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Absorption of Hydrophobic Contaminants from Ingested *Chlamydomonas rheinhardtii* and *Chlorella vulgaris* by Zebra Mussels, *Dreissena polymorpha*

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ABSTRACT. *The zebra mussel,* Dreissena polymorpha, *has the potential to influence contaminant cycling in freshwater systems because of its large population density, high lipid content, and high filtering rate. Ingestion of contaminated particles such as algae dominates exposure routes for the zebra mussel for strongly particle-associated contaminants. However, the data on absorption efficiency are limited and models to predict contaminant accumulation for the lower food web have identified the absence of such data as limiting and necessary to improve predictions. Accumulation of 2,2′,4,4′-tetrachlorobiphenyl (TCBP), 3,3′,4,4′,5-pentachlorobiphenyl (PCBP), 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP) and 1,1-dichloro-2,2-bis[4-chlorophenyl] ethylene(DDE) was determined at two algal concentrations from exposures to contaminated* Chlamydomonas rheinhardtii *and* Chlorella vulgaris. *The contaminant absorption efficiencies were determined based on a chemical-mass-balance model. Mussel absorption efficiencies for the four chemicals at the two different algal concentrations for the two algal species ranged from 68.3% to 95.4% and were independent of algal concentrations and algal species for the same chemical.*

KEY WORDS: *Zebra mussel, contaminant, absorption efficiency, contaminant transfer.*

INTRODUCTION

The zebra mussel is a dominant benthic organism in portions of Lake Erie and Lake St. Claire and many shallow water areas of the rest of the Great Lakes since their invasion in 1986. Because of their high filtering rate and high population density, the mussels have been responsible for significant changes in the energy flow from pelagic to benthic disrupting the amount of phytoplankton available to pelagic zooplankton and the rest of the food web (Nalepa and Fahnenstiel 1995, Stoeckmann and Garton 1997). These changes in energy flow are producing major shifts in the ecology of the lakes (Nalepa *et al.* 1996, Dermott and Kerec 1997).

Several studies have demonstrated the capability of the zebra mussel to accumulate non-polar organic contaminants including laboratory studies (Fisher *et al.* 1993; Bruner *et al.* 1994a,b; van Haelst *et al.* 1996; Gossiaux *et al.* 1998) and field measurements (Secor *et al.* 1993; Morrison *et al.* 1995, 1996; Roper *et al.* 1996; Chevreuil *et al.* 1996; Robertson and Lauenstein 1998). Accumulation of organic contaminants from some sources are high enough to produce realistic hazards for organisms that feed on them (Roper *et al.* 1996). Furthermore, zebra mussels can transfer contaminants to feces and pseudofeces, which are predicted to be a contaminant rich source to benthic organisms living in or near mussel colonies (Bruner *et al.* 1994b). Therefore, zebra mussels may play a potentially important role in contaminant cycling in aquatic ecosystems

Accumulation of contaminants by lower organisms can occur via several potential routes: gills, diet, and integument. In order to understand the ex-

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posure of organisms from various sources, an assessment of the accumulation from each route is needed. Such an assessment permits interpretation of the importance of the route and provides potential information for controls on exposure. One of the major difficulties in making such assessment is evaluating the efficiency of absorption of the contaminants from ingested diet. Absorption efficiency is defined as the fraction of ingested contaminant accumulated by the organism (Penry 1998). Models that have attempted to define the accumulation of contaminants from multiple sources for zebra mussels have used average values to represent the absorption efficiency from ingested materials (Morrison *et al.* 1996). In this case, the observed accumulation was about two to four times greater than the predicted values. This could have occurred in part from the use of average absorption efficiency values because the model output is most sensitive to diet-related parameters. Thus, there is a recognized need for improved information on the absorption efficiency for ingested contaminants for all invertebrates because of the importance of particle ingestion as a route of exposure (Morrison *et al.* 1996, Thomann *et al.* 1992).

Contaminant accumulation from ingested particles is an important exposure route for mussels for strongly sorbed contaminants and accounts for upwards of 80% of the total accumulation of contaminants by zebra mussels (Bruner *et al.* 1994b, Gossiaux *et al.* 1998). Different types of contaminated food particles presumably have varying abilities to serve as contaminant sources to zebra mussels due to differences in their chemical composition, their corresponding ability to sorb chemicals, and their digestibility by zebra mussels. For instance, the absorption of contaminants associated with algae was two to three fold greater than from suspended sediment particles. (Bruner *et al.* 1994b, Gossiaux *et al.* 1998). Differences in absorption efficiencies have also been observed with more subtle differences in food quality. Goldfish had greater absorption efficiencies for chlorinated hydrocarbons when fed a low lipid diet (Gobas *et al.* 1993). Thus, differences in feeding preference and digestability could alter zebra mussel exposure depending on particle composition.

In addition to particle composition differences, when varying amounts of food are fed, the absorption efficiency appears to be lower with larger amounts of ingested food (Clark and Mackay 1991). This presumably occurs in part because of change in gut residence time. Since the algal concentration in water will vary with season, light, and nutrient conditions, the amount and type of algae available for ingestion will change. This could lead to differences in the amount of algal material ingested and thus to differences in absorption efficiency. Zebra mussels are known to consume the green algae, such as *Chlamydomonas rheinhardtii* (Ma 1996) and *Chlorella vulgaris* (Berg *et al.* 1996). These species are found in the Great Lakes and have different characteristics. *C. rheinhardtii* is slightly elliptical, 9 to 12 μm in diameter, is diflagellated, and its cell is composed of hydroxyproline-rich glycoproteins and lacks cellulose (Adair and Apt 1990). *C. vulgaris* is a spherical alga, 1 to 5 μm in diameter, and its cell wall is composed of cellulose (Millamena *et al.* 1990). These two species of algae, *C. rheinhardtii* and *C. vulgaris*, were chosen for study because they differed in physiological characteristics which might affect the ability to sorb contaminants. Thus, differences in the amount ingested due to feeding preferences, the characteristics of interaction between the contaminants and the algae, or the ability to digest the different species could result in differential exposure of the zebra mussels.

To expand the data on absorption efficiencies of contaminants from ingested particles, the absorption efficiencies of three PCB congeners, 2,2′,4,4′-tetrachlorobiphenyl (TCBP), 3,3′,4,4′,5-pentachlorobiphenyl (PCBP), 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP), and 1,1-dichloro-2,2-bis[4-chlorophenyl] ethylene (DDE) were investigated at two algal concentrations for *C. rheinhardtii* and *C. vulgaris*.

MATERIALS AND METHODS

Chemicals

^{14}C-labeled 2,2′,4,4′-tetrachlorobiphenyl (TCBP, log Kow 5.85, Hawker and Connell 1988), 3,3′,4,4′,5-pentachlorobiphenyl (PCBP, log Kow 6.89, Hawker and Connell 1988), 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP, log Kow 6.92, Hawker and Connell 1988), and 1,1-dichloro-2,2-bis[4-chlorophenyl] ethylene (DDE, Log Kow 6.96, De Bruijn *et al.* 1989) were obtained from Sigma Chemical Company (St. Louis, MO). The specific activity of each chemical was 12.6 mCi/mmol (TCBP), 18.5 mCi/mmol (PCBP), 21.2 mCi/mmol (HCBP), and 12.7 mCi/mmol (DDE). Compound purity was greater than 97% as determined by thin layer chromatography and radiometric analysis on

silica gel plates using a non-polar solvent system, benzene:hexane (20:80) (Leversee *et al.* 1982).

Organisms

Zebra mussels (*Dreissena polymorpha*) were collected by SCUBA divers from the littoral zones in North Bay, Kelly's Island, Lake Erie. The mussels were transported to the laboratory in Columbus, OH, wrapped in wet paper towels. The mussels were held in 200-L aquaria filled with aerated carbon-filtered tap water at 12ºC. A suspension of mixed diatoms (Oyster Diet B, Coast Seafood Company) or live algal mixture (*C. vulgaris, C. rheinhardtii* and *Ankistrodesmus falcatus*) was fed to zebra mussels on alternate days (approximately 3.3 g dry weight food per 1,000 zebra mussels). Temperature, pH, and dissolved oxygen in each aquarium containing zebra mussels were monitored daily. Sixty percent of the water in the aquaria was changed weekly. To prevent the dissemination of veligers into local waterways, discarded water was treated with chlorine bleach (50 ppm) for 24 h before disposal. At least 2 weeks before the experiments, zebra mussels were transferred to aquaria containing hard standard reference water (HSRW, pH 8.3, alkalinity = 130 mg/L as $CaCO_3$, hardness = 170 mg/L as $CaCO_3$) (USEPA 1975) at 22°C. The HSRW was made with 1.0 M Na_2HPO_4 in substitution for K_2HPO_4 because potassium containing compounds are toxic to zebra mussels (Fisher *et al.* 1991).

Algae

The initial populations of *C. vulgaris* and *C. rheinhardtii* were obtained from Carolina Biological Supply. Monocultures of each algal species were maintained in 9:1 Bold's Basal Medium (Nichols and Bold 1965) under a light regime of 16:8 hour (light: dark). The purity of the cultures were checked weekly and prior to each experiment under a light microscope.

Media Preparation

For each algal species and for each contaminant, a large batch of algae containing a sufficient amount for both the high and low algal cell concentrations was spiked with a contaminant. A subfraction of each batch of spiked algae was taken to produce the two algal cell concentrations to be studied. The high and low algal cell concentrations were approximately 4 and 8 µg dry weight algae/mL for *C. rheinhardtii.* For *C. vulgaris,* the high and low concentrations were about 2.5 and 5 µg dry weight algae/mL. To dose the algae, the required amount of algal culture was sedimented by centrifugation for 30 min. at 2,000 rpm, the supernatant was decanted, and algal pellets were resuspended in 80 to 100 mL HSRW. The algal suspensions were spiked with a radiolabeled chemical and agitated overnight in the darkened incubator at 4ºC.

On the day of each experiment, the spiked algae were sedimented by centrifugation and the supernatant removed. The pellet was then washed three times with HSRW to displace loosely bound contaminant but resuspending the algae in fresh HSRW, sedimenting the algae and decanting the supernatant. After rinsing, the algal pellets were resuspended in 200 mL HSRW. Then, the algal cell concentration was determined with a Coulter Counter to determine the amount of algae required to give the high and low algal concentrations.

Zebra Mussel Contaminant Absorption Efficiency Experiments

On the day of the experiment, for both the high and low algal concentration experiments, eight zebra mussels were placed in each of the three replicate aquaria containing spiked algae. A fourth aquarium containing the same amount of spiked algae but without zebra mussels was used as a control to monitor for gravitational settling of algae. At the beginning of the experiment (T_0), algal solution samples (50 mL) were taken from each aquarium and filtered through preweighed 0.5 µm glass filters (Fisher Scientific, Pittsburgh, PA), then dried in the oven for at least 24 h at 60°C to determine algal mass concentration. The amount of radioactivity associated with the algae was determined as described below by liquid scintillation counting (LSC). After taking these samples, aquaria containing the zebra mussels were placed in a darkened incubator at 22°C for 2 h.

After 2 h, aquaria were removed from the incubator and triplicate algal solution samples were taken from each aquarium to determine the algal mass density and contaminant mass (T_2) in water and algae as at T_0. Then, zebra mussels were transferred to uncontaminated HSRW without feeding. The pseudofeces and feces produced during the 2 h exposure were collected separately, filtered through preweighed 0.5 µm filters, aspirated at a rapid rate for 5 min., and dried in a dessiccator until a steady

dry weight was obtained. Pseudofeces and feces were collected by hand pipetting the material from the beakers. The pseudofeces were distinguished from the feces by characteristics: the pseudofeces are loose amophorous clumps of material while the feces are more well formed and consolidated materials. The contaminant mass in the pseudofeces and feces was determined as described below by LSC.

During 72 h following the exposure, HSRW in the beakers containing mussels was changed every 24 h. The pseudofeces and feces were collected separately at 24 h, 48 h, and 72 h and dried, weighed, and analyzed by LSC.

Liquid Scintillation Analyses

All samples were analyzed in scintillation cocktail made from 1,000 mL 1,4-dioxane, 100 g naphthalene and 5 g 2,5-diphenyloxazole (PPO) on a Beckman LS 6000IC Scintillation Counter (^{14}C efficiency > 95%) with automatic quench control. Tissue solubilizer (0.5 to 1 mL) was added to each algae, feces, and pseudofeces sample before analyzing. After 30 min., acetic acid (1 mL) was added to neutralize the solubilizer. Scintillation cocktail (15 mL) was added for LSC. The contaminant mass was determined from the total activity in the sample and the specific activity of the contaminant. Concentrations for each of the solid samples was determined based on the amount contaminant and the algal or fecal material dry weight.

Contaminant Percent Absorption Efficiency (%AE) Calculation

The percent absorption efficiency (%AE) for each contaminant is calculated using a chemical mass-balance model:

$$\%AE = [(B\text{-}P\text{-}F) / (B\text{-}P)] \times 100 \qquad (1)$$

where B is the total mass of contaminant on algae filtered (μg) from the water by the zebra mussels over the course of the exposure (2 h), P is the cumulative mass of contaminant found in pseudofeces (μg) over the course of the experiment, and F is the cumulative amount of contaminant found in feces (μg) over the course of the experiment. The model assumptions are: (1) contaminants absorbed to algae do not desorb appreciable amounts of compound during the course of the exposure and have three potential fates: a) absorption into tissues, b) expulsion in the feces, and c) expulsion with pseudofeces, (2) the loss of contaminants from tested system is due to zebra mussel filtration, and (3) 72 h is long enough for mussels to accumulate chemicals completely. Thus, the efficiency of absorption is based on the amount accumulated by the mussels (the amount ingested less the amount egested as feces) divided by the total amount ingested (amount filtered less that ejected as pseudofeces) times 100 to yield a percent. Any material accumulated into tissue but lost to water during the elimination phase could sorb to feces or pseudofeces and would serve to lower the overall measured absorption efficiency. However, the exchange of water every 24 h during the elimination phase was an attempt to insure that this effect was kept to a minimum.

Data Analysis

Single-factor ANOVA (analysis of variance) F-test was employed to analyze significant differences among different variables and differences were considered significant at $p \leq 0.05$. Also, Tukey's multiple comparison was applied to further investigate the difference between individual variables ($p \leq 0.05$).

RESULTS

With both algal species, the gravitational control indicated that very little of the algae settled, as the concentration of algae did not vary in the control over the time required for the exposure. Thus, it was expected that the algae remained available to the zebra mussels during the exposure period.

The model follows the amount of contaminant associated with the algae to estimate the absorption efficiency. This model does not directly measure the amount of the compounds in the zebra mussels. This model tracks the total mass of contaminant passing through each process, filtration of algae, ejection of algae as pseudofeces, and elimination as feces. Thus, the amount ingested is determined by the difference between the total contaminant mass removed by filtration over 2 h and the cumulative mass ejected with pseudofeces during the 3 d of the experiment. The potential for desorption of contaminant from the algae was considered small and no measurable difference in the concentration on the algae was detected from the beginning of the exposure to the end of the exposure for either algal species or any compound. Thus, the first assumption was validated. The fraction absorbed is the mass of contaminant ingested less the cumulative mass found in the feces over 3 d of the experiment.

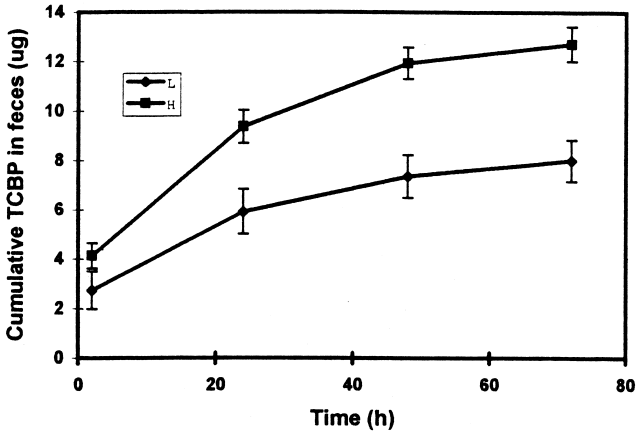

FIG. 1. Cumulative TCBP in zebra mussel feces (n = 8) collected after exposure to spiked* Chlamydomonas rheinhardtii. *Error bars represent standard error. L and H represent low (4 μg algal dry weight/mL) and high (8 μg algal dry weight/mL) algal concentration, respectively.

The appearance of a plateau in the cumulative mass of contaminant in the feces after 3 d (Fig. 1) indicates that the absorption is essentially complete, thus the third assumption was validated.

Zebra Mussel Contaminant Absorption Efficiencies from Chlamydomonas rheinhardtii and Transfer of Contaminants to the Feces and Pseudofeces

Zebra mussels cleared the ingested contaminants from their gut and passed the unassimilated contaminants to feces. Zebra mussels eliminated all of the contaminants rapidly during the first 24 h and the total elimination approached a plateau by about 48 h (Fig. 1). This plateau represents the cumulative elimination via the fecal route. This plateau was observed for all compounds at both high (8 μg algal dry weight/mL) and low (4 μg algal dry weight/mL) algal concentrations. Also, for each chemical, zebra mussels fed a higher concentration of *C. rheinhardtii* expelled more contaminants in feces at every time interval than those fed a lower concentration of *C. rheinhardtii,* except for DDE. For DDE (Table 1), mussels egested a similar amount of chemicals in feces when fed either the high or low concentration of contaminated *C. rheinhardtii* (Fig. 2).

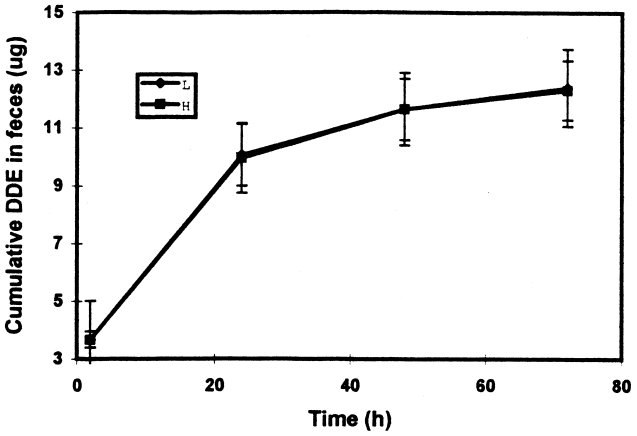

FIG. 2. Cumulative DDE in zebra mussel feces (n = 8) collected after exposure to spiked* Chlamydomonas rheinhardtii. *Error bars represent standard error. L and H represent low (4 μg algal dry weight/mL) and high (8 μg algal dry weight/mL) algal concentration, respectively.

***TABLE 1. Contaminant concentrations in feces for zebra mussels exposed to* Chlamydomonas rheinhardtii.**

Compound	Conc. Algae	Conc. In Algae (ng/mg dry wt.)	Chemical Concentration In Feces (ng/mg dry wt.)			
			2h	24h	48h	72h
TCBP	H	22.1	21.6 (0.2)	14.0 (1.1)	11.1 (1.2)	4.2 (1.8)
	L	23.6	12.5 (1.3)	8.8 (0.8)	7.7 (0.7)	3.9 (0.5)
PCBP	H	27.7	25.3 (0.5)	23.6 (1.5)	17.5 (2.5)	12.4 (1.7)
	L	29.1	8.7 (1.5)	24.8 (1.9)	16.9 (0.9)	9.8 (1.0)
HCBP	H	26.9	23.8 (1.1)	19.5 (1.6)	4.5 (0.2)	3.7 (0.1)
	L	23.6	20.8 (1.0)	12.4 (1.4)	4.2 (0.9)	2.7 (0.5)
DDE	H	33.5	24.4 (2.4)	22.3 (1.2)	8.6 (0.6)	6.5 (0.8)
	L	29.1	22.6 (2.0)	17.2 (0.5)	6.4 (0.2)	6.3 (0.3)

Numbers in parentheses represent standard error (n = 3). H and L represent high (8 μg algal dry weight/mL) and low (4 μg algal dry weight/mL) algae concentration respectively.

TABLE 2. Concentration of contaminants in pseudofeces when zebra mussels were exposed to **Chlamydomonas rheinhardtii.**

Compound	Concentration of Algae	Concentration In Algae (ng/mg dry wt.	Concentration in Pseudofeces (ng/mg dry wt.) 2h	24h
TCBP	H	22.1	22.2 (1.4)	10.5 (3.6)
	L	23.6	15.8 (1.3)	6.3 (2.5)
PCBP	H	27.7	36.9 (2.1)	28.9 (2.0)
	L	29.1	15.0 (1.9)	20.4 (1.4)
HCBP	H	26.9	30.5 (2.2)	23.6 (5.6)
	L	23.6	29.5 (0.6)	21.2 (3.1)
DDE	H	33.5	46.1 (4.5)	27.5 (4.6)
	L	29.1	37.7 (4.3)	21.0 (3.3)

Numbers in parentheses represent standard error (n = 3). H and L represent high (8 μg algal dry weight/mL) and low (4 μg algal dry weight/mL) algae concentration respectively.

Contaminant concentrations in the feces were lower than measured in ingested algae. In addition, the contaminant concentrations in the feces decreased with time. In general, the contaminant concentrations in the feces produced within 24 h were higher than those produced at 72 h (Table 1).

When mussels were fed different concentrations of *C. rheinhardtii* with identical chemical concentrations, contaminant concentrations in feces produced by mussels at the high concentration of *C. rheinhardtii* were usually higher than those produced at a low concentration of *C. rheinhardtii* measured at for the same sampling time (Table 1).

Zebra mussels produced pseudofeces only within 24 h after exposure to *C. rheinhardtii*. Like the feces, the contaminant concentration in the pseudofeces decreased with time, and the contaminant concentrations in pseudofeces found at 24 h were lower than those of ingested algae. However, the pseudofeces contaminant concentrations at 2 h were higher than for ingested algae except for TCBP and PCBP for mussels exposed at the low algal concentrations (Table 2).

The %AEs were apparently independent of algal concentrations, except for TCBP, where the contaminant %AE at the low concentration of *C. rheinhardtii* was higher than at the higher concentration of *C. rheinhardtii* (Fig. 3). Comparing contaminant absorption efficiencies among the four chemicals at low and high algal concentrations respectively, PCBP absorption efficiency was significantly lower than the contaminant %AEs for the other contaminants, and contaminant %AEs of the other three contaminants were not significant from each other.

Zebra Mussel Contaminant Absorption Efficiencies from *Chlorella vulgaris* and Transfer of Contaminants to the Pseudofeces and Feces

Zebra mussel gut clearance of ingested TCBP, PCBP, HCBP and DDE after exposure to the high (5 μg algal dry wt/mL) and low (2.5 μg algal dry wt./mL) algal concentrations of contaminated *C. vulgaris* was slower than with *C. rheinhardtii*. With *C. vulgaris*, elimination barely approached a plateau by 72 h (e.g., for TCBP Fig. 4). At each time interval, zebra mussels expelled significantly higher amounts of contaminants in feces when fed higher concentrations of *C. vulgaris*.

Algal contaminant concentrations were higher than fecal contaminant concentrations for all chemicals. Although there are some discrepancies in the data sets, the tendency for contaminant concentration in the feces to decrease with time was consistent. For each chemical at low or high algal concentrations, the contaminant concentrations in the feces at 2 h and 24 h were usually significantly higher than that at 72 h (Table 3). The fecal contaminant concentrations also varied with the ingested algal concentrations. Generally, for each time interval, mussels had lower fecal contaminant concentrations when fed a lower concentration of *C. vulgaris* than when fed a higher cell concentration of *C. vulgaris* (Table 3).

The contaminant concentrations in the pseudofeces produced when mussels were fed high and low algal concentrations for each chemical at different time intervals were always lower than the original algal concentration and tended to decline with increased sampling time. Unlike the mussels fed contaminated *C. rheinhardtii,* which produced

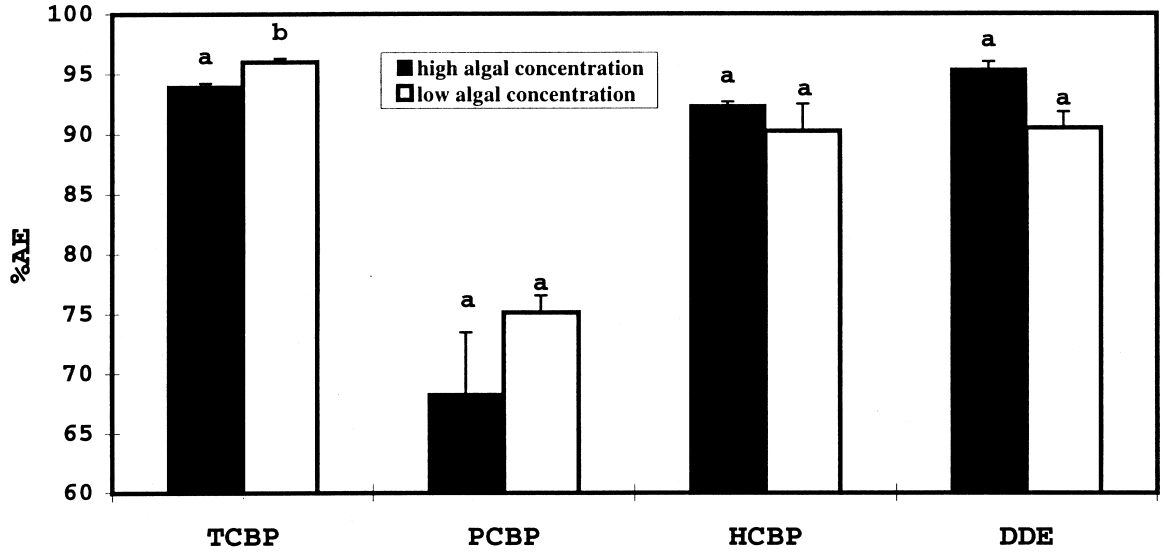

FIG. 3. Comparison of percent absorption efficiencies (%AE) for the zebra mussel exposed to contaminated **Chlamydomonas rheinhardtii** *at high (8 µg algal dry weight/mL) and low (4 µg algal dry weight/mL) algal concentrations. For each chemical, bars with the same letter are not significantly different.*

pseudofeces only during the first 24 h, mussels fed *C. vulgaris* produced pseudofeces during the 48 h period following exposure to spiked algae (Table 4).

With the exception of PCBP, the contaminant %AEs were independent of ingested algal concentration (Fig. 5). Comparing %AEs for the four contaminants, at the low algal concentration, PCBP %AE was significantly lower than TCBP and DDE %AEs. Further, the %AEs of contaminants other than PCBP were not significantly different from each other. At the high algal concentration, no significant differences existed in %AEs among the four chemicals.

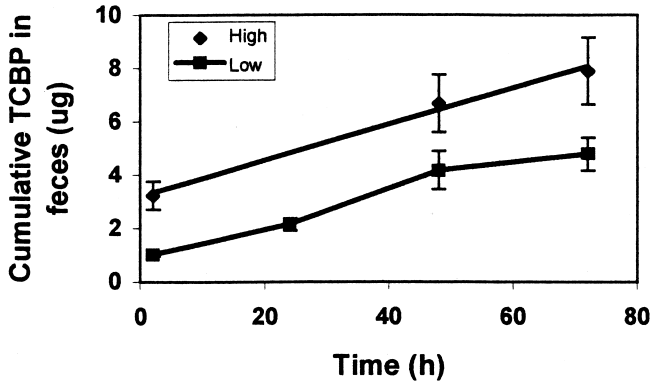

FIG. 4. Cumulative TCBP in zebra mussel feces (n = 8) collected after exposure to spiked **Chlorella vulgaris.** *Error bars represent standard error (L and H represent low (2.5 µg algal dry weight/mL) and high (8 µg algal dry weight/mL) algal concentration respectively.*

DISCUSSION

Absorption Efficiency

For small organisms, several methods have been proposed for evaluating absorption efficiency. Among these are the pulse chase method (Luoma *et al.* 1992, Bruner *et al.* 1994b), mass balance method (Gossiaux *et al.* 1998), and a dual tracer method (Calow and Fletcher 1972, Klump *et al.* 1987, Lopez and Elmgren 1989, Lee *et al.* 1990, Forbes and Forbes 1997). Each of these techniques has strengths and limitations for determining absorption efficiency. The mass balance method should be the most accurate when the amount of ingested and egested material can be tracked. However, it is often difficult to get accurate measures of the amount of ingested and egested material particularly for small organisms. Further, in some cases, it is impossible to determine exactly what particles are being ingested, as might occur in sediment exposures, thus performing a mass balance would not be possible. The pulse chase method relies on a rapid elimination of the intestinal contents with lit-

***TABLE 3. Concentration of contaminants in feces for zebra mussels exposed to* Chlorella vulgaris.**

Compound	Conc. Algae	Conc. in Algae (ng/mg dry wt.)	Chemical Concentration In Feces (ng/mg dry wt.)			
			2h	24h	48h	72h
TCBP	H	17.6	4.0 (0.7)	ND	2.4 (0.8)	2.5 (0.2)
	L	16.9	1.5 (0.2)	2.5 (0.4)	2.1 (0.7)	1.3 (0.1)
PCBP	H	25.2	9.0 (1.1)	7.5 (1.1)	5.7 (0.2)	3.7 (0.2)
	L	27.8	9.7 (0.6)	8.8 (1.8)	5.0 (0.7)	2.5 (0.2)
HCBP	H	20.6	9.9 (0.2)	7.3 (0.8)	6.5 (0.1)	5.3 (0.5)
	L	20.3	5.0 (0.02)	7.8 (0.4)	4.2 (0.2)	3.8 (0.1)
DDE	H	26.3	11.7 (0.5)	16.0 (1.0)	9.7 (0.7)	4.0 (0.3)
	L	24.8	8.2 (0.8)	10.3 (0.4)	5.8 (0.8)	2.4 (0.3)

Numbers in parentheses represent standard error (n = 3). H and L represent high (5 μg algal dry weight/mL) and low (2.5 μg algal dry weight/mL) algal concentration respectively. ND indicates no feces detected at a given sampling time.

***TABLE 4. Contaminant concentrations in pseudofeces from zebra mussels exposed to* Chlorella vulgaris.**

Compound	Conc. Algae	Conc. in Algae (ng/mg dry wt.)	Chemical Concentration in Pseudofeces (ng/mg dry wt.)		
			2h	24h	48h
TCBP	H	17.6	ND	2.4 (0.3)	2.5 (0.8)
	L	16.9	9.4 (0.6)	6.9 (0.4)	5.4 (1.9)
PCBP	H	25.2	ND	9.3 (1.3)	13.2 (3.4)
	L	27.8	16.8 (4.5)	9.4 (2.8)	6.3 (0.5)
HCBP	H	20.6	ND	8.0 (0.5)	5.0 (0.4)
	L	20.3	14.2 (0.6)	8.8 (0.04)	6.1 (1.6)
DDE	H	26.3	ND	11.5 (1.7)	5.5 (0.7)
	L	24.8	23.1 (1.7)	14.3 (1.3)	10.3 (2.6)

Numbers in parentheses represent standard error (n = 3). H and L represent high (5 μg algal dry weight/mL) and low (2.5 μg algal dry weight/mL) algal concentration respectively. ND indicates no feces detected at a given sampling time.

tle elimination of the absorbed contaminant. The accumulation from other sources such as accumulation of desorbed contaminant via the water must also be accounted for as a part of the method. It is possible to make corrections for both the amount of elimination occurring during the chase phase of the experiment and the extent of uptake of desorbed compound (Bruner *et al.* 1994b). However, the corrections can lead to under estimates of the absorption efficiency depending of the magnitude of the two processes and the accuracy of determining the magnitude of the water uptake and elimination loss. The dual labeled method relies on the relative ratio of the contaminant and a non-absorbed tracer in food and fecal material. There must be a correction for selectivity of the two tracers during the ingestion process (Forbes and Forbes 1997). Initial accumulation of desorbed contaminant can result in an over estimate of the measure of selectivity because the amount of contaminant accumulated from a desorbed phase could dominate early measures of the ratio of absorbable and non-absorbable tracer. This would lead to an error in determination of the relative selectivity of the two tracers and could result in an over estimate of the absorption efficiency. Thus, all of these methods have limitations that could lead to errors in determining the true absorption efficiency and at best bracket the true absorption efficiency with the highest estimates resulting from mass balance and dual labeled techniques while lower end estimates come from the pulse chase method.

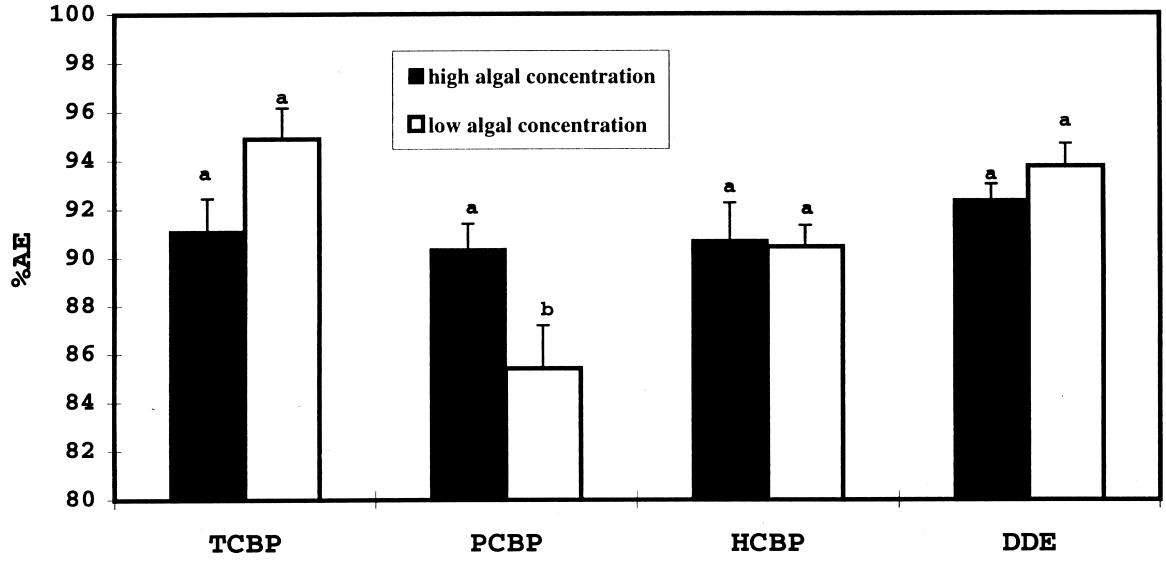

FIG. 5. Comparison of percent absorption efficiencies (%AE) for the zebra mussel exposed to contaminated* Chlorella vulgaris *at high (5 µg algal dry weight mL) and low (2.5 µg algal dry weight mL) algal concentrations. For each chemical, bars with the same letter are not significantly different.

Two models have been proposed to illustrate the process of absorption of ingested hydrophobic chemicals in the gut (Gobas *et al.* 1993). The first is a co-absorption model, which assumes that the absorption of chemicals in the gastrointestinal tract (GIT) is through the co-absorption with lipids. The second model assumes that chemicals are assimilated individually in the GIT by means of passive diffusion. The flux of the contaminants from food into the organisms is primarily governed by the fugacity gradient between the GIT and food. In this model chemicals diffuse from high fugacity to low fugacity until equilibrium is reached, with the fugacity equal in all phases (Gobas *et al.* 1988). In the GIT, food digestion and absorption results in increasing fugacity of chemicals as food is absorbed from the GIT (Gobas *et al.* 1993). This fugacity gradient serves as a pump for the transfer of contaminants from food to the GIT. Although several authors have pointed out the importance of lipid co-transport in the dietary uptake of hydrophobic contaminants (Vetter *et al.* 1985), the passive diffusion of hydrophobic chemicals in the GIT has been considered to be the major route for the absorption of hydrophobic contaminants by organisms (Gobas *et al.* 1993).

This study suggests that the contaminants and algal cells are assimilated individually in the GIT of the zebra mussel. Comparing the algal absorption efficiencies measured in bioprocessing experiments (Berg *et al.* 1996, Ma 1996) to contaminant absorption efficiencies, contaminant absorption efficiencies are higher. This suggests that contaminants are not co-assimilated with food, but diffuse from food to organisms. Otherwise, the contaminant absorption efficiencies would be equal to or lower than the algal absorption efficiencies.

Despite the slower processing rate for *C. vulgaris* compared to *C. rheinhardtii* based on the duration required for complete fecal elimination, the absorption efficiencies for the range of compounds were not different except for PCBP. PCBP was an anomaly in both algal exposures and was far was less absorbed than would be expected, based on its log Kow, in comparison with the other compounds. Compounds with lower and higher log Kow values, TCBP and HCBP (this work, Gossiaux *et al.* 1998), exhibited substantially greater absorption efficiencies than PCBP. This may result from its particular chemical configuration, which is planar due to the absence of ortho substituted chlorines. Such structures could result in stronger association with the algal material and more of the compound remaining with the egested feces.

The absorption efficiencies for HCBP from *C. rheinhardtii* in this work were 92.4 and 90.3% at high and low algal concentrations, respectively, which was similar to that measured independently

with a mass balance model using *Chlamydomonas* spp., 97.6% (Gossiaux *et al.* 1998). Both of these studies employed a mass balance model for estimating the absorption efficiencies, which should lead to very similar measurements. In contrast, when a pulse-chase model was employed for absorption efficiency determination with *C. rheinhardtii* as the algal food, both HCBP (68.6%) and TCBP (77.6%) had lower absorption efficiencies compared to those measured with the mass balance model (Bruner *et al.*1994b). The two models work on different assumptions and have different potential measurement errors. The pulse chase model must account for contaminant associated with uptake from water during the accumulation phase and for elimination from tissue during the elimination phase. The %AEs determined by these two different methods are thought to describe the boundaries of the true absorption efficiency for a compound.

Despite the expectation that food concentration can affect the absorption of contaminants (Clark and Mackay 1991), there was no consistent evidence that the algal concentration affected the absorption efficiency of the contaminants for either algal species. Since zebra mussels can alter the production of pseudofeces and regulate the amount of algae actually ingested, the actual amount ingested may not have varied greatly between concentrations of algae. In bioprocessing studies over a wide range of algal concentrations, the actual ingestion rate increased to a plateau even while the filtering rate (volume of water cleared of algae per time) remained constant or even declined (Ma 1996). Thus, changes in absorption rate might not occur if the actual amount ingested and the absorption efficiency for the algal biomass remains constant (Ma 1996). This would create a relatively constant fugacity gradient as suggested by the second model for contaminant absorption. This constant absorption efficiency with algal concentration allows for comparison between algal species even though the concentrations of algae varied between species tested.

The absorption efficiencies for the PCB congeners, except PCBP, were in the range of 90% which was greater that that used by Morrison *et al.* (1996) in their modeling exercise. This may, in part, be the reason for the differences observed between model predicted values and observed values from field collected organisms. Equally important in such consideration is the ability of the zebra mussel to select particles for ingestion which could also have contributed to observed versus model differences. Overall improving data on absorption efficiencies should lead to improved models.

Contaminant Transfer and Elimination

The contaminants found in feces and pseudofeces support the hypothesis that the zebra mussels could pass the unassimilated contaminants to other benthic organisms (e.g., amphipods) that will utilize them as a food source. Unassimilated contaminants from zebra mussel feces and pseudofeces were found to provide a contaminant-rich source for gammarid amphipods (Bruner *et al.* 1994b). For this study, the concentrations in the fecal material were lower than that for the incoming algae and this was particularly true for *C. vulgaris*. This suggests that the risk from this source to other benthos would be expected to be less than ingesting algae. However, these mussels were not at steady state, as would be expected in the field. In the field situation, the concentrations in the fecal materials could well be as high or higher than the incoming algae depending on the dominant routes and processes for contaminant accumulation and elimination. Further, many benthic organisms do not feed directly on algae, rather the more usual food source is sedimenting fecal pellets from pelagic zooplankton or other sediment detritus. Thus, large quantities of pseudofeces and feces are likely an enriched contaminant source and result in enhanced food chain bioaccumulation.

Feces were collected at different intervals during the gut clearing period. Visual inspection of the feces indicated that some differences existed in the feces collected at different sampling times. Feces produced within 24 h were usually green and moderately uncompacted, while, the feces produced from 24 h to 72 h were dark-green and more compacted. It is very possible that the feces produced at different times were the result of different digestive processes, either extra cellular digestion in the gut (Widdows *et al.* 1979) or glandular digestion (Morton 1983). Consequently, the degree of absorption of contaminants may change over time, but such potential changes could not be determined with the current experimental procedure.

Algal cell concentrations somewhat affected contaminant concentrations in the feces resulting in lower fecal contaminant concentration with mussels fed low algal concentrations. The gut passage time affects the contaminant absorption; thus, mussels fed higher algal concentrations may have processed more material producing feces more rapidly than at

lower algal concentrations. This difference in concentrations was often only observed at early time points. The difference should have lead to lower absorption efficiencies at the higher algal concentrations but since the difference was generally only noticed early in the elimination phase, it was not of sufficient magnitude to result in an overall significant difference in absorption efficiency except for TCBP with *C. rheinhardtii* and PCBP with *C. vulgaris*.

Compared to feces, more pseudofeces were produced at the higher algal concentrations. The chemical concentrations in pseudofeces were usually lower than ingested algae, but occasionally the 2 h sample of pseudofeces was more contaminated than the algae when fed high algae concentrations of *C. rheinhardtii* and low algae concentrations of DDE and HCBP-contaminated *C. rheinhardtii*. Because pseudofeces are not digested, they are expected to have similar chemical concentrations to that of the ingested algae. However, lower chemical concentrations in pseudofeces may result from some desorption of chemicals from algae into the zebra mussels when the particles are in contact with the gill surface. It is not clear why the pseudofeces sometimes had higher chemical concentrations than that of ingested algae. However, Reeders and Vaate (1992) have reported that zebra mussel pseudofeces were more polluted than ingested particles. In the field, this may result from the selection process where the zebra mussel selectively ingests algae and rejects sediment or other particles.

The filtering activity of the zebra mussel may alter the contaminant fate in aquatic systems. The zebra mussel not only assimilates the contaminant into tissue, but also passes the unassimilated contaminants to the feces and pseudofeces. This redirection of contaminants may result in biomagnification of contaminants, e.g., gammarid amphipods fed hexachlorobiphenyl contaminated zebra mussel feces contained 20 times higher tissue concentrations than found for the zebra mussel tissue exposed to contaminated algae (Bruner *et al.* 1994b). Because gammarids are an important food source, the transfer of contaminants to feces and pseudofeces may lead to the high contaminant concentration in organisms at upper trophic levels through food web transfer.

In addition to their impact through filtering and delivering contaminants in feces and pseudofeces to the benthic component of food webs, zebra mussels are prey organisms for both crayfish (Martin and Corkum 1994) and some fish species (French and Bur 1993). Further, zebra mussels are not exposed to just a single species of algae but rather to mixtures of particles and a variety of algal species. Since the algal route has been estimated to be a significant route of exposure (Bruner *et al.* 1994b, Gossiaux *et al.* 1998), what then would be the impact of exposure under natural conditions? Using these two species as examples, perhaps some light can be shed on the process. If zebra mussels were exposed to each of these algae alone, because the absorption efficiencies are similar, zebra mussels would likely obtain a higher dose from *C. rheinhardtii* than from *C. vulgaris*. This occurs not because of differences in absorption efficiencies but because of differences in ingestion rate. At the same filtration rate, zebra mussels ingest about twice as much *C. rheinhardtii* compared to *C. vulgaris* (Ma 1996). If the two species were fed together, it is postulated that the dose of contaminant could be lower than if just *C. rheinhardtii* was fed because the *C. vulgaris* apparently induces satiation at lower ingestion rates (Ma 1996). This hypothesis needs to be tested not only with pairs of algae but also with addition of suspended sediment particles for which the absorption efficiencies of contaminants are much lower (Bruner *et al.* 1994b, Gossiaux *et al.* 1998).

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REFERENCES

Adair, W.S., and Apt, K.E. 1990. Cell wall regeneration in *Chlamydomonas*: Accumulation of mRNAs encoding cell wall hydroxyproline-rich glycoproteins. *Proc. Natl. Acad. Sci.* 87:7355–7359.

Berg, D.J., Fisher, S.W., and Landrum. P.F. 1996. Clearance and processing of algal particles by zebra mussels (*Dreissena polymorpha*.). *J. Great Lakes Res.* 22:779–788

Bruner, K.A., Fisher, S.W., and Landrum, P.F. 1994a. The role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling: I. The effect of body size and lipid content on the bioconcentraion of PCBs and PAHs. *J. Great Lakes Res.* 20:725–734.

_____, Fisher, S.W., and Landrum, P.F. 1994b. 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*. *J. Great Lakes Res.* 20:735–750.

Calow, P., and Fletcher, C.R. 1972. A new radiotracer

technique involving ^{14}C and ^{51}Cr for estimating the assimilation efficiencies of aquatic, primary consumers. *Oecologia* 9:155–170.

Chevreuil, M., Blanchard, M., Teil, M-J., Carru, A-M., Testard, P., and Chesterikoff, A. 1996. Evaluation of the pollution by organochlorinated compounds (polychlorobiphenyls and pesticides) and metals (Cd, Cr, Cu, and Pb) in the water and in the zebra mussel (*Dreissena polymorpha* Pallas) of the River Seine. *Wat., Air Soil Pollut*. 88:371–381.

Clark, K.E., and Mackay, D. 1991. Dietary uptake and biomagnification of four chlorinated hydrocarbons by guppies. *Environ. Toxicol. Chem.* 10:1205–1217.

De Bruijn, J, Busser, R., Seinen, W., and Hermens. J. 1989. Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the "slow-stirring" method. *Environ. Toxicol. Chem.* 8:499–512.

Dermott, R., and Kerec, D. 1997. Changes to the deepwater benthos of eastern Lake Erie since the invasion of *Dreissena*: 1979–1993. *Can. J. Fish. Aquat. Sci*. 54:922–930.

Fisher, S.W., Trongberg, P., Bruner, K.A., and Boulet, L.D. 1991. Molluscicidal activity of potassium to the zebra mussel, *Dreissena polymorpha*: toxicity and mode of action. *Aquat. Toxicol.* 20:219–234.

_____, Gossiaux, D.C., Bruner, K.A., and Landrum, P.F. 1993. Investigations of the toxicokinetics of hydrophobic contaminants in the zebra mussel (*Dreissena polymorpha*). In *Zebra mussel:Biology, Impact and Control,* eds. T.F. Nalepa and D.W. Schloesser, pp. 465–490. Ann Arbor, MI: Lewis Publishers.

Forbes, V.E., and Forbes, T.L. 1997. Dietary absorption of sediment-bound fluoranthene by a deposit-feeding gastropod using the ^{14}C:^{51}Cr dual-labeled method. *Environ. Toxicol. Chem*. 16:1002–1009.

French III, J.R.P., and Bur, M.T. 1993. Predation of the zebra mussel (*Dreissena polymorpha*) by freshwater drum in western Lake Erie. In *Zebra mussels: Biology, Impacts, and Control*, eds. T.F. Nalepa and D.W. Schloesser, pp. 453–464. Chelsea, MI: Lewis Publishers.

Gobas, F.A.P.C., Muir, D.C.G., and Mackay, D. 1988. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. *Chemosphere* 17:943–962.

_____, McCorquodale, J.R., and Haffner, G.D. 1993. Intestinal absorbtion and biomagnification of organochlorines. *Environ. Toxicol. Chem*. 12:567–576

Gossiaux, D.C., Landrum, P.F., and Fisher, S.W. 1998. The assimilation of contaminants from suspended sediment and algae by the zebra mussel, *Drissena polymorpha*. *Chemosphere* 36:3181–3197.

Hawker, D.W., and Connell, D.W. 1988. Octanol-water Partition coefficients of polychlorinated biphenyl congeners. *Environ. Sci. Technol*. 22:382–387.

Klump, J.V., Krezoski, J.R., Smith, M.E., and Kaster, J.L. 1987. Dual tracer studies of the assimilation of an organic contaminant from sediment by deposit feeding oligochaetes. *Can J. Fish. Aquatic. Sci*. 44: 1574–1583.

Lee, H. II, Boese, B.L., Randall. R.C., and Pelletier, J. 1990. A method for determining gut uptake efficiencies of hydrophobic pollutants in a deposit-feeding clam. *Environ. Toxicol. Chem*. 9:215–219.

Leversee, G.J., Geisy, J.P., Landrum, P.F., Gerould, S., Bowling, J.W., Fannin, T.E., Haddock, J.D., and Bartell, S.M. 1982. Kinetics and biotransformation of benzo(a)pyrene in *Chironomus riparius*. *Arch. Environ. Contam. Toxicol.* 11:25–31.

Lopez, G., and Elmgren, R. 1989. Feeding depths and organic assimilation for the deposit-feeding benthic amphipods, *Pontoporeia affinis* and *Pontoporeia femorata. Limnol. Oceanogr*. 34:982–991.

Luoma, S.N., Johns, C., Fisher, N.S., Steinberg, N.A., Oremland, R.S., and Reinfleder, J. 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. *Environ. Sci. Technol*. 26:485–491.

Ma, X. 1996. Bioprocessing of algae and transfer of hydrophobic contaminants by zebra mussels (*Dreissena polymorpha)*. Masters thesis, The Ohio State University, Columbus, OH.

Martin, G.W., and Corkum, L.D. 1994. Predation of zebra mussels by crayfish. *Can. J. Zool*. 72:1867–1871.

Millamena, O.M., Peaflorida, V.D., and Subosa, P.F. 1990. The macronutrient composition of natural food organisms mass cultured as larval feed for fish and prawns. *Isr. J. Aquac.* 42:77–83.

Morrison, H., Yankovich, T., Lazar, R., and Haffner, G.D. 1995. Elimination rate constants of 36 PCBs in zebra mussels (*Dreissena polymorpha*) and exposure dynamics in the Lake St. Clair—Lake Erie corridor. *Can. J. Fish. Aquat. Sci.* 52:2574–2582.

_____, Gobas, F.P.C, Lazar, R., and Haffner, G.D. 1996. Development and verification of a bioaccumulation model for organic contaminants in benthic invertebrates. *Environ. Sci. Technol.* 30:3377–3384.

Morton, B. 1983. Feeding and digestion in bilvalvia. In *The Mollusca*, Vol. 5 Physiology, Part 2, K.M. Wilbur ed., pp. 65–147. New York, NY: Academic Press.

Nalepa, T.F., and Fahnenstiel, G.L. 1995. *Drissena polymorpha* in the Saginaw Bay, Lake Huron ecosystem, overview and perspective. *J. Great Lakes Res*. 21:411–416.

_____, Hartson, D.J., Gostenik, G.W., Fanslow, D.L., and Lang, G.A.. 1996. Changes in the freshwater mussel community of Lake st. Clair: from Unionidae to *Dreissena polymorpha* in eight years. *J. Great Lakes Res*. 22:354–369.

Nichols, H.W., and Bold, H.C. 1965. *Trichosarcina polymorpha* gen. et. sp. nov. *J. Phycology* 1:34–38.

Penry, D.L. 1998. Applications of efficiency measurements in bioaccumulation studies: Definitions, clarifications and a critique of methods. *Environ. Toxicol. Chem*. 17:1633–1639.

Reeders, H.H., and bij de Vaate, A. 1992. Bioprocessing of polluted suspended matter from the water column by the zebra mussel (*Dreissena polymorpha* Pallas). *Hydrobiologia* 239:53–63.

Robertson, A., and Lauenstein, G.G. 1998. Distribution of chlorinated organic contaminants in dreissenid mussels along the southern shores of the Great Lakes. *J. Great Lakes Res.* 24:608–619.

Roper, J.M., Cherry, D.S., Simmers, J.W., and Tatem, H.E. 1996. Bioaccumulation of toxicants in the zebra mussel, *Dreissena polymorpha*, at the times beach confined disposal facility, Buffalo, New York. *Environ. Pollut.* 94:117–129.

Secor, C.L., Mills, E.L., Harshbarger, J., Kuntz, H.T., Gutenmann, W.H., and Lisk, D.J.. 1993. Bioaccumulation of toxicants, element, and nutrient composition and soft tissue histology of zebra mussels (*Dreissena polymorpha*) from New York State waters. *Chemosphere* 26:1559–1575.

Stoeckmann, A.M., and Garton, D.W. 1997. A seasonal energy budget for zebra mussels (*Dreissena polymorpha*) in Western Lake Erie. *Can. J. Fish. Aquat. Sci.* 54:2743–2751.

Thomann, R.V., Connolly, J.P., and Parkerton, T.F.. 1992. An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. *Environ. Toxicol. Chem*. 11: 615–629.

USEPA. 1975. *Methods for acute toxicity testing with fish, macroinvertebrates and amphibians*. EPA-6601/3-75-009. National Technical Information Service, Springfield, VA.

Van Haelst, A.G., Loonen, H., van Wielen, F.W.M., and Govers, H.A.J. 1996. Comparisons of bioconcentration factors of tetrachlorobenzyltoluenes in the guppy (*Poecilia reticulata*) and the zebra mussel (*Dreissena polymorpha*). *Chemosphere* 32: 1117–1122.

Vetter, R.D., Carey, M.C., and Patton, J.S. 1985. Coassimilation of dietary fat and benzo(a)pyrene in the small intestine: An assimilation model using killifish. *J. Lipid Res*. 26:428–434.

Widdows, J., Fieth, P., and Worrall, C.M. 1979. Relationship between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar. Biol*. 50:195–207.

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