in mussels (tissue and gut) (µg/kg wet wt)
   C_w = contaminant load from water-borne compound (µg/kg wet wt).

In the calculation, it was assumed that C_0 represented the total contaminant exposure in the pulse period. This was a reasonable assumption for the

algal experiments as the contaminant concentration of the feces was less than 3% of the total exposure. However, in the suspended sediment experiments, the amount of contaminant in the feces produced during the 2-hour exposure period was as much as that in the mussels at the end of the exposure period and represented a significant amount of the total exposure. To correct for this loss, the amount (μg) of contaminant in the feces collected during the sediment exposure was added to the amount (μg) of contaminant in the mussel tissues at T_2 and then divided by the tissue wet wt to determine the total contaminant concentration in the mussels, C_0.

Gammarids

AEs for gammarids also were determined using a pulse-chase protocol similar to that described for the zebra mussels. To eliminate complications due to egg-bearing and deposition, only adult, male gammarids were used in the experiments. Because female gammarids are smaller than the males (Clements 1950), an adult male was operationally defined as any gammarid $\geq$ 7 mm. One day prior to an experiment, adult male gammarids (7–15 mm) were held in aquaria filled with hard SRW and fed uncontaminated zebra mussel feces. The aquaria were aerated and held in an environmental chamber at 16:8 light:dark (17°C).

On the day of the experiment, gammarids (16 gammarids/aquarium) were exposed in three replicate 2-L glass beakers filled with 1 L of hard SRW to which 0.04–0.05 g contaminated zebra mussel feces had been added (exposure concentration: HCBP = 12 μg/g wet wt; TCBP = 36 μg/g wet wt). A fourth beaker filled with 1 L of SRW and contaminated feces but lacking gammarids was used as a control for compound desorption from mussel feces. Additional aliquots of the spiked feces were used to determine fecal contaminant concentration and a fecal dry/wet ratio. After a 4–6 h exposure, five gammarids from each beaker were processed for liquid scintillation counting (LSC) to determine the contaminant concentration in the gammarid (tissue and gut), C_0. The gammarids were blotted dry, weighed, and placed in individual glass scintillation vials filled with 5 mL of tissue solubilizer for 3 days. The solubilizer was then neutralized with 0.5 mL of glacial acetic acid and 5 mL of scintillation cocktail (1,000 mL dioxane, 500 g naphthalene, 5 g diphenyloxazole) were added. The gammarid and water samples were analyzed by LSC using a Beckman LS 6000IC liquid scintillation system (^{14}C efficiency >95%)

with automatic quench control. Contaminant concentration was determined on a wet-weight basis. In addition, 15 mL of the test water were centrifuged and the supernatant analyzed by LSC for dissolved contaminant. Uneaten zebra mussel feces were filtered from the water, dried for 3 d in a desiccator, weighed, and analyzed by LSC.

The remaining gammarids were rinsed with clean SRW, transferred to uncontaminated water, and fed unlabeled filamentous algae (*Cladophora* sp.) for 48 h to "chase" the unassimilated contaminants from the gut. The gammarids were screened from the bottom 8 cm of the beakers to prevent them from consuming their own contaminated feces. Feces were collected 2–3 times per day during this portion of the experiment to monitor gut passage of the contaminated food. Contaminant concentration in the purged gammarids (C_{24}) was determined after 24 h and was corrected for elimination of assimilated contaminant by equation 1.

The amount of water-borne contaminant taken up by the gammarids during the pulse-chase experiment was determined by centrifuging water from the control aquarium to remove the spiked zebra mussel feces. A clean gammarid was then placed in each of two replicate 250 mL of the supernatant for 1 h after which contaminant levels in the gammarids were determined. The tissue concentration in these gammarids, multiplied by the length of the pulse-chase exposure was used to correct for uptake of water-borne compound (C_w).

Gammarid contaminant AE was determined using equation 2. In this calculation, C_0 is assumed to be the total gammarid contaminant exposure received during the "pulse" period of the experiment.

Zebra Mussel Feeding Efficiency

Zebra mussel feeding efficiency (fraction of the filtered particles that are ingested) of suspended sediments was determined for 21 ± 1 mm long, Lake Erie western basin zebra mussels. The test solution was prepared by adding a $\leq$20 μm size fraction of suspended sediment to 12 L of soft SRW (20°C) to make a particle concentration of 0.8 × 10^5 particles/mL (12 mg dry wt/L). Individual mussels were placed into 20 of 23 beakers filled with 500 mL of the suspended sediment solution and were allowed to filter for 3 h. The remaining beakers were used as a control for particle settling. All beakers were held in an environmental chamber (20°C) and mussel filtering was visually monitored every h. After 3 h, the mussels that filtered over the entire

exposure period were removed from the test beakers and placed in clean water to clear their guts. Pseudofeces produced during the exposure period and the gut clearance period were collected, dried, and weighed. The gut clearance period was terminated at 48 h, when fecal production had ceased. Zebra mussels were eviscerated and the tissue wet wt was determined. The suspended sediment feeding efficiency was determined by the following equation:

Feeding efficiency = [(mg sediment cleared −
    mg pseudofeces)/ (mg sediment cleared)].   (3)

Gammarid Feeding Rate

Gammarid feeding rate on zebra mussel feces was determined for adult male gammarids (10–15 mm long). Individual gammarids were placed into 9 of 12 beakers filled with 250 mL of hard SRW to which 0.005 g wet wt of uncontaminated zebra mussel feces were added, and the gammarids were allowed to feed on it for 4 h. The remaining beakers were used as a control for feces recovery efficiency and all beakers were held in an environmental chamber at 17°C. Additional aliquots of feces were used to determine the feces dry/wet ratio. After 4 h, the gammarids were removed from the test beakers, blotted dry, and weighed. The remaining zebra mussel feces were filtered from the test beakers onto preweighed filter papers, dried 24 h in an oven (56 ± 2°C), cooled in a desiccator, and weighed. Gammarid feeding rate was calculated using the following equation:

Feeding rate (FR) = (T_0 mg dry wt mussel feces −
T_4 mg dry wt mussel feces)/g wet wt gammarid/4 h.
(3)

Gammarid Bioconcentration

Uptake Experiment

Individual adult male gammarids were exposed to contaminants in uncovered 1-L glass beakers containing 250 mL of spiked water. Spiked water was prepared for gammarid experiments using the methods described in Bruner *et al.* (1994). Exposure concentrations were: HCBP = 0.2 µg/L; TCBP= 0.3 µg/L. After 0.5, 1, 1.5, 2, 4, or 6 h of exposure, five beakers were removed from the chamber. Three 1-mL water samples were taken from each beaker for radiometric analysis and the gammarids were processed for LSC as described above. To complete the mass-balance analysis, the amount of compound adsorbed to the glassware was

determined. Empty beakers were rinsed with clean water, then with 20 mL of acetone. A 1-mL sample of the acetone rinse was analyzed for radioactivity.

Elimination Experiment

Fifty adult male gammarids were exposed for 6 h in five replicate aquaria filled with spiked SRW. After 6 h, the gammarids were removed from the spiked water, rinsed thoroughly with clean SRW, and one gammarid from each aquarium was sampled for radiometric analysis. The remaining gammarids were placed in five replicate glass elimination aquaria (4 L) filled with hard SRW. The elimination aquaria were aerated and maintained in an environmental chamber at 17°C. Five gammarids were removed from each chamber for analysis at 24, 48, 72, 96, 120, 144, and 168 h. The water in the elimination chambers was changed daily and the gammarids were fed filamentous algae (*Cladophora* sp.). Contaminant levels in the water were monitored by taking three 1-mL water samples at each sampling period.

Kinetic Model

A mass-based kinetic model was used to estimate gammarid contaminant uptake clearance, k_u (Fisher *et al.* 1993). The basic assumptions of the model are: 1) the mass of compound does not change in the system; and 2) no biotransformation of contaminant takes place. Because sorption to glassware was <3% of the total mass and significant elimination did not occur over the exposure time (6 h), these parameters were eliminated to give a final equation:

$$k_1 = [-\ln(1 - Q_a/A)]/t \qquad (4)$$

where Q_a = amount of contaminant in the tissue
            (µg)
        A = amount of contaminant in the treatment beaker at T_0 (µg).

The system independent k_u was calculated as

$$k_u = k_1 \text{ (Volume of water/Wet mass of tissue). (5)}$$

The elimination data were fit to a first order elimination model with the following integrated form:

$$\ln C_a = \ln C_{ao} - k_d t \qquad (6)$$

where C_{ao} = Contaminant concentration in organism at beginning of elimination period (µg kg^{-1} wet wt).
        C_a = Contaminant concentration in organism at time t (µg kg^{-1} wet wt).

The elimination rate constant (k_d) is determined from the slope of the regression line for ln C_a vs t.

Bioconcentration factors (BCFs) were calculated from the ratio of the uptake clearance and elimination rate constants:

$$BCF = k_u/k_d. \qquad (7).$$

Statistics

Analysis of variance (ANOVA, $p < 0.05$) was performed on % AE values (defined below) using the NCSS statistical program (NCSS 1991). Log transformed k_u and BCF values were analyzed by a general linear analysis of variance (GLM ANOVA NCSS 1991). Significance was determined at $p < 0.05$. Differences in k_d values were significant if 95% confidence intervals did not overlap. Individuals from systems with a mass-balance <70% were not included in the data analysis.

RESULTS

Zebra Mussel

Assimilation Efficiency and Feeding Efficiency

For ease of discussion, AEs are presented as % AE (AE × 100). % AEs from spiked algae to zebra mussels ranged from 45 to 77% (Table 1). Significant differences in % AEs for the algal exposure were found between DDT and the two PCB congeners and between BaP and TCBP. With the exception of HCBP, contaminant % AEs from spiked algae increased with decreasing compound log octanol:water partition coefficient (log K_{ow}). The correlation of contaminant % AE with compound log K_{ow} was negative (% AE = 645.2 − 97.4 log K_{ow}, $r^2 = 0.52$, $p < 0.05$, n = 4) but was not statistically significant.

Contaminant % AE from spiked sediment particles ranged from 1 to 30% and, except for TCBP, % AEs from spiked sediment were not significantly different from each other (Table 1). Consequently, contaminant % AEs were not significantly correlated to log K_{ow} (% AE = 19.4 − 103.1 log K_{ow}, $r^2 = 0.56$, $p < 0.05$, n = 4). When contaminant % AEs for the same compound were compared between routes of exposure, % AEs from contaminated algae were statistically greater than those determined from contaminated suspended sediment for all compounds (Table 1). Zebra mussel feeding efficiency of suspended sediments (12 mg dry wt/L) was determined to be 0.46% SE ± 0.04%.

Elimination of contaminants ingested from spiked algae was much slower than that from ingested suspended sediment with fecal elimination leveling off 36 h after ingestion of the spiked algae (Fig. 1A). Contaminants ingested with suspended sediments particles were rapidly cleared from the gut and by 24 h after ingestion, fecal elimination of contaminants had reached a plateau (Fig. 1B).

Gammarids

Feeding Rate

Recovery efficiency of zebra mussel feces from the controls was 100% and gammarid feeding rate was determined to be 0.0026 mg dry wt/mg wet wt/h. Previously published *Gammarus fasciatus* feeding rates were not found. However, when our feeding rate was converted to mg dry wt/mg dry wt gammarid/h (0.0115) it fell near the top end of a range of feeding rates reported for two freshwater populations of *Gammarus pulex* (0.0042–0.0104 mg/mg animal/h) (Crane and Maltby 1991).

Assimilation Efficiency

Gammarid gut clearance of TCBP and HCBP after exposure to contaminated zebra mussel feces in the "pulse" period of the experiment was very slow after 24 h and elimination of HCBP had reached its highest level at this time (Fig. 2). Based

TABLE 1. **Zebra mussel contaminant percent assimilation efficiencies (% AE) from spiked algae and suspended sediment particles. The numbers in parenthesis are standard errors.**

| Compound | % AE | | | Log K_{OW} * |
	Algae	Sediment		
HCBP	68.6 [b] (2.9)	29.6 [a] (7.6)	**	6.90 [1]
DDT	44.9 [a] (7.6)	22.9 [a] (2.8)	**	6.19 [2]
BaP	53.0 [ab] (6.2)	20.6 [a] (3.8)	**	5.98 [3]
TCBP	77.6 [c] (5.5)	1.0 [b] (0.5)	**	5.90 [1]

* Within a column, values with different letters are significantly different from each other ($p < 0.05$).
** Between columns, values with double asterisks are significantly different from each other ($p < 0.05$).
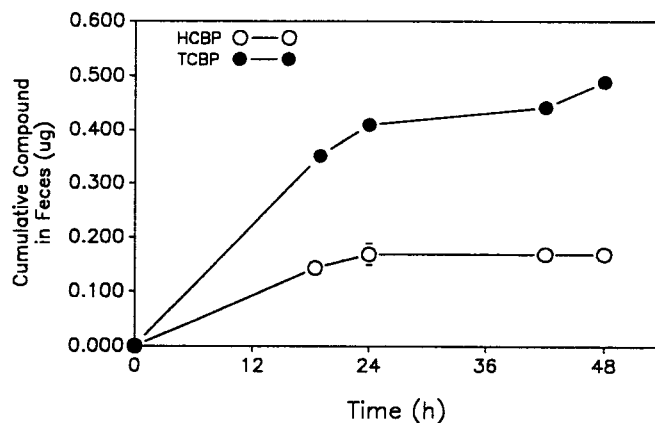[1] Shiu and Mackay (1986)
[2] Chiou et al. (1977)
[3] Miller et al. (1985)
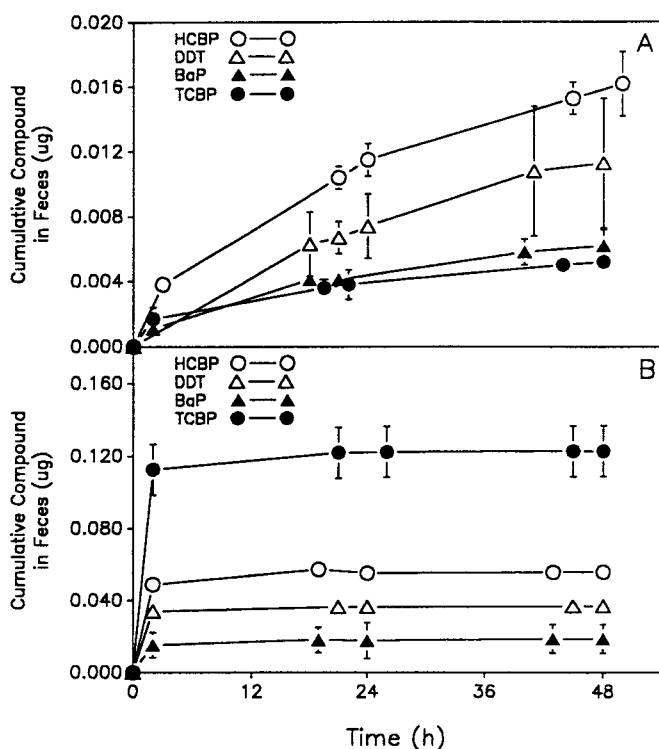
FIG. 1. *Cumulative contaminant (μg) in zebra mussel's feces collected after exposure to spiked algae (A) or spiked suspended sediments (B). Error bars represent standard errors.*

FIG. 2. *Cumulative contaminant (μg) in gammarid feces during the "chase" period of the assimilation experiment. Error bars represent standard errors.*

on these data, the length of the "chase" period of the experiment was set at 24 h. Reported gut clearance time for *Gammarus fasciatus* was not available, however, gut clearance for *Gammarus pseudolimnaeus*, a similar sized freshwater gammarid, is 6–10 h (Marchant and Hynes 1981). Gammarid contaminant% AE of HCBP (89.4%) was greater than for TCBP (79.0%), however, the two values were not statistically different (Table 2).

Bioconcentration

Uptake clearances were significantly higher for HCBP (780 mL g^{-1} h^{-1}), the more hydrophobic compound (log K_{ow} 6.9), than for TCBP (430 mL g^{-1} h^{-1}, log K_{ow} 5.9) (Table 2). Elimination rate constants (k_d) were identical for the two compounds while BCFs were significantly greater for HCBP (195,000 mL g^{-1}) than for TCBP (96,000 mL g^{-1}). The BCF differences reflect differences in uptake rather than elimination.

DISCUSSION

Assimilation Efficiency

Contaminant assimilation from food reported for a number of aquatic organisms, including fish, bivalves, and benthic invertebrates, is affected by food digestibility, gut retention time, size, and feeding behavior of the aquatic organisms (Ekelund 1989, Jahan-Parwar *et al.* 1990). If % AE values between food sources are compared, the percent assimilation of PCBs from highly digestible food was greater than 50% for the guppy (51–61%)(Gobas *et al.* 1988) and the marine gastropod *Aplysia californica* (84–94%)(Jahan-Parwar *et al.* 1990). PCB assimilation from less digestible sediment particles is considerably lower: the marine bivalve, *Macoma nasuta* (38%); freshwater oligochaetes (36–15%); and mysids (53%) (Klump *et al.* 1987, Lee *et al.* 1990, Klump *et al.* 1991).

For zebra mussels, contaminant assimilation from spiked algae was greater than from sediment supporting the above finding that contaminant% AEs are greater for the more digestable particles. An explanation for this result may be found in the mechanics of contaminant absorption. In the zebra mussels' gut, algal cell membranes are lysed during the digestive process and the cellular components of the lysed cells are further broken down and assimilated. This removal of mass by assimilation of cellular components will increase the contaminant concentration (fugacity) resulting in an increase in contaminant assimilation as material moves down

TABLE 2. Contaminant percent assimilation efficiencies (% AE), kinetic and physiological parameters used in the steady-state model to determine gammarid PCB concentrations from exposure to contaminanted zebra mussel feces. Numbers in parenthesis are standard errors.

Compound	% AE	k_u mL g^{-1} h^{-1}	k_d h^{-1}	BCF* mL/g	C^w ng/L	C^f mg/kg dry wt	FR mg dry wt/ mg wet wt/h
HCBP	89.4 [a] (7.2)	780 [a] (58)	0.004 [a] (0.001)	195,000 [a] (14,500)	0-0.5	0.877	0.0026
TCBP	79.0 [a] (14.0)	430 [b] (64)	0.004 [a] (0.001)	96,000 [b] (16,800)	0-0.5	0.076	0.0026

* Within a column, values with the same letter are not significantly different.

the gut (Gobas *et al.* 1993). The highly digestible algae is more amenable to the influence of the digestive process than the sediment particles. Further, the algae were retained longer in the zebra mussel gut than the sediment particles, allowing more time for contaminant assimilation (Fig. 1).

The gammarid % AEs for PCBs (79 and 89.4%) also fell within the range of % AEs listed above for PCBs accumulated from highly digestible food (Jahan-Parwar *et al.* 1990, Gobas *et al.* 1988). % AEs of PCBs for other freshwater gammarid species were not available for comparison. However, the marine gammarid, *Gammarus tigrinus*, accumulated 64.3% of the PCBs taken up from food after 24 h of elimination in uncontaminated water (Pinkney *et al.* 1985). Thus, for gammarids living in zebra mussel colonies, ingestion of contaminated zebra mussel feces can be a significant route of PCB exposure.

A negative correlation between compound log K_{ow} and contaminant % AEs has been found for highly hydrophobic compound assimilation in fish and marine molluscs (Means *et al.* 1980, Muir and Yarechewski 1988, Jahan-Parwar *et al.* 1990). In our study, compounds sorbed to algae were, with the exception of HCBP, more readily assimilated as the log K_{ow} of the compound decreased but the correlation to log K_{ow} was not statistically significant. Why HCBP, with the highest log K_{ow} was so readily assimilated is not known. The assimilation of compounds from spiked suspended sediments had, with the exception of TCBP, similar assimilation efficiencies despite differing log K_{ow} values. Gammarid % AEs of TCBP and HCBP from feces were not significantly different even though HCBP has a greater log K_{ow} value than TCBP. More compounds must be tested before a definite relationship between log K_{ow} and % AEs can be determined for these species.

The extremely low zebra mussel % AE of TCBP from suspended sediments is due more to deficiencies in the the experimental design (batch cultures) than a lack of assimilation of TCBP in the mussel gut. Because of rapid desorption of TCBP from the sediment particles or because unbound TCBP was inadvertently introduced into the aquaria with the spiked sediment, the dissolved fraction of TCBP in the water was much higher than for the other chemicals. Consequently, correcting for the dissolved fraction accumulation reduced TCBP % AEs from spiked sediment.

Gammarid Bioconcentration

The kinetics of gammarid uptake from water were characteristic of nonpolar, nonmetabolized, hydrophobic compounds in invertebrates; compound uptake is rapid and elimination is slow resulting in significant bioconcentration (Spacie and Hamelink 1985). A direct comparison of our BCFs to BCFs reported in the literature was not possible because the experimental methodologies were different. However, the freshwater *Gammarus psuedolimnaeus* exposed 60 d to water-borne PCBs had significant BCFs ranging from 16,000 to 108,000 depending on water concentration and PCB congener (Nebeker and Puglisi 1974). In addition, *Gammarus pseudolimnaeus* exposed to HCBP-contaminated water for 192 days had BCFs of 160,000 and 460,000, again depending on the water concentration (Lynch and Johnson 1982). Thus, our BCF values for *Gammmarus fasciatus* are similar to those of other freshwater gammarids and demonstrate significant bioconcentration.

Route of Exposure

Zebra Mussels

Based on contaminant assimilation efficiencies, the algal route of exposure is the more important route of zebra mussel dietary contaminant exposure, assuming mussels are exposed to equal concentrations of algae and suspended particles. However, differences in the mass of algae and suspended sediment in the field as well as potential differences between field contaminant concentrations of algae and suspended sediment may alter the relative importance of these vectors. In addition, water-borne contaminants will contribute to the zebra mussel's contaminant load.

To estimate whether contaminated algae consumption is an important route of dietary exposure for the Lake Erie zebra mussel and to measure the contribution of all routes of exposure on zebra mussel contaminant concentrations, a steady-state model was constructed. The model allowed us to calculate the relative contribution of each exposure route to the steady-state contaminant concentration of a representative organochlorine compound, HCBP, in zebra mussels (equations 8–10).

$$dC_a/dt = 0 = (k_u \cdot C_w) +$$
$$(Fr \cdot AE \cdot FE \cdot C_p \cdot C_e) - (k_d \cdot C_a) \qquad (8)$$

$$k_d \cdot C_a = (k_u \cdot C_w) + (Fr \cdot AE \cdot FE \cdot Cp \cdot C_e) \qquad (9)$$

$$C_a = [(k_u \cdot C_w) + (Fr \cdot AE \cdot FE \cdot C_p \cdot C_e)] / k_d \qquad (10)$$

where C_a = steady-state contaminant concentration in the animal (μg kg^{-1} wet wt)
  k_u = uptake clearance constant (mL g^{-1} h^{-1})
  C_w = contaminant concentration in water (ng L^{-1})
  Fr = filtering rate (mL g^{-1} wet wt h^{-1})
  FE = feeding efficiency
  AE = compound assimilation efficiency
  C_p = contaminant concentration of algae or suspended sediment (ng g^{-1} dry wt)
  C_e = concentrations of algae or suspended sediment (mg dry wt L^{-1})
  k_d = elimination rate constant (h^{-1})

The basic assumptions of the model are: 1) there is a steady-state condition in which contaminant flux out of the organism is equal to the flux into the organism; 2) contaminant uptake is both from water and particulate sources; 3) contaminant uptake and elimination rates will not vary; and 4) filtering rates, feeding efficiencies, and toxicant assimilation efficiencies are conditional. The model term for the

dietary route of exposure was calculated for both algae and suspended sediments.

Literature and measured values for the model parameters used in the HCBP simulation are presented in Table 3. Zebra mussel filtering rates of algae and feeding efficiencies of algae and suspended sediment of ≤ 20 μm were determined using Lake Erie western basin zebra mussels (Table 3) (this study; D. Berg, pers. comm., Miami University, Oxford, Ohio, 1994). Contaminant clearances (k_u) and elimination rate constants (k_d) were predetermined from Lake Erie zebra mussel populations (Bruner *et al.* 1994, Fisher *et al.* 1993). The zebra mussel algal feeding rate (1158 mL/g wet wt/h) was used in calculations for both algae and sediment routes of exposure because it was a conservative estimate of zebra mussel environmental filtering rate (2,952 mL/g wet wt/h using 0.01 g mussel wet wt) (MacIsaac *et al.* 1992).

Suspended sediment concentrations were estimated from reported contaminant concentrations in Lake Erie sediment and from the percent of congeners present in the suspended sediment (Table 3). Because field contaminant concentrations for phytoplankton in Lake Erie were not available in the literature, the phytoplankton concentrations of HCBP and TCBP (TCBP data used in the gammarid model below) were estimated from regressions of phytoplankton BCFs vs log K_{ow} from Green Bay, Lake Michigan (Swackhamer and Armstrong 1987, Swackhamer and Skoglund 1993). Because BCFs are the ratio of the algae contaminant concentration to the ambient water contaminant concentration, the BCFs were divided by the Green Bay water concentration for each congener to arrive at the phytoplankton contaminant concentrations. Further, since Lake Erie PCB water concentrations were approximately four times lower than those of Green Bay, the estimated phytoplankton HCBP and TCBP concentrations were divided by this factor so that the phytoplankton estimates reflected HCBP and TCBP water concentrations in western Lake Erie.

Reported levels of contaminants in Lake Erie water samples were difficult to interpret because it was not always evident if suspended material had been removed from the water samples before analysis. In addition, water concentrations of individual PCB congeners were not available. Therefore, the contaminant water concentration was varied from zero to ten times the detection limit of HCPB (Table 3). The percent of the total zebra mussel steady-state HCBP concentration contributed by algae, suspended sediment, or by water-borne

TABLE 3. **Parameters used in the steady-state model to determine mussel steady-state HCBP concentrations.**

Media	k_u mL g^{-1} h^{-1}	k_d h^{-1}	C_w ng/L	C_p ng/g dry wt	C_e mg/L	% AE	Fr mL/g wet wt/h	% FE
Sediment	895 [a]	0.005 [a]	0 – 0.5	25.3 [b]	11.1 [c]	29.6	1,158 [d]	47
Algae	895 [a]	0.005 [a]	0 – 0.5	165.0	0.2 [e]	68.9	1,158 [d]	100 [d]

[a] Bruner *et al.* (1994)
[b] Estimated from sediment concentrations of total PCB [1] × % of congener in total PCB[1] (HCBP = 12%) × % of congener in bottom sediments[2] (HCBP = 46%).
[c] Rowan and Rasussen (1992)
[d] D. Berg (pers. comm., Miami University, Oxford, Ohio, 1994)
[e] Makarewicz *et al.* (1989)
[1] Oliver and Bourbonniere (1985)
[2] Oliver and Charlton (1984)

routes of exposure was then determined at each water concentration. The effect of changes in contaminant water concentrations on algae and suspended sediment contaminant concentrations was not modeled. Reported Lake Erie water concentrations for HCBP fell within the range of water concentrations modeled.

Model estimates indicated that when HCBP concentrations in the water were near detection limits, dietary exposure was the primary route of contaminant accumulation (61.5% of the total contaminant concentration)(Table 4). Water was the most important route of contaminant exposure (89.5% of the total contaminant concentration) when HCBP concentrations in water were 10 times greater than the compound detection limit. Suspended sediment was the major dietary source of HCBP at all water concentrations. Zebra mussel steady-state HCBP concentrations determined by the model were compared to those reported for a nearshore, western Lake Erie zebra mussel population (Kries *et al.* 1991). Contaminant concentrations in the field mussels were reported as total PCB (520 µg/kg wet wt). Thus, the amount of HCBP in the field mussels had to be estimated from the percent of the total each congener represented (HCBP = 12%). The calculated HCBP concentration of the field collected zebra mussels (62.4 µg/kg) fell within the range of HCBP steady-state values estimated from the model (14.0–104.0 µg/kg). The model estimates of zebra mussel steady-state HCBP levels were surprisingly similar to concentrations in field collected zebra mussles.

The model results suggests that contaminant AEs alone cannot predict the primary route of dietary ex-

posure when contaminant concentrations of the media, the media particle concentrations, and other physiological processes are considered. In addition, the model simulations indicated that the steady-state estimates may be modified by variations in contaminant levels in the water source. Further, zebra mussel filtering rates and feeding efficiency will vary with food concentration, size and palatability (Ten Winkel and Davis 1982; Sprung and Rose 1988; D. Berg, pers. comm., Miami University, Oxford, Ohio) and contaminant AEs may change with fluctuations in food concentration or with the contaminant concentration of the ingested material. Thus, incorporation of variations in the physiological parameters of the model will help to make the model estimates more representative of the actual contaminant concentration in western Lake Erie mussels.

Gammarids

Based on the kinetic data and assimilation efficiencies, gammarids can readily assimilate PCBs from water and contaminated food. To determine the relative importance of each exposure route, a steady-state model similar to that used for the zebra mussel was constructed to calculate the relative contribution of each exposure route to the steady-state contaminant concentration in the gammarid. This model was identical to equation 10 described above for the zebra mussel except that the term for the food compartment consisted of only the gammarid AE, feeding rate (FR, ng mg^{-1} wet wt h^{-1}) and the contaminant concentration of food (C_f, mg g^{-1} dry wt).

TABLE 4. Model estimates of zebra mussel steady-state (SS) HCBP concentration from algal, suspended sediment and water routes of exposure for three water concentrations (ng/L) and the percent contribution to the total mussel HCBP concentration from each route of exposure.

| | Contribution to Zebra Mussel SS Contaminant Concentration Route of Exposure | | | | | | |
| | Sediment | | Algae | | Water | | |
Compound	Concn. μg/kg wet wt	%	Concn. μg/kg wet wt	%	Concn. μg/kg wet wt	%	Water Concn. ng/L
HCBP	9.10	63.3	5.20	36.7	0	0	0
	9.10	38.9	5.20	22.6	8.95	38.5	0.05
	9.10	8.7	5.20	5.0	89.50	86.3	0.50

The gammarid feeding rate, PCB congener AEs, uptake clearances, and elimination rate constants used in the model were determined in this study (Table 2). PCB congener concentrations in zebra mussel feces were estimated from PCB concentrations in zebra mussel feces produced by mussels fed algae spiked to estimated field phytoplankton concentrations (HCBP = 165 ng/g dry wt, TCBP = 46.3 ng/g dry wt). As with the zebra mussel model described above, we varied the water concentrations of HCBP and TCBP from zero to ten times the detection limit for PCBs and then calculated the percent of the total gammarid steady-state contaminant concentration contributed by food or water at each water concentration. The effect of changes in contaminant water concentrations on zebra mussel feces contaminant concentrations were not modeled.

Gammarid steady-state HCBP concentrations were higher than those of TCBP (Table 5). Lower TCBP assimilation efficiency and lower TCBP concentrations in mussel feces accounted for the lower steady state concentrations of TCBP. For both PCB congeners, at low water concentrations (0.05 ng/L), ingestion of contaminated zebra mussel feces contributed the greater percentage of the total contaminant load. At high water concentrations (0.5 ng/L), HCBP contaminated feces remained the primary source of HCBP in gammarids while TCBP spiked feces contributed only 42% to the total contaminant concentration.

The model also was used to generate gammarid steady-state concentrations from feeding on an algal detritus in contrast to zebra mussel feces. This gives an estimate of the particle contribution to gammarid

body burdens in the absence of the zebra mussel. The PCB congener concentration in the algal detritus was assumed to be the same as concentrations in the live algae (HCBP = 165 ng/g dry wt, TCBP = 46.3 ng/g dry wt). Gammarid PCB concentrations were 2–5 times greater when gammarids fed on mussel feces than when they fed on contaminated algal detritus (0.04–0.51 vs 0.0.024–0.10 mg/kg wet wt) (Table 6). The results suggest that gammarids feeding in mussel colonies are at greater risk of contaminant accumulation than gammarids feeding on lake detritus.

Model Characteristics

Each of the model calculations made were deterministic and used the mean value for the conditional kinetic coefficient. These calculations permitted evaluation of the relative importance of the various routes of accumulation. However, the errors associated with these values were not considered in the model calculations. Normally, the uncertainty of the model would be determined by Monte Carlo methods to assess the role of each parameter's error on C_a. However, because the concentrations in the water and on the particles could only be approximated and the kinetic constants were determined under only one set of conditions, it did not seem appropriate to do a full uncertainty analysis at this time.

The sensitivity analysis examining the relative role of the water concentrations in determining C_a was simplified also due to the very approximate nature of the water concentration data. However, even

TABLE 5. *Model estimates of gammarid steady-state (SS) contaminant concentrations from dietary and water routes of exposure for two water concentrations (ng/L) and the percent contribution to the total gammarid contaminant level from each route of exposure.*

	Contribution to Gammarid SS Contaminant Concentration Route of Exposure				
	Zebra Mussel Feces		Water		
Compound	Concn. µg/kg wet wt	%	Concn. µg/kg wet wt	%	Water Concn. ng/L
HCBP	509.6	98.1	9.7	1.9	0.05
	509.6	84.0	97.4	16.0	0.50
TCBP	39.0	88.0	5.4	12.0	0.05
	39.0	42.4	54.0	57.6	0.50

TABLE 6. *Steady-state concentrations of HCBP and TCBP in gammarids feeding on contaminated feces, in gammarids feeding on contaminated algae, in zebra mussel feces, and in algal detritus.*

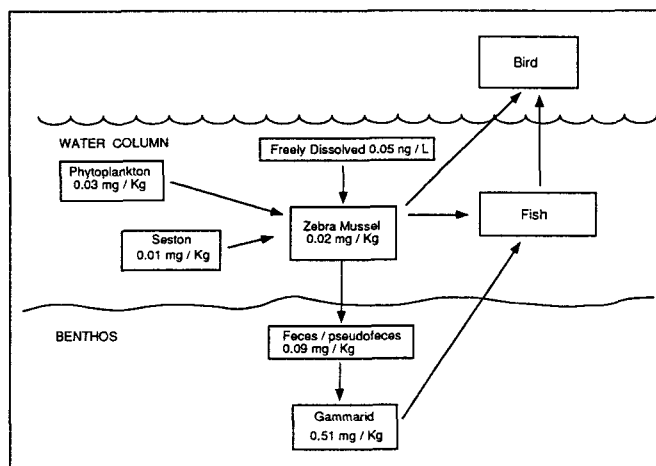|	Steady State Concentration			
Compound	Gammarid Feeding on Feces mg/kg wet wt	Gammarid Feeding on Algae mg/kg wet wt	Zebra Mussel Feces mg/kg wet wt	Algal Detritus mg/kg wet wt
HCBP	0.51	0.10	0.088	0.026
TCBP	0.04	0.024	0.008	0.007

these simple simulations in which the water concentrations were altered demonstrate the importance of the aqueous phase in accumulation of nonpolar contaminants. When the various model parameters are better described, e.g., when AEs have been determined with several algal species and suspended sediment sources, the effects of environmental and physiological parameters on the kinetics are defined, and the contaminant concentrations are better measured, a complete sensitivity and uncertainty analyses should be performed.

FIG. 3. *HCBP concentrations in various compartments of a lake ecosystem. HCBP concentrations were taken from the steady-state model.*

Ecological Significance

Biomagnification is the process in which tissue contaminant concentrations increase as the chemical moves through two or more trophic levels (Rand and Petrocelli 1985). To describe the effects of zebra mussels on contaminant transfer through aquatic food chains, it is necessary to have estimates of contaminant concentrations in the various trophic levels that comprise the food chain. Figure 3 depicts a simplified lake ecosystem and illustrates a pelagic (particle-mussel-fish) and a benthic (particle-mussel feces-gammarid-fish) food chain. Excluding the vertebrate trophic levels, the estimated steady-state concentration of HCBP is listed next to the relevant food chain components of the aquatic ecosystem. The HCBP concentrations in the water,

phytoplankton, suspended sediments, and zebra mussel feces were the same as those used in the steady-state model and, except for the water concentration, converted to mg of contaminant per kg wet wt of the media. Steady-state HCBP concentrations in gammarids and zebra mussels were estimated from the models and are expressed as organism wet wt.

The model estimates indicated HCBP biomagnification only in the zebra mussel detrital food chain. In this food chain there was a 4 to 5-fold increase in HCBP concentrations for gammarids which fed on contaminated zebra mussel feces. This 5-fold increase was consistent with that of Rasmussen *et al.* (1990) who found a general 3-fold increase in PCBs

with each trophic level of a freshwater, pelagic food chain. The HCBP did not biomagnify in the zebra mussel pelagic food chain as there was no increase of HCBP in the zebra mussel relative to its algal food source. To conclusively determine if PCBs will biomagnify in zebra mussel-dominated food chains, field measurements of PCB concentrations at additional trophic levels must be made.

The model results also indicated that the filtering activity of the zebra mussel may fundamentally alter contaminant fate in Lake Erie. For instance, comparison of the gammarid concentration of HCBP to that in the zebra mussel indicated that gammarids which fed on zebra mussel feces had HCBP concentrations 20 times higher than the zebra mussel tissue (Fig. 3). Consequently, if a fish were to ingest 1 g of gammarids it will be exposed to 20 times more HCBP than if it ingested 1 g of zebra mussel tissue (assuming equal contaminant assimilation efficiency by the fish). In addition, the direct predation of zebra mussels by fish in western Lake Erie is limited to one species, the freshwater drum (French and Bur 1993), while gammarids are a food source for almost all Lake Erie fish species at some point in their life cycle. Thus, the indirect transfer of HCBP via the detrital food chain is expected to funnel significantly more contaminant to higher trophic levels than through the pelagic food chain. Finally, model estimates of the gammarid steady-state PCB concentrations is probably conservative since it did not include HCBP associated with mussel pseudofeces and dead mussel tissue. Once the contribution of these sources to gammarid HCBP levels are determined, the biomagnification process in the detrital food chain may be enhanced.

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