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Bioconcentration and trophic transfer of lipophilic contaminants by the zebra mussel, *Dreissena polymorpha*: The role of lipid content, body size, and route of exposure

Bruner, Kathleen Ann, Ph.D.
The Ohio State University, 1993

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BIOCONCENTRATION AND TROPHIC TRANSFER OF LIPOPHILIC CONTAMINANTS BY THE ZEBRA MUSSEL, DREISSENA POLYMORPHA:

THE ROLE OF LIPID CONTENT, BODY SIZE, AND ROUTE OF EXPOSURE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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* * * * *

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To Helen, Reta, Mary, Margaret and Susan
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Control. eds. T.F. Nalepa and D.W. Schloesser. Lewis publishers, Ann Arbor,
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TABLE OF CONTENTS

ACKNOWLEDGMENTS .......................................................... iii
VITA ............................................................... iv
LIST OF TABLES .................................................. viii
LIST OF FIGURE ................................................... xi
INTRODUCTION ................................................... 1

CHAPTER PAGE

I. BIOCONCENTRATION OF LIPOPHILIC CONTAMINANTS BY THE ZEBRA MUSSEL, DREISSENA POLYMORPHA: EFFECTS OF LIPID CONTENT AND BODY SIZE ............. 12
   Introduction ......................................................... 12
   Materials and Methods ........................................ 14
   Results .............................................................. 21
   Discussion ........................................................ 31

II. ACCUMULATION OF LIPOPHILIC CONTAMINANTS BY THE ZEBRA MUSSEL, DREISSENA POLYMORPHA: ALGAL AND PARTICULATE ROUTES OF EXPOSURE ............. 37
   Introduction ......................................................... 37
   Materials and Methods ........................................ 39
   Results .............................................................. 45
   Discussion ........................................................ 47

III. TROPHIC TRANSFER OF LIPOPHILIC CONTAMINANTS TO GAMMARUS FASCIATUS VIA CONTAMINATED ZEBRA MUSSEL FECES ................................. 64
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>64</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>66</td>
</tr>
<tr>
<td>Results</td>
<td>75</td>
</tr>
<tr>
<td>Discussion</td>
<td>78</td>
</tr>
<tr>
<td>IV. Summary</td>
<td>88</td>
</tr>
</tbody>
</table>

**APPENDICES**

A. Data and Protocols Relative to Chapter I .......................... 94

B. Data and Protocols Relative to Chapter II .......................... 107

**LIST OF REFERENCES** ...................................................... 116
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Kinetic parameters for the high lipid, 21 mm size class zebra mussels. Numbers in parenthesis are standard errors. Within a column, values with the same letter(s) are not significantly different.</td>
<td>24</td>
</tr>
<tr>
<td>2. Kinetic parameters for the low lipid, 21 mm size class zebra mussels. Numbers in parenthesis are standard errors. Within a column, values with the same letter(s) are not significantly different.</td>
<td>25</td>
</tr>
<tr>
<td>3. Kinetic parameters for the high lipid, 15 mm size class zebra mussels. Numbers in parenthesis are standard errors. Within a column, values with the same letter(s) are not significantly different.</td>
<td>28</td>
</tr>
<tr>
<td>4. Zebra mussel contaminant % assimilation efficiencies (% AE) from spiked algae and suspended sediment particles. The numbers in parenthesis are standard errors.</td>
<td>46</td>
</tr>
<tr>
<td>5. Parameters used in the steady-state model to determine the concentration and percent of zebra mussel body burden from contaminated suspended sediment</td>
<td>55</td>
</tr>
<tr>
<td>6. Parameters used in the steady-state model to determine the concentration and percent of zebra mussel body burden from contaminated algae</td>
<td>56</td>
</tr>
<tr>
<td>7. Model estimates of zebra mussel contaminant concentrations from algal, suspended sediment and water routes of exposure for three water concentrations (ng/L) and the percent contribution to the mussel body burden from each route of exposure. The concentration of algae and suspended sediments were identical</td>
<td>59</td>
</tr>
<tr>
<td>8. Model estimates of zebra mussel contaminant concentrations from algal, suspended sediment and water routes of exposure for three water</td>
<td></td>
</tr>
</tbody>
</table>
TABLE PAGE

<table>
<thead>
<tr>
<th>Table Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Contaminant assimilation efficiencies (% AE) and kinetic parameters for uptake and elimination of compounds by <em>Gammarus fasciatus</em>. Numbers in parenthesis are standard errors</td>
</tr>
<tr>
<td>10</td>
<td>Parameters used in steady-state model to determined gammarid contaminant concentration from zebra mussel feces route of exposure</td>
</tr>
<tr>
<td>11</td>
<td>Model estimates of gammarid contaminant concentrations from fecal and water routes of exposure for three water concentrations (ng/L) and the percent contribution to the mussel body burden from each route of exposure</td>
</tr>
<tr>
<td>12</td>
<td>Steady-state concentrations of HCBP and TCBP for gammarids feeding on contaminated feces, for gammarids feeding on contaminated algae, for zebra mussel feces, and for algae detritus</td>
</tr>
<tr>
<td>13</td>
<td>Regression equations and 95% confidence intervals calculated for comparison of elimination rates ($k_d$)</td>
</tr>
<tr>
<td>14</td>
<td>Percent lipid by dry weight, % lipid by dry weight corrected for lipid and % lipid wet wt corrected for lipid for all zebra mussels used in the kinetic experiments</td>
</tr>
<tr>
<td>15</td>
<td>Comparison of kinetic parameters between two lipid levels for four compounds</td>
</tr>
<tr>
<td>16</td>
<td>Comparison of kinetic parameters for two size classes of high lipid zebra mussels</td>
</tr>
<tr>
<td>17</td>
<td>$k_u$, $k_d$, BCF and Log $k_u$, $k_d$ and BCF values used in regression analysis</td>
</tr>
<tr>
<td>18</td>
<td>Results of regression analysis of Log $K_{ow}$ vs $k_u$, $k_d$, or BCF for two lipid levels and two size classes of mussels</td>
</tr>
<tr>
<td>19</td>
<td>Results of regression analysis of Log $K_{ow}$ vs Log $k_u$, $k_d$, or BCF for two lipid levels and two size classes of mussels</td>
</tr>
<tr>
<td>20</td>
<td>Media particle or cells concentration (particles/mL), by weight (ug</td>
</tr>
</tbody>
</table>
particles or algae/mL, and water contaminant concentration (ug/mL) at T₀ and T₂ for algae and suspended sediment pulse-chase experiments. DPM's of feces at T₂ is also listed.

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>21. Compound mass-balance for pulse-chase experiments used to determine zebra mussel contaminant assimilation efficiencies from spiked algae and suspended sediments</td>
<td>109</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

FIGURES PAGE

1. The zebra mussel's potential influence on lipophilic contaminant cycling in the Great Lakes ................................................................. 3

2. Seasonal variation in lipid levels (% dry wt) of 15 and 21 mm size classes of zebra mussels collected from western basin of Lake Erie, 1991 and 1992. Error bars indicate standard errors ................................. 22

3. Comparison of kinetic parameters between the high and low lipid levels, 21 mm size class zebra mussels. For each compound, values with the same letter are not significantly different. Error bars indicate standard errors. ......................................................... 26

4. Comparison of kinetic parameters between the 21 and 15 mm size class, high lipid level zebra mussels. For each compound, values with the same letter are not significantly different ........................................ 29

5. Log BCF vs log $K_{oc}$ for 21 and 15 mm, high lipid mussels and 21 mm low lipid mussels .............................................................................. 30

6. Comparison of contaminant assimilation efficiencies from spiked algae and sediment. Error bars represent standard errors .................. 48

7. Cumulative contaminant (ug) in zebra mussel's feces collected after exposure to spiked algae (A) or spiked suspended sediments (B). Error bars represent standard errors ...................................................... 49

8. Cumulative compound in gammarid feces during the "chase" period of the assimilation experiment .............................................................. 76

9. HCBP (A) and TCBP (B) concentrations in various compartments of a lake ecosystem. PCB concentrations were estimated from field data and from steady-state models ......................................................... 93
INTRODUCTION

The zebra mussel, *Dreissena polymorpha*, was introduced into the Great Lakes ecosystem in 1986 (Hebert *et al.* 1989), and has successfully established extensive populations in littoral zones of four of the major Great Lakes, with highest numbers in the western basin of Lake Erie (Sea Grant 1991). Major factors that have contributed to the zebra mussels' colonization of Lake Erie are: 1) a mobile veliger larva; 2) high reproductive rates; and 3) byssus attachment (Hebert *et al.* 1989). In addition, the ability of adult zebra mussels to filter approximately 1 L/ind/d of lake water (O’Neill and MacNeill 1989) allows it to be a formidable competitor for algal stocks in Lake Erie. With zebra mussel densities as great as 175,000 ind/m² (Leach 1993), a substantial amount of water and associated seston can be filtered by these organisms. Biologists are concerned that zebra mussels will exploit algal populations currently utilized by fish (Mackie *et al.* 1989; MacIsaac *et al.* 1992). Those particles and food that are filtered but not ingested by the zebra mussel are bound into a mucus bolus and eliminated as pseudofeces (Morton 1969). There is additional concern that the sedimentation of this material as pseudofeces, coupled with fecal production, will funnel water-borne nutrients to the benthos at a rate great enough to disrupt the present nutrient cycling in Lake Erie (O’Neill and MacNeill 1989; Griffiths 1993).

These same filtering and sedimentation processes of the zebra mussel may be
significant in altering the cycling of environmental contaminants common in the Great Lakes (Fig. 1). Large populations of zebra mussels may filter toxicants dissolved in the water or associated with algal or suspended material and accumulate these compounds in their tissues. Contaminants sequestered in the mussels' tissue would then be transferred to zebra mussel predators such as fish or birds. This may pose a hazard to aquatic and terrestrial predators by contributing to chronic problems of reduced fitness, cancers or reproductive failure (Neff 1979; D’itri and Kamrin 1983). In addition, movement of contaminated feces, pseudofeces or dead mussel tissue to the benthos may increase contaminant exposure for benthic detritivores.

**Zebra Mussel Contaminant Concentration**

Numerous studies have demonstrated the abilities of bivalves to accumulate toxins to concentrations far exceeding ambient water concentrations (Lee et al. 1972; Vreeland 1974; Clark and Findley 1975; Dunn and Stich 1976; Neff et al. 1976; Ernst 1977; Langston 1978; Fortner and Sick 1985; Pruell et al. 1986; Mersch et al. 1992). This ability has led researches to use bivalves as biomonitoring organisms for both fresh and marine aquatic systems (Kraus and Hamdy 1985; Renberg et al. 1986; Doherty 1990). Thus, *Dreissena* can be expected to accumulate toxins, though the extent of this accumulation is not presently known. From preliminary studies it has been demonstrated that *Dreissena* rapidly accumulates chlorinated lipophilic compounds such as polychlorinated biphenyls (PCBs), polycyclic hydrocarbons (PAHs) and the insecticide DDT from water with contaminants uptake clearance
Figure 1. The zebra mussel's potential influence on lipophilic contaminant cycling in the Great Lakes.
constants of 167 to 424 mL g\(^{-1}\) h\(^{-1}\). In addition, Fisher et al. (1993) reported uptake clearance constants for the same compounds by a Lake St. Claire population of zebra mussels. Like the Lake Erie population, these zebra mussels rapidly took up organic contaminants with uptake clearance constants of 300-800 mL g\(^{-1}\) h\(^{-1}\). Compound elimination in both mussel populations was slow (0.004-0.0169 h\(^{-1}\)). Because accumulation of a compound occurs when uptake exceeds elimination (Spacie and Hamelink 1985), the preliminary results indicate that zebra mussels have the potential to concentrate water-borne lipophilic contaminants orders of magnitudes higher than ambient water concentrations.

The most widely used measure of contaminant accumulation from water is the bioconcentration factor (BCF). The BCF is the ratio of an organism’s steady-state contaminant concentration to the contaminant concentration in the water (Chiou 1985). The larger the BCF the greater the bioconcentration potential. In an attempt to establish a predictive relationship between the physical-chemical properties of contaminants and organism bioconcentration, BCF’s have been repeatedly correlated with a compound’s octanol-water partition coefficient (\(K_{ow}\)). (\(K_{ow}\) is a measure of a compound’s lipid solubility.) For aquatic organisms, including bivalves, BCF values were positively correlated to log \(K_{ow}\) for compounds with log \(K_{ow}\) in the range of 3-6 (Chiou et al. 1977; Bruggerman 1982; Geyer et al. 1982, 1985; Veith 1980). Therefore, PCBs and PAHs are contaminants of great concern for bioconcentration because of their high lipophilicity (log \(K_{ow}\) > 3). Indeed, these compounds have been classified as priority pollutants because of their persistence in detectable
concentrations in the biota of the Great Lakes and because of their carcinogenic properties (Fitchko 1986). In addition, the high lipid solubility of these compounds allows them to sorb readily to particulate organic matter in the water and sediment. When ingested, the compounds may desorb from the contaminated particles and partition into the lipid rich tissues of aquatic organism (Neff 1979; D’Itri and Kamrin 1983). Further, dissolved PAHs and PCBs can enter the aquatic organisms through the permeable membrane of the gills.

Uptake of lipophilic contaminants by aquatic organisms is dependent on the bioavailability of that chemical. As mentioned above, water-borne contaminants can be taken across the gills and absorbed directly into the blood stream (Zitko 1980) while contaminants associated with food or ingested sediment must desorb and pass through the gut barrier before assimilation (Klump et al. 1987; Gobas et al. 1993). The direct absorption of compounds from water is thought to be the most rapid and predominant mode of contaminant uptake (Stegeman and Teal 1973; Pruell et al. 1986; Muncaster et al. 1990). Uptake through contact with contaminated sediments is considered to be of lesser importance for sediment-associated animals because sorption of contaminants to sediment reduces bioavailability (Schuytema et al. 1990). However, there is evidence that sediment ingesting organisms are at risk when contaminants desorb from ingested materials (Langston 1978; Landrum and Scavia 1983; Ekelund et al. 1987; Klump et al. 1987; Swindoll and Applehans 1987; Boese et al. 1990). In addition, contaminated sediment particles can be resuspended and, along with contaminated food particles, become potential sources of exposure to non-
selective filter feeders such as zebra mussels.

Agreement as to which route of contaminant exposure is most importance for bivalves has not been reached. Pruell et al. (1986) reported that in comparison to particulate material, water-borne PCBs and PAHs were the primary source of contamination for the filter-feeding marine mussel, *Mytilus edulis*. In contrast, results from laboratory experiments by Langston (1978) demonstrated that for the suspension-feeding bivalve, *Cerastoderma edule*, ingestion of contaminated sediment particles can be a major source of PCBs. He noted that this route of exposure can be especially important in aquatic systems with high particulate loads. Ekelund et al. (1987) reported similar results for the filter-feeding, marine bivalve, *Abra nitida*. Fortner and Sick (1985) found that contaminated algae was either of greater or equal importance than contaminated water to compound accumulation in filter-feeding oysters. The lack of consensus on the principle route of contaminant exposure in bivalves suggests that all routes discussed above may represent potential sources of exposure, depending on the contaminant concentration and the relative amount of food or suspended particles available. Because all modes of exposure may contribute to the contaminant body burden in zebra mussels, each potential route of uptake should be examined.

The uptake of contaminants from water (bioconcentration) is affected by a number of physical and physiological parameters (Muncaster et al. 1990). For aquatic organisms, lipid content is an important regulator for partitioning of lipophilic contaminants into body tissues and influences the uptake and elimination of these
compounds (Ernst et al. 1976; Dunn and Stich 1976; Ingebrigtsen et al. 1988). In a study of uptake of petroleum hydrocarbons by oysters, Stegeman and Teal (1973) reported oysters with a high fat content (1.6 % lipid/wet wt) had approximately twice the tissue concentration of hydrocarbons than low fat (0.93 % lipid/wet wt) oysters after 35 days of exposure. Comparison of zebra mussel bioconcentration factors to those of the marine mussel, *Mytilus edulis*, indicates that zebra mussels have the potential for concentration of lipophilic compounds about an order of magnitude higher than for *Mytilus* (Fisher et al. 1993). This difference in bioconcentration was presumed to be the result of a higher percent lipid (11%) for zebra mussels as compared to *Mytilus edulis* (1.8%). Thus, a greater lipid content may allow zebra mussels to sequester higher concentrations of lipophilic contaminants than similarly sized marine mussels.

The storage and mobilization of lipid reserves in response to the demands of spawning can contribute to seasonal variation in lipophilic contaminant concentrations in bivalves (Hummel et al. 1990; McDowell-Capuzzo et al. 1989). Garton and Haag (1993) have reported seasonal changes in the body weight of *Dreissena* collected from the Western Basin of Lake Erie. These changes in body mass were highly correlated to the spawning activities of the mussels suggesting that the zebra mussels' lipid pool will vary over a reproductive season. Seasonal variations in contaminant concentrations due to spawning have not been reported for zebra mussels, but a large loss of lipids in the form of gametes may influence zebra mussels' lipophilic contaminant concentration.
Another factor that will influence contaminant bioconcentration in bivalves is body size. Size-dependent accumulation of trace metals and lipophilic contaminants such as PCBs has been reported for both freshwater and marine bivalves (Boyden 1974; Cossa et al. 1980; Strong and Luoma 1981; Muncaster et al. 1990). Smaller individuals generally have faster rates of uptake and greater contaminant body burdens than larger individuals (Cossa et al. 1980; Muncaster et al. 1990). Physiological differences in metabolic demand between large and small individuals is given as the primary cause for size differences in contaminant accumulation (Boyden 1974). For example, smaller bivalves have faster filtration rates and larger gill surface to volume ratios than larger mussels (Vahl 1973; Bayne and Widdows 1978) both contributing to the greater assimilation of contaminants in smaller individuals (Muncaster et al. 1990). Consequently, size-specific predation on smaller individuals may expose predators to greater risk of contaminant uptake than if they choose larger individuals. For zebra mussels, the effect of body size on contaminant bioconcentration has not been rigorously studied. While, contaminant uptake clearance constants for PAHs and PCBs were found to be positively correlated to mussel mass (Fisher et al. 1993), the effect of size on compound elimination and BCF values has yet to be determined.

**Zebra Mussel Contaminant Sedimentation**

As mentioned previously, contaminated material in the form of zebra mussel feces, pseudofeces, or dead mussel tissue will follow the sedimentation route to the benthos (Fig. 1). The effect of bivalve biodeposition on contaminant sedimentation
has been poorly studied, particularly in freshwater systems. Reynoldson (1987) in his discussion of biodeposition of contaminated particles by oysters and marine invertebrates, remarked that these filter feeders with their "active removal of fine particles ... may have particular significance for the movement of contaminants which tend to adsorb to fine particulate material." In a more detailed study of trace metal cycling in an estuary system, the fecal pellets and pseudofeces of four bivalve species contained metal concentrations higher than surface sediment concentrations (Brown 1986). These results suggest that zebra mussel biodeposition of contaminated filtered material will cause enrichment of the contaminated material in the sediments. Zebra mussel biodeposition of contaminants will expose benthic detritivores that ingest or contact contaminated feces or pseudofeces to a higher than normal risk of contaminant uptake (Pinkney et al. 1985). This flux of contaminants to the benthos will be especially important in areas where zebra mussel populations are large (Negus 1966; Reynoldson 1987). Further, the zebra mussel's preference for water currents means mussels will colonize near outfalls that may discharge contaminated effluent. Hence, the sedimentation abilities of the zebra mussel may be more efficient at moving contaminants from the water to the sediment than the normal processes currently operating in the Great Lakes. Predation on these contaminated benthic species could then lead to increased rates of contaminant transfer to higher trophic levels.

Large populations of the benthic amphipod, *Gammarus fasciatus* have been found in association with zebra mussels colonies (Dermott and Barton 1991; Griffiths 1993). Because of the close association with zebra mussel colonies, gammarids will
ingest or contact excrement and detritus generated by the zebra mussels. If these materials are contaminated with lipophilic compounds, the gammarids' risk of contaminant uptake will increase. Because gammarids are prey for many fish species (Oster 1980; Weisberg and Janicki 1990), gammarids will be an important vector for transfer of lipophilic contaminants to predators.

Objectives

The zebra mussels' influence on contaminant cycling in the Great Lakes is a complex problem. Three aspects of the problem were chosen for this study. The first was to measure the zebra mussels' potential for accumulation of selected priority pollutants and to assess the affect of body size and lipid content on zebra mussel contaminant bioconcentration. The second was to identify the relative importance of the major routes of zebra mussels contaminant exposure i.e. water and dietary exposure. An understanding of the relative importance of each route of contaminant exposure will help in quantifying the sources of mussel contaminant exposure. Finally, zebra mussel sedimentation of contaminated material to a benthic detritivore was examined. The specific objectives of the entire study are listed by chapter below.

Chapter 1. The objective of this portion of the study was to assess the effect of varying lipid content and body size on the uptake and elimination of radiolabeled, nonpolar, lipophilic PCBs and PAHs congeners by adult zebra mussels. The effect of compound lipophilicity on zebra mussel bioconcentration of these selected PCBs and PAHs was also determined.
Chapter 2. The objective of this portion of the study was to determine the relative importance of the algal and suspended sediment routes of exposure to zebra mussel accumulation of nonpolar, lipophilic compounds by comparison of contaminant assimilation efficiencies. In addition, the relative importance of the dietary and water routes of exposure were determined using a steady-state model.

Chapter 3. The objective of this portion of the study was to examine trophic transfer of selected PCBS from contaminated zebra mussel feces to a benthic detritivore, *Gammarus fasciatus*. In addition, a steady-state model was used to determine the relative importance of the fecal, detrital and water routes of exposure on gammarid accumulation of the nonpolar, lipophilic PCBs.

Chapter 4. The final chapter presented a summary of the results of the preceeding chapters and included a discussion of *Dreissena polymorpha's* influence on priority pollutant cycling in Lake Erie.
CHAPTER I

BIOCONCENTRATION OF PCBS AND PAHS BY THE ZEBRA MUSSEL,

DREISSENSA POLYMORPHA: EFFECTS OF BODY SIZE AND LIPID CONTENT

INTRODUCTION

Dreissena polymorpha, the zebra mussel, is a recent invader of the North American Great Lakes ecosystem (Hebert et al. 1989). Zebra mussels are reported to be excellent candidates for bioaccumulation of lipophilic contaminants such as polychlorinated biphenyls (PCBs), chlorinated insecticides (DDT) and polycyclic aromatic hydrocarbons (PAHs) (Fisher et al. 1993). Their large bioaccumulation capacity, coupled with high densities in littoral areas associated with contaminated outfalls (Yankovich and Haffner 1993) and their low position in the food chain puts zebra mussels in an ideal situation to affect contaminant cycling in the Great Lakes. Two ways in which zebra mussels could affect contaminant cycling are by 1) increasing the contaminant concentration in sediments through biodeposition of contaminated feces and pseudofeces and 2) increasing trophic transfer of contaminants to mussel predators. The degree to which mussels will accumulate contaminants is a major factor that will influence the zebra mussel’s impact on contaminant trophic transfer.
Contaminant accumulation in bivalves is regulated by several physiological parameters such as age, size, and lipid content (Stegeman and Teal 1973; Boyden 1974; Ernst et al. 1976; Cossa et al. 1980; Strong and Luoma 1981; Muncaster et al. 1990). Because lipid reserves are a primary site of non-polar, hydrophobic xenobiotic storage, lipid content is an important factor for determining lipid-soluble compound accumulation (Geyer et al. 1985). The high lipid content of zebra mussels has been cited as a major reason for the order of magnitude difference in bioconcentration factors (BCFs) between zebra mussels and *Mytilus edulis*, a related marine mussel (Fisher et al. 1993). In addition, lipid levels in bivalves vary throughout the season, particularly after spawning when lipid levels drop significantly (Sprung 1993). Seasonal variations in lipid levels have modified contaminant accumulation in field populations of marine bivalves (McDowell-Capuzzo et al. 1989; Hummel et al. 1990). Presently, the influence of seasonal variations in zebra mussel lipid reserves on lipophilic contaminant accumulation is unknown.

Another factor that influences contaminant accumulation in bivalves is body size. Size-dependent accumulation of trace metals and lipophilic contaminants such as PCBs has been reported for both freshwater and marine bivalves (Boyden 1974; Cossa et al. 1980; Strong and Luoma 1981; Muncaster et al. 1990). Smaller individuals generally have faster rates of uptake and greater contaminant body burdens than larger individuals (Cossa et al. 1980; Muncaster et al. 1990). Physiological differences in metabolic demand are the primary cause for the differential contaminant accumulation, although size-dependent feeding behavior, surface to volume ratios or
concentrations of enzymes that influence uptake may also play a role (Boyden 1974; Newman and Mitz 1988).

Because of the possible influence of body size and lipid reserves on lipophilic xenobiotic accumulation, the effects of these variables on zebra mussel contaminant biconcentration were examined. Using kinetic methodology, bioconcentration factors (BCFs) and kinetic parameters for contaminant bioconcentration in two size classes of zebra mussels were determined. In addition, the accumulation kinetics in pre- (high lipid) and post-spawning (low lipid) mussel populations were examined for mussels collected from the western basin of Lake Erie. The compounds studied were from the chemical classes identified as Great Lakes priority pollutants: polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (Fitchko 1986). The compounds varied in lipid solubility as measured by log octanol:water partition coefficients (log $K_{ow}$) and allowed examination of the relationship between compound lipophlicity and xenobiotic bioconcentration.

MATERIALS AND METHODS

Chemicals

$^{14}$C-labeled PCBs were obtained from Sigma Chemical Company (St. Louis, Mo.) and tritiated PAHs were obtained from NCI Radiochemical Carcinogen Repository, Chemsyn Science Laboratories (Lenexa, Kansas). The specific activity for each chemical was: 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP, 20 mCi/mmol); 2,2',4,4'-tetrachlorobiphenyl (TCBP, 13.8 mCi/mmol); benzo(a)pyrene (BaP, 380
mCi/mMol); and pyrene (507 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 (Dreissena polymorpha) were collected from littoral zones of Lake Erie by SCUBA divers off-shore of Kelly’s Island, Ohio. Mussels recovered from the lake were scraped from rocks and transported within 3 h (in moist air) to holding facilities at the Ohio State University, Columbus, Ohio. Cultures were maintained in 200 L tanks filled with aged, aerated tap water at 20 ± 2 °C and fed 150 ml of a Tetra Min (fish food) solution (10.5 g/L) per 3000 individuals. Temperature, dissolved oxygen, and ammonia concentration were checked daily and water was replaced 1-2 times weekly. All waste water was treated with chlorine bleach (50 ppm) before disposal to prevent introducing mussels or veligers into inland waters. Zebra mussels were acclimated to laboratory conditions for at least 1 wk prior to experimentation and only conditioned mussels were used in experiments. Zebra mussels were considered suitable for use if, after severing of byssal threads, they reattached to a substrate within 48 h. The two size classes used were 21 ± 1 mm and 15 ± 1 mm mussels.

Media

Soft (hardness 40-48 mg/L as CaCO₃ and total alkalinity 30-35 mg/L as CaCO₃), buffered, standard reference water (SRW) adjusted to pH 8 (USEPA 1975)
was used in all experiments. Because potassium-containing compounds can be toxic to zebra mussels (Fisher et al. 1991), Na$_2$HPO$_4$ was substituted for the recommended K$_2$HPO$_4$ buffer.

Media Preparation

One day prior to initiation of either an uptake or elimination experiment, SRW (15 L) was spiked with radiolabeled compound dissolved in 44-284 μL of acetone and stirred for 1 h. After equilibration (4-5 h), the spiked water was divided among the test containers and the foil-covered containers were placed overnight in a darkened environmental chamber (Forma Scientific N37422, Marietta, Ohio) at 20 °C. On the test day, the initial toxicant concentration was determined for each container before placing zebra mussels into the test containers.

Uptake Clearance Rates

Three days before an uptake experiment, individual zebra mussels were placed in 40 L aquaria filled with SRW (pH 8). The bottoms of the aquaria were covered with 2.5 x 7.5 cm glass microscope slides to which the zebra mussels reattached. The aquaria were maintained at experimental conditions and the mussels were fed daily (10 mL TetraMin solution/aquarium).

On the day of an experiment, mussels individually attached to glass microscope slides were rinsed thoroughly and placed in 1-L glass beakers containing 500 mL of SRW spiked with a radiolabeled PCB or PAH congener. Zebra mussel
filtration was monitored visually every 0.5 h throughout the experiment. Mussels were assumed to be filtering if both incurrent and excurrent siphons were extended. After 0.5, 1, 1.5, 2, 4, or 6 h of exposure, 5 beakers were removed from the chamber. Three 1 mL water samples were taken from each beaker for radiometric analysis. The zebra mussels were then processed for liquid scintillation counting (LSC). 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 sample of the acetone rinse (1 mL) was analyzed for radioactivity.

Elimination Experiment

Three days prior to an elimination experiment, zebra mussels were allowed to attach to the bottom of 100 mm glass petri dishes as described for the uptake experiments.

On the day of an experiment, 100 mussels attached to 10 petri dishes were exposed for 6 h in 5 replicate aquaria filled with spiked SRW. The filtering of each mussel was monitored visually every hour during the exposure period. After 6 h, the petri dishes were removed from the spiked water and rinsed thoroughly with unlabeled SRW. Mussels that had failed to filter over the entire exposure period were removed with forceps and the remaining mussels were placed in 5 replicate glass elimination chambers (4 L) filled with aerated SRW. The elimination chambers were maintained in an environmental chamber at 20 °C. Five zebra mussels were removed
for analysis at 12, 24, 48, 72, 96, 120, 144, and 168 h. The water in the elimination chambers was changed daily, after the mussels were fed (TetraMin solution). Contaminant levels in the water were monitored by taking 3-1 mL water samples at each of the sampling periods.

All mussels removed for analysis were blotted dry, weighed and eviscerated. The rinsed valves and viscera were blotted dry, weighed, and placed in separate glass scintillation vials with 5 mL of scintillation cocktail (1000 mL dioxane, 500 g naphthalene, 5 g diphenyloxazole). The mussel samples were extracted for 24 h in the cocktail and then all water and mussel samples were analyzed by LSC using a Beckman LS 6000IC liquid scintillation system ($^{14}$C efficiency > 95%; $^{3}$H efficiency > 60%) with automatic quench control. Contaminant concentration was determined on a wet-weight basis (Appendix A).

Lipid Analysis

Forty mussels from each size class were eviscerated, the tissue blotted dry and the wet weight recorded. Tissues from 20 of the mussels were placed in individual test tubes and homogenized by hand in 6 mL (21 mm size class) or 3 mL (15 mm size class) of chloroform/methanol 2:1. The test tubes were sealed with aluminum foil and refrigerated overnight. A 0.5 mL sample of each mussel extract was then analyzed for total lipids using the colorimetric method of van Handel (1985). The 20 remaining mussel viscera were dried 24 h in an oven (56 ± 2 °C) and desiccated for 24 h before weighing. A dry to wet weight ratio was determined to convert total
lipids to % dry mussel weight.

Kinetic Models

A mass-based model was used to estimate uptake contaminant clearance, $k_u$ (Fisher et al. 1993). $k_u$ describes the volume of water cleared of contaminant per mass of organism per time and has units of mL g$^{-1}$ h$^{-1}$. This coefficient is conditional with respect to the experimental parameters under which the $k_u$ is measured. In this calculation, the organism is defined as wet tissue only. Contaminants are assumed to partition between the organism and the water such that:

$$\frac{dQ_a}{dt} = k_1 Q_w - k_d Q_a. \quad (1)$$

Assuming mass balance in the system:

$$A = Q_w + Q_a \quad (2)$$

where $Q_a = \text{quantity of contaminant in the animal (\mu g)}$

$k_1 = \text{conditional uptake rate constant (h}^{-1})$

$Q_w = \text{quantity of contaminant in the water (\mu g)}$

$k_d = \text{the conditional elimination rate constant (h}^{-1})$

$A = \text{total amount of compound in the system (\mu g).}$

The basic assumptions of the model are that the mass of contaminant in the system does not change and that no biotransformation of contaminant takes place.

Zebra mussel biotransformation of PAHs was examined using thin layer chromatography (TLC) and radiometric analysis (Leversee et al. 1982) and no biotransformation was found after a 24 h exposure.
Because sorption to glassware and shells was < 3% of the total mass and significant elimination did not occur over the exposure time (6 h) these parameters were eliminated from the equation. The following simplified integrated initial rates equation resulted:

\[ k_i = \frac{-\ln(1 - Q_i/A)}{t} \quad (3) \]

This conditional rate constant \(k_i\) is a system dependent value and must be converted to a system independent clearance \(k_u\) by the following equation:

\[ k_u = k_i \text{ (volume of water/wet mass of tissue).} \quad (4) \]

The elimination data were fit to a first order elimination model:

\[ \frac{dC_a}{dt} = k_d C_a \quad (5) \]

Integration of equation 5 yields:

\[ \ln C_a = \ln C_{ao} - k_d t \quad (6) \]

where 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:

\[ \text{BCF} = \frac{k_u}{k_d}. \quad (7) \]

Statistical Analysis

Log transformed \(k_u\) and BCF values were analyzed by a general linear analysis of variance (GLM ANOVA SAS 1985) and means were separated using least squares means test (GLM SAS 1985). Significance was determined at \(p < 0.05\). \(k_d\) values
were tested for homogeneity of slopes (SAS 1985) and significant slopes were separated using 95 % confidence intervals (NCSS 1991). Differences in $k_d$ values were significant if 95 % confidence intervals did not overlap. Individuals with uptake clearance rates $< 100 \text{ mL g}^{-1} \text{ h}^{-1}$ or from systems with a mass-balance $< 70$ % were not included in the data analysis.

RESULTS

Monitoring of Lipid Levels

Before we could collect mussels in the desired reproductive state, it was necessary to define the lipid levels for a pre- and post-spawning zebra mussel field population. Therefore, lipid levels of a western basin Lake Erie mussel population were determined from March through October for the 1991 and 1992 reproductive seasons (Fig. 2). Lipid levels measured in both years followed the spawning cycle of zebra mussels in the western basin (Nichols and Kollar 1991). Lipid levels for the 21 mm size class were highest in late May (1991) or mid June (1992) corresponding to the high percent of gravid mussels in the population at these times. Lipid levels then declined over the summer as the mussels spawned. Based on these data, the high lipid or pre-spawning population was defined as one in which the % lipid by dry weight was $\geq 9.0$ % and the post-spawning population was defined as having % lipid $< 7.0$ %. Mussels used in the high lipid experiments were collected in late May (1991) or mid June (1992) and post spawning mussels were collected in early September (1991). Lipid levels were determined in cultured zebra mussels prior to
Figure 2. Seasonal variation in lipid levels (% dry wt) of 15 and 21 mm size classes of zebra mussels collected from the western basin of Lake Erie, 1991 and 1992. Error bars indicate standard errors.
experimentation. Lipid levels were 2 times greater in the high lipid mussels (avg = 13.5 %) than the low lipid groups (avg = 5.9 %). In both 1991 and 1992, lipid levels in the 15 mm size class were greater than 7.0 % on the dates collected, thus the effect of varying lipid content on accumulation were not determined for this size class. In addition, the lipid level of the 1992, 21 mm size class did not fall below 7.0 % restricting these populations to the high lipid kinetic experiments.

Effect of Differences in Lipid Levels on Bioconcentration

Overall, zebra mussel contaminant bioconcentration, as measured by BCF, was high regardless of compound or lipid level (Tables 1 and 2). Uptake clearances were rapid (323 to 895 mL g\(^{-1}\) h\(^{-1}\)) while elimination rate constants were slow (0.005 to 0.024 h\(^{-1}\)) (Tables 1 and 2). In general, uptake clearance rates were higher and elimination rate constants were lower for the high lipid mussels (Fig. 3). However, only BCFs of the more lipophilic compounds, HCBP and BaP, were significantly affected by changes in lipid levels (Fig. 3). This difference between lipid levels for HCBP and BaP bioconcentration was due to a significantly greater uptake clearance rate in the high lipid mussels. Elimination rate constants for these compounds did not vary with lipid content. Differences in lipid levels did not result in significant differences in uptake or elimination of the less lipophilic compounds, TCBP and pyrene. Consequently, BCF values for these compounds were statistically identical. When BCF values were lipid normalized, the correlation of BCFs with compound lipid solubility did not change significantly within a lipid level (Tables 1 and 2).
Table 1. Kinetic parameters for the high lipid, 21 mm size class zebra mussels. Numbers in parenthesis are standard errors. Within a column, values with the same letter(s) are not significantly different.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>$k_u$ mL g$^{-1}$ h$^{-1}$</th>
<th>$k_d$ h$^{-1}$</th>
<th>BCF mL g$^{-1}$</th>
<th>BCF lipid normalized mL g$^{-1}$</th>
<th>Log $K_{ow}$</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCBP</td>
<td>895 $^a$ (111)</td>
<td>0.005 $^a$ (0.001)</td>
<td>173,370 $^a$ (21,539)</td>
<td>9,556,738 $^a$ (1,187,324)</td>
<td>6.90 $^1$</td>
<td>24</td>
</tr>
<tr>
<td>BaP</td>
<td>759 $^a$ (91)</td>
<td>0.009 $^a$ (0.001)</td>
<td>84,316 $^b$ (9,880)</td>
<td>4,668,952 $^b$ (538,960)</td>
<td>5.98 $^2$</td>
<td>25</td>
</tr>
<tr>
<td>TCBP</td>
<td>423 $^b$ (43)</td>
<td>0.016 $^{bc}$ (0.002)</td>
<td>27,449 $^c$ (2,781)</td>
<td>1,372,431 $^c$ (139,037)</td>
<td>5.90 $^1$</td>
<td>26</td>
</tr>
<tr>
<td>PYRENE</td>
<td>352 $^b$ (34)</td>
<td>0.021 $^c$ (0.003)</td>
<td>16,028 $^d$ (1,561)</td>
<td>835,756 $^d$ (81,396)</td>
<td>5.20 $^2$</td>
<td>26</td>
</tr>
</tbody>
</table>

$^1$ Shiu and Mackay (1986)
$^2$ Miller et al. (1985)
Table 2. Kinetic parameters for the low lipid, 21 mm size class zebra mussels. Numbers in parenthesis are standard errors. Within a column, values with the same letter(s) are not significantly different.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>$k_u$ mL g$^{-1}$ h$^{-1}$</th>
<th>$k_d$ h$^{-1}$</th>
<th>BCF mL g$^{-1}$</th>
<th>BCF Lipid normalized mL g$^{-1}$</th>
<th>Log $K_{ow}$*</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCBP</td>
<td>380* (69)</td>
<td>0.006* (0.001)</td>
<td>63,304* (11,551)</td>
<td>7,072,427* (1,292,432)</td>
<td>6.90</td>
<td>14</td>
</tr>
<tr>
<td>BaP</td>
<td>331* (68)</td>
<td>0.008* (0.001)</td>
<td>41,239* (8,485)</td>
<td>4,563,601* (925,608)</td>
<td>5.98</td>
<td>11</td>
</tr>
<tr>
<td>TCBP</td>
<td>443* (52)</td>
<td>0.021 b (0.001)</td>
<td>21,114 b (2,477)</td>
<td>2,892,280 b (339,267)</td>
<td>5.90</td>
<td>18</td>
</tr>
<tr>
<td>PYRENE</td>
<td>323* (34)</td>
<td>0.024 b (0.002)</td>
<td>12,956 c (1,307)</td>
<td>1,843,929 cb (192,429)</td>
<td>5.20</td>
<td>18</td>
</tr>
</tbody>
</table>
Figure 3. Comparison of kinetic parameters between the high and low lipid levels, 21 mm size class zebra mussels. For each compound, values with the same letter are not significantly different. Error bars indicate standard errors.
Effect of Size on Bioconcentration

A comparison of kinetic parameters between the 15 and 21 mm mussel size classes, indicated that bioconcentration of all compounds was greater for the smaller mussels but this effect was statistically significant only for TCBP and pyrene (Fig. 4)(Tables 1 and 3). Smaller mussels had significantly greater uptake clearance rates for all four compounds than the 21 mm sized mussels whereas elimination rate constants were statistically identical between sizes except for BaP (Fig 4). The effect of size on bioconcentration was not significantly altered when lipid normalized BCFs were compared.

Correlation between Compound Lipophilicity and Bioconcentration

BCF values increased with increasing compound $K_{ow}$ regardless of lipid level or size. This corresponded to decreasing elimination rate constants with increasing compound $K_{ow}$ (Table 1-3). Although differences in BCF values among compounds within a size or lipid class were not always statistically different from each other, the regression of the log BCF with log $K_{ow}$ was positively correlated (Fig. 5). As with the BCF values, uptake clearance rates among compounds in the various size and lipid classes were not always significantly different from each other; however the log $k_u$ for the high lipid mussels of both 21 and 15 mm size classes were highly correlated to log $K_{ow}$ ($\log k_u = 0.024\log K_{ow} - 1.31 r^2 = 0.75$ and $\log k_u = 0.25\log K_{ow} + 1.57 r^2 = 0.90$ respectively)(Appendix A). The relationship of elimination rate constants with log $K_{ow}$ was negatively correlated for both size classes ($\log k_d = -0.37\log K_{ow} +$
Table 3. Kinetic parameters for the high lipid, 15 mm size class zebra mussels. Numbers in parenthesis are standard errors. Within a column, values with the same letter(s) are not significantly different.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>$k_a$ mL g$^{-1}$ h$^{-1}$</th>
<th>$k_d$ h$^{-1}$</th>
<th>BCF mL g$^{-1}$</th>
<th>BCF Lipid normalized mL g$^{-1}$</th>
<th>Log $K_{ow}$</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCBP</td>
<td>$1,683^a$ (215)</td>
<td>0.007$^a$ (0.001)</td>
<td>$240,362^a$ (30,699)</td>
<td>$9,096,750^a$ (1,217,068)</td>
<td>6.90</td>
<td>25</td>
</tr>
<tr>
<td>BaP</td>
<td>$1,305^a^b$ (186)</td>
<td>0.017$^b$ (0.002)</td>
<td>$76,771^b$ (10,946)</td>
<td>$3,133,514^b$ (446,781)</td>
<td>5.98</td>
<td>25</td>
</tr>
<tr>
<td>TCBP</td>
<td>$989^b$ (118)</td>
<td>0.014$^b$ (0.002)</td>
<td>$71,154^b$ (8,515)</td>
<td>$2,883,498^b$ (345,048)</td>
<td>5.90</td>
<td>27</td>
</tr>
<tr>
<td>PYRENE</td>
<td>$640^c$ (73)</td>
<td>0.021$^b$ (0.004)</td>
<td>$35,117^c$ (4,807)</td>
<td>$1,909,490^c$ (571,745)</td>
<td>5.20</td>
<td>27</td>
</tr>
</tbody>
</table>
Figure 4. Comparison of kinetic parameters between the 21 and 15 mm size class, high lipid level zebra mussels. For each compound, values with the same letter are not significantly different.
Figure 5. Log BCF vs log $K_{ow}$ for 21 and 15 mm, high lipid mussels and 21 mm low lipid mussels. (Log BCF = 0.62 Log $K_{ow}$ + 0.95, $r^2 = 0.94$, $p < 0.05$, 21 mm, high lipid) (Log BCF = 0.41 Log $K_{ow}$ + 2.02, $r^2 = 0.85$, $p < 0.05$, 21 mm, low lipid) (Log BCF = 0.49 Log $K_{ow}$ + 1.97, $r^2 = 0.99$, $p < 0.05$, 15 mm, high lipid).
0.3, $r^2 = 0.85$, 21 mm; $\log k_d = -0.29\log K_{ow} - 0.15, r^2 = 0.91, 15$ mm) and lipid levels ($\log k_d = -0.37\log K_{ow} + 0.3, r^2 = 0.85$, high lipids; and $\log k_d = -0.36\log K_{ow} + 0.27, r^2 = 0.72$, low lipids).

**DISCUSSION**

**Effect of Differences in Lipid Levels on Bioconcentration**

As in other organisms, the lipid reserve of bivalves is important in the accumulation and storage of hydrophobic contaminants (Hansen *et al.* 1978; Tanabe and Tatsukawa 1987). The relative size of the lipid pool affects the movement of lipophilic chemicals into the pool such that bivalves with higher lipid levels have faster uptake and slower elimination of lipophilic contaminants. This results in a greater total contaminant accumulation in the high lipid bivalves than in those with smaller lipid reserves (Stegeman and Teal 1973; Hansen *et al.* 1978). Consequently, the high lipid, pre-spawning zebra mussels were expected to have greater contaminant bioconcentration as measured by BCF and it was expected that their bioconcentration kinetics would follow the kinetics described above for other bivalves. As predicted, pre-spawning zebra mussels had significantly greater bioconcentration of the more lipophilic HCBP and BaP than post-spawning mussels. The high lipid mussels also exhibited faster uptake of these two compounds relative to the low lipid, post-spawning mussels. Lipid normalized BCFs values for HBCP and BaP were statistically identical for both the high and low lipid mussels suggesting that the differential accumulation between the two populations resulted from differences in
Contrary to expectations, HCBP and BaP elimination rate constants were not significantly different between high and low lipid mussels; uptake mechanisms thus appear to be driving accumulation in the high lipid animals. The lack of differences in elimination rate constants between the two populations argues against the lipid storage theory presented above. A more probable explanation is that the lipid pool in the mussels was not completely saturated by the contaminants, a condition that is necessary to see effects due to differences in size of lipid pools (Bickel 1984). The short exposure time, prior to elimination, insured that lipid saturation did not occur even in the post-spawning mussels. The difference in HCBP and BaP uptake clearance rates also could be due to differences in condition between the two populations rather than differences in the size of the lipid pool. Quigley et al. 1993 reported that the seasonal reduction in food availability caused a slowing of metabolic rates in zebra mussels collected in late summer from Lake St. Clair as compared to metabolic rates measured for mussels collected in the spring when food was plentiful. The post-spawning mussels used in the kinetic experiments were collected in late summer. It is possible that the feeding regime and length of the acclimation period prior to experimentation may not have been sufficient to allow the mussels to recovery from starvation. Hence, lower filtration rates due to suppressed metabolic could account for the reduced uptake of HCPB and BaP in the post-spawning population.

Differences in lipid levels between the two populations did not affect the
accumulation of the less lipophilic TCBP and pyrene. In addition, both TCBP and pyrene were accumulated to a lesser extent that the more lipid soluble HCBP and BaP. A number of studies have reported that low chlorinated biphenyls and smaller molecular weight PAHs are not retained in bivalves as readily as are the highly lipophilic HCBP and BaP and therefore are not as readily influenced by lipid content (Neely et al. 1974; Vreland 1974; Geyer et al. 1982; McDowell-Capuzzo et al. 1989). Hence, the physicochemical properties of TCBP and pyrene account for lack of significant difference in their accumulation kinetics and bioconcentration between the two populations.

Effect of Size on Bioconcentration

Field studies have shown that contaminant body burdens in mussels and body size are negatively correlated (Boyden 1974; Cossa et al. 1980; Muncaster et al. 1990; Fisher et al. 1993). Size-dependent contaminant bioconcentration is attributed to a link between metabolic rates and rates of uptake or loss of contaminant; smaller individuals compensate for higher metabolic demands by increasing their respiration (filtering) rates thereby increasing their exposure to water-borne contaminants (Boyden 1974; Muncaster et al. 1990). Contaminant uptake rates are reported to be greater in smaller individuals for a wide range of aquatic organisms (Anderson and Spear 1980; Newman and Mitz 1988) and for some invertebrates have been positively correlated with oxygen clearance rates (Landrum et al. 1992). In this study, contaminant uptake clearance rates were significantly higher for the 15 mm zebra
mussels for all compounds tested. There is a twofold increase in oxygen consumption for 15 mm vs 21 mm zebra mussels (Fisher et al. 1993) suggesting that contaminant uptake is linked to the greater oxygen demands of the 15 mm mussels. However, Fisher et al. (1993) reported that high filtration rates in zebra mussels reflect food requirements rather than oxygen demand. Since filtration rates in mussels are inversely related to size (Vahl 1973; Bayne and Widdows 1978), size-dependent uptake of water-borne contaminants in zebra mussels is more likely related to nutrient requirements than oxygen demands. Because, both size classes were pre-spawning mussels, the condition and lipid levels of the 15 and 21 mm size classes were similar (avg = 13.5 and 15.5 % dry wt respectively) and did not contribute to differences in uptake. The similarity in elimination rate constants between the two size classes supports the argument that size-dependent uptake of contaminants drives bioconcentration.

The implication for contaminant bioconcentration in field mussels is that smaller mussels will have greater accumulation of contaminants than larger mussels as evidenced by BCF values. Consequently, predators that prey on smaller individuals are more likely to get a larger dose of contaminant than if they ingested the same mass of larger zebra mussels. However, there is conflicting evidence that suggests that the elevated contaminant concentrations in small animals reported in many transfer studies reflects a short term contaminant exposure. In long term exposures, larger animals will have greater contaminant concentrations than smaller individuals despite their reduced contaminant uptake (Strong and Luoma 1981). Both size classes
of zebra mussels had significant contaminant accumulation from water as measured by BCFs. Therefore, the potential to bioconcentrate PCBs and PAHs in either size class will increase transfer of contaminants to predators.

Correlation of Compound Lipophilicity with Bioconcentration

The positive correlation between compound lipid solubility (log $K_{ow}$) and an organism’s BCF is highly predictive of lipophilic contaminant bioconcentration (Geyer et al. 1982, 1985; Hawker and Connell 1986). Significant correlations were found for the regression of log $K_{ow}$ with log $k_e$ and BCFs for both size classes of the pre-spawning mussels and the BCF values of the 21 mm post-spawning mussels. Similar positive correlation of PCB and PAH $K_{ow}$’s with uptake clearance rates or BCFs have been reported for zebra mussels (Fisher et al. 1993) and the blue mussel, Mytilus edulis (Pruell et al. 1986). The negative correlation of lipid solubility with elimination rate constants found in both the 21 and 15 mm size classes confirms similar results reported for the zebra mussel (Fisher et al. 1993) and for the green lipped mussel (Tanabe et al. 1987). The absence of a correlation with uptake clearance rates or elimination rate constants in the post-spawning zebra mussels may be due to their poor condition. Hence, compound lipid solubility appears to accurately predict accumulation of highly lipophilic contaminants such as PCBs and PAHs. However, the predictive value of $K_{ow}$ may be modified by physiological changes in condition due to reproductive state.

In sum, zebra mussels in both size classes and lipid levels significantly
accumulated lipophilic non-polar contaminants. If water-borne contaminants are a primary source of contaminant exposure then the large potential for zebra mussel contaminant bioconcentration may alter contaminant cycling in the Great Lakes by increasing contaminant transfer to mussel predators. In addition, selective predation on small, pre-spawning (high lipid) mussels may present a greater hazard to predators than predation on larger, post-spawning (low lipid) mussels. Finally, the lipid solubility of a compound appears to accurately predict zebra mussel accumulation of highly lipophilic contaminants such as PCBs and PAHs.
CHAPTER II

ACCUMULATION OF NONPOLAR, LIPOPHILIC CONTAMINANTS BY THE ZEBRA MUSSEL, \textit{(Dreissena polymorpha)}: ALGAL AND SUSPENDED SEDIMENT ROUTES OF EXPOSURE.

INTRODUCTION

Significant amounts of lipophilic contaminants such as polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs) are present in the water, sediment and biota of the Great Lakes ecosystems (Fitchko 1986). \textit{Dreissena polymorpha}, the zebra mussel, a recent European invader to the North American Great Lakes, has the potential to alter the cycling of these contaminants due to its high filtration rate, its large population numbers and its preference for littoral zones and out-falls that may have large contaminant loads (Griffiths \textit{et al.} 1989; O’Neil and MacNeil 1989). The impact on contaminant cycling can be two-fold: 1) a significant accumulation of contaminants in zebra mussel tissue; and 2) sedimentation of unassimilated contaminated material in the form of feces and pseudofeces. To adequately determine the zebra mussels’s impact on contaminant cycling through tissue accumulation of compounds, all possible routes of zebra mussel contaminant exposure must be investigated.
Uptake of any chemical is dependent on the bioavailability of that chemical to the organisms (Spacie and Hamelink 1985). Freely dissolved, nonpolar, lipophilic organic molecules can readily diffuse across the lipid bilayer of biological membranes in aquatic organisms (Zitko 1980; Spacie and Hamelink 1985). For filter-feeding bivalves such as the zebra mussel, freely dissolved, nonpolar, lipophilic contaminants are readily available for uptake across the gill membrane (Fisher et al. 1993). Contaminants associated with ingested material must desorb from that medium and then diffuse through the gut lining before being assimilated into the mussel tissue (Gobas et al. 1993; Klump et al. 1987). Therefore, the direct absorption of compounds from water is the fastest and possibly 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 low compared to contaminant concentrations in suspended solids and phytoplankton (Great Lakes Water Quality Board 1987; Swackhammer and Skoglund 1993). Thus, ingested material may present a route of contaminant exposure for zebra mussels which may be more biologically significant than contaminant uptake from water. This may be particularly true in waters with high particle loads (Langston 1978; Ekelund et al. 1987).

To determine the contribution of ingested material to zebra mussel contaminant accumulation, contaminant assimilation efficiencies (% of total contaminant exposure that is assimilated into tissue) from spiked algae and suspended sediment particles
were measured. Assimilation efficiencies of benzo(a)pyrene, the insecticide DDT and selected PCB congeners were compared between the two dietary components. Zebra mussel contaminant assimilation efficiencies determined in this study, coupled with other physiological and environmental parameters were used in a steady-state model to determine the relative importance of the algal, suspended sediment and water-borne routes of exposure to zebra mussel steady-state contaminant concentrations.

MATERIALS AND METHODS

Chemicals

\(^{14}\text{C}\)-labeled compounds were obtained from Sigma Chemical Company (St. Louis, Mo.). The specific activity for each chemical was: 2,2',4,4',5,5'-hexachlorobiphenyl (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: Mussels were collected from littoral zones of Lake Erie by SCUBA divers off-shore of Kelly’s Island, Ohio. Mussels recovered from the lake were scraped from rocks and transported in moist air to holding facilities at the Ohio
State University, Columbus, Ohio. Cultures were maintained in 200 L tanks filled with aged, aerated tap water at 20 ± 2 °C and fed 150 mL of a Tetra Min solution (10.5 g/L) per 3000 individuals. Water temperature, dissolved oxygen and ammonia were checked daily and water was replaced 1-2 times weekly. All waste water was treated with chlorine bleach (50 ppm) before disposal to prevent introducing mussels or veligers into inland waters. Zebra mussels were acclimated to laboratory conditions for at least 1 wk prior to experimentation and only conditioned mussels were used in experiments. Zebra mussels were considered suitable for use if, after severing of byssal threads, they reattached to a substrate within 48 h. Zebra mussels used in this study were 21 ± 1 mm in length.

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

Water: Soft (hardness 40-48 mg/L, and alkalinity 30-35 mg/l as CaCO₃), buffered, standard reference water (SRW) adjusted to pH 8 was used in all experiments (USEPA 1975). Because potassium-containing compounds can be toxic to zebra mussels (Fisher *et al.* 1991), Na₂HPO₄ was substituted for the recommended K₂HPO₄ buffer.
Sediment particles: Olentangy River sediment (organic content = 0.9 %) was sieved and then separated by suspension to yield particle sizes of \( \leq 20 \) um. PCB and PAH contaminant levels in the sediment were below detection limits (REALab, Wooster, Ohio).

Media Preparation

Algae (0.3 \( \times 10^7 \) cells/mL) suspended in 90 mL SRW were spiked with a radiolabeled chemical (4-8 \( \mu \)L) and gently agitated for 3 h. The algae were then centrifuged, the supernatant removed and the algal pellet rinsed with clean SRW. The algae were centrifuged and rinsed three times then resuspended in 80 mL of SRW. Equal volumes (20 mL) of the algae solution were added to each of 4-4 L aquaria.

SRW also was added to a volume of sediment slurry having sediment particles of diameter \( \leq 20 \) um so that the final volume was 90 mL SRW and particle concentration was approximately 2.0 \( \times 10^6 \) particles/mL. This slurry was spiked with the appropriate radiolabeled chemical (4-10 \( \mu \)L) 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 aquarium.

Contaminant Assimilation Efficiencies

Contaminant assimilation efficiencies were determined using a pulse-chase experimental protocol developed from methods of Luoma et al. (1992). For 2-3 days
prior to an experiment, zebra mussels attached to glass petri dishes were held in aquaria filled with SRW and fed low concentrations of noncontaminated suspended sediments or algae (approximately 0.3 x 10^3 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 3 replicate aquaria filled with low concentrations (0.3 to 0.1 x 10^5 particles/mL) of spiked algae or sediment particles suspended in 2 L of SRW adjusted to pH 8. The low particle concentration prevented the formation of pseudofeces. A fourth aquarium filled with spiked media (algae or sediment particles) but lacking mussels was used as a control. After a 2 h exposure, 4 mussels from each aquarium were processed for liquid scintillation counting (LSC) to determine the contaminant concentration in the animal (tissue and gut), C_0. The medium concentration, particle number, and mass in each aquarium were determined at the beginning and end of the exposure period (Appendix B). Feces produced during the experiment were collected and their contaminant concentration was determined.

The remaining mussels were rinsed with clean SRW, transferred to clean water and fed low concentrations of unlabeled algae or sediment particles (0.3 x 10^5 cells or particles/mL) in 2-3 pulses per day for 48 h to "chase" the unassimilated material from 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 at time 48 h 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 \( T_{48} \) (\( \mu g/Kg \) wet wt)

\( C_E \) = contaminant concentration assimilated by mussels (\( \mu g/KG \) 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 (Chapter 1; Fisher et al. 1993).

The amount of water-borne contaminant accumulated up by the experimental mussels 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 water-borne compound (\( C_w \)).

Percent assimilation efficiencies were determined using the following equation;

\[
\% \text{ Contaminant Assimilation Efficiency (AE)} = \left( \frac{C_E - C_w}{C_0 - C_w} \right) \times 100
\tag{2}
\]

where \( C_E \) = contaminant concentration assimilated into the mussel tissue (\( \mu g/Kg \) wet wt)

\( C_0 \) = contaminant concentration in mussels (tissue and gut) (\( \mu g/Kg \) wet wt)

\( C_w \) = contaminant load from water-born compound (\( \mu g/Kg \) wet wt).

In the calculation, it was assumed that \( C_0 \) represented the total contaminant exposure in the pulse period. However, in the suspended sediment experiments, the
amount of contaminant in feces produced during the exposure 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 error, the amount of contaminant in the feces
collected during the sediment exposure was added to $C_0$ concentration to determine
the total mussel exposure.

Analysis of variance (ANOVA, $p < 0.05$) was performed on assimilation
efficiencies and means were separated (Newman-Keuls, alpha = 0.05) using the
NCSS statistical program (NCSS 1991).

Feeding Efficiency

Zebra mussel % feeding efficiency of suspended sediments ($\leq 20$ um) was
determined for $21 \pm 1$ mm long, Lake Erie Western basin zebra mussels. The test
solution was prepared by adding a $\leq 20$ um size fraction of suspended sediment to 12
L of soft SRW ($20^\circ C$) to make a particle concentrations of $0.8 \times 10^5$ 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 ($20^\circ C$) 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:

\[
\% \text{ Feeding efficiency} = \frac{[\text{mg sediment cleared} - \text{mg pseudofeces}]}{\text{mg sediment cleared}} \times 100. \quad (3)
\]

RESULTS

Assimilation Efficiencies

Contaminant assimilation efficiencies from spiked algae ranged from 45 to
77 % (Table 4). Significant differences in assimilation efficiencies for the algal route
of exposure were found between DDT and the two PCB congeners and between BaP
and TCBP. With the exception of HCBP, contaminant assimilation efficiencies from
spiked algae increased with decreasing compound octanol:water partition coefficient
\((K_{ow})\). The correlation of contaminant assimilation efficiency with compound log \(K_{ow}\)
was negative \((\% \text{ AE} = 645.2 - 97.4 \log K_{ow}, r^2 = 0.52, p < 0.05, n = 4)\). This
relationship was improved by removing HCBP \% AE from the analysis \((\% \text{ AE} = \\
647.1 - 97.7 \log K_{ow}, r^2 = 0.74, p < 0.05, n = 3)\).

Contaminant assimilation efficiency from spiked sediment particles ranged
from 1.0 to 30 % and, except for TCBP, assimilation efficiencies from spiked
sediment were not significantly different from each other (Table 4). Contaminant
assimilation efficiencies were negatively correlated to log \(K_{ow}\) but this relationship was
weak \((\% \text{ AE} = 19.4 - 103.1 \log K_{ow}, r^2 = 0.56, p < 0.05, n = 4)\).
Table 4. Zebra mussel contaminant % assimilation efficiencies (% AE) from spiked algae and suspended sediment particles. The numbers in parenthesis are standard errors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% AE</th>
<th>Log K&lt;sub&gt;ow&lt;/sub&gt; *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algae</td>
<td>Sediment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCBP</td>
<td>68.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>29.6&lt;sup&gt;a&lt;/sup&gt; ** 6.90&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(2.9)</td>
<td>(7.6)</td>
</tr>
<tr>
<td>DDT</td>
<td>44.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.9&lt;sup&gt;a&lt;/sup&gt; ** 6.19&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(7.6)</td>
<td>(2.8)</td>
</tr>
<tr>
<td>BaP</td>
<td>53.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>20.6&lt;sup&gt;a&lt;/sup&gt; ** 5.98&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(6.2)</td>
<td>(3.8)</td>
</tr>
<tr>
<td>TCBP</td>
<td>77.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt; ** 5.90&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(5.5)</td>
<td>(0.5)</td>
</tr>
</tbody>
</table>

* 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 Choiu et al. (1977)
3 Miller and Wasik (1985)
When contaminant assimilation efficiencies for the same compound were compared between routes of exposure, assimilation efficiencies from contaminated algae were statistically greater than those determined from contaminated suspended sediment for all compounds (Fig. 6).

Contaminants introduced into zebra mussels from ingested sediment particles were rapidly cleared from the gut and by 24 h after ingestion, fecal elimination of contaminants had reached a plateau (Fig. 7). Elimination of contaminants ingested from spiked algae was much slower than that from ingested suspended sediment (Fig. 7) as fecal elimination of compound did not level off until 36 h after ingestion of the spiked algae.

Feeding Efficiencies

Zebra mussel feeding efficiency of suspended sediments (12 mg dry wt/L) was determined to be 46.5 %.

DISCUSSION

Nonpolar, lipophilic chemicals can be readily absorbed in the gastrointestinal tract of aquatic organisms via diffusion processes (Spacie and Hamelink 1985; Gobas et al. 1993). The assimilation efficiency of compounds associated with ingested material is dependent on the partitioning of the chemical between the ingested material and the organism (Klump et al. 1987). The greater the chemical affinity to the ingested material, the less likely the contaminant will desorb and become assimilated
Figure 6. Comparison of contaminant assimilation efficiencies from spiked algae and sediment. Error bars represent standard errors.
Figure 7. 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.
into the organism (Jahan-Parwar et al. 1990; Gobas et al. 1988; Muir and Yarechewski 1988).

The affinity of nonpolar, lipophilic compounds for the ingested material is affected by chemical and physical properties of the sorbent. Sorbent size and organic content have been two important parameters regulating sorption of neutral, nonpolar lipophilic compounds (Voice and Weber 1983; Knezovich et al. 1987). Small sized particles have a larger surface/volume ratio and therefore more sorption sites than larger sorbent factions (Karickhoff et al. 1979). In addition, a sorbent with a high organic content will have more binding sites for nonpolar, lipophilic compounds than sorbents with lower organic content (Knezovich et al. 1987). Therefore, large surface/volume ratios of small particles and high organic content of sorbents favor strong sorption of nonpolar lipophilic compounds. Based on the above, lipophilic chemicals would be expected to sorb more strongly to algae than the suspended sediment particles because 1) the algal cells have higher organic content than the suspended sediment particles (40 % vs 0.9 % respectively) and 2) a higher percentage of algae cells were smaller (6 um) than the sediment particles (< 20 um). Indeed, fresh water, unicellular algae do accumulate nonpolar, lipophilic compounds readily via sorption and absorption processes (Sodergren 1968; Autenrieth and DePinto 1991). In addition, when chemical sorptive ability was compared between unicellular algae and suspended sediments, (Autenrieth and DePinto 1991) or algae and glassware (Day and Kaushik 1987) the algae had higher sorption and slower desorption of organic chemicals than the sediment and greater sorptive ability than the glassware.
Based on assimilation efficiencies, contaminant assimilation from spiked algae was greater than from sediment suggesting greater contaminant sorption to suspended sediment particles than from the algae cells. In the experiments, most likely algal cells remained more sorptive than sediment particles as expected, but algae cells were more digestible than the sediment particles. In the zebra mussels gut, the digestive process broke apart algae cells disrupting cell membranes and hydrophobic bonds that held organic contaminants. The freed contaminants could then be assimilated across the gut membrane (Gobas et al. 1993). In addition, the algae were retained longer in the zebra mussel gut than the sediment particles, allowing more time for contaminant assimilation. Conversely, assimilation of contaminants from sediment was lower than from algae because compounds sorbed to the suspended sediment were not as strongly affected by the digestive process. Contaminants remained sorbed to the suspended sediment particles and quickly passed through the gut relative to the contaminated algae.

A negative correlation between compound log $K_{ow}$ and contaminant assimilation efficiencies is predictive of highly lipophilic compound assimilation in fish and marine molluscs (Means et al. 1980; Muir and Yarechewski 1988; Jahan-Parwar et al. 1990). In our study, assimilation efficiencies determined from spiked algae were negatively correlated with contaminant log $K_{ow}$. With the exception of HCBP, compounds were more readily assimilated as the lipid solubility (log $K_{ow}$) of the compound decreased. Why HCBP, with the higher 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.

The extremely low assimilation efficiency of TCBP measured for suspended sediments is due more to deficiencies in the experimental design (batch cultures) than to greater binding of TCBP to sediment. The dissolved fraction of TCBP in the water was much higher than for the other chemicals either because of rapid desorption of TCBP from the sediment particles or because unbound TCBP was introduced into the aquaria with the spiked sediment. Consequently, correcting for uptake from the dissolved fraction deflated TCBP assimilation efficiencies from spiked sediment.

Zebra mussel assimilation efficiencies of TCBP from spiked suspended sediments may be greater if the compound were tested in a flow-through situation.

Zebra mussel contaminant assimilation efficiencies for PCBs ranged from 69-78 % when fed spiked algae. These values were slightly lower than contaminant assimilation efficiencies determined from PCB spiked algae for the marine gastropod, *Aplysia californica* (84-94 %)(Jahan-Parwar et al. 1990). Species differences in assimilation efficiencies are due to differences in gut retention time, feeding behavior and size (Jahan-Panwar et al. 1990). The greater assimilation efficiencies for *Aplysia* could be due to food palatability or to the larger size of *Aplysia* as gut passage time in the two species were of similar length (24-48 h) (Jahan-Parwar et al. 1990). Zebra mussel assimilation efficiencies from suspended sediments were slightly lower than assimilation efficiencies measured for the deposit feeding clam, *Malcoma nasuta* (38 - 56 %)(Lee et al. 1990). In addition, zebra mussel contaminant assimilation
efficiencies from suspended sediment particles were lower than contaminant assimilation efficiencies determined from spiked sediments for mysids (53%) and but fell within the range reported for freshwater oligochaetes (36-15%) (Klump et al. 1987; Klump et al. 1991).

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 algae and suspended suspended sediment concentrations as well as differences in contaminant concentrations between algae and suspended sediment may alter the relative importance of these vectors. In addition, water-borne contaminants may contribute to the zebra mussel contaminant load.

To determine if the algal route of exposure is the primary route of dietary exposure for the Lake Erie zebra mussel and to determine the contribution of all routes of exposure on zebra mussel contaminant concentrations, a steady-state model was constructed to estimate the relative steady-state contaminant concentration of the zebra mussel from each route of contaminant exposure (equation 4-6).

\[
\frac{dC_a}{dt} = 0 = (k_u \times C_w) + (F_r \times \% \ AE \times FE \times C_p \times C_e) - (k_d \times C_a) \quad (4)
\]

\[
k_d \times C_a = (k_u \times C_w) + (F_r \times \% \ AE \times FE \times C_p \times C_e) \quad (5)
\]

\[
C_a = (k_u \times C_w) + (F_r \times \% \ AE \times FE \times C_p \times C_e) \quad (6)
\]

where \( C_a \) = contaminant concentration in the animal (\( \mu g/kg \) wet wt)

\( k_u \) = uptake clearance constant (\( mL \ g^{-1} h^{-1} \))
\[ C_w = \text{contaminant concentration in water (ng L}^{-1}) \]

\[ Fr = \text{filtering rate (mL g}^{-1} \text{ wet wt h}^{-1}) \]

\[ FE = \text{feeding efficiency (\%)} \]

\[ AE = \text{compound assimilation efficiency (\%)} \]

\[ C_p = \text{contaminant concentration of algae or suspended sediment (ng g}^{-1} \text{ dry wt)} \]

\[ C_e = \text{concentrations of algae or suspended sediment (ng dry wt/L)} \]

\[ k_d = \text{elimination rate constant (h}^{-1}) \]

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 individually for both algae and suspended sediments.

Literature and measured values for the model parameters are presented in Tables 5 and 6. Zebra mussel filtering rates of algae and feeding efficiencies of algae and suspended sediment of \(\leq 20 \) um were determined using Lake Erie, western basin zebra mussels (Tables 5 and 6) (this study, Berg et al. 1993). Contaminant clearance constants \((k_w)\) and elimination rate constants \((k_d)\) were predetermined from Lake Erie zebra mussel populations (Chapter 1; Fisher et al. 1993). The zebra mussel algal feeding rate \((1527 \text{ mL/g wet wt/h})\) was used in calculations for both algae and sediment routes of exposure because it was a conservative estimate of zebra mussel
Table 5. Parameters used in the steady-state model to determine the concentration and percent of zebra mussel body burden from contaminated suspended sediment.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_u$</th>
<th>$k_d$</th>
<th>$C_w$</th>
<th>$C_p$</th>
<th>$C_e$</th>
<th>% AE</th>
<th>Fr</th>
<th>% FE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mL g(^{-1}) h(^{-1})</td>
<td>ng/L</td>
<td>ng/g</td>
<td>ng/L</td>
<td>mL/mg</td>
<td>wet wt/h</td>
<td></td>
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</tr>
<tr>
<td>HCBP</td>
<td>895</td>
<td>0.005</td>
<td>0-0.5</td>
<td>25.3</td>
<td>11.1</td>
<td>29.6</td>
<td>1527</td>
<td>47</td>
</tr>
<tr>
<td>DDT</td>
<td>736</td>
<td>0.007</td>
<td>0-0.02</td>
<td>0.37</td>
<td>&quot;</td>
<td>22.9</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>BaP</td>
<td>759</td>
<td>0.009</td>
<td>0-0.3</td>
<td>1.0</td>
<td>&quot;</td>
<td>20.6</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>TCBP</td>
<td>423</td>
<td>0.016</td>
<td>0-0.5</td>
<td>29.4</td>
<td>&quot;</td>
<td>1.0</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

\(^{a}\) Chapter 1; Fisher et al. (1993)

\(^{b}\) Estimated from sediment concentrations of total PCB or DDT \(^{(1)}\) x % of congener in total PCB \(^{(1)}\) (TCBP =25 and HCBP = 12 % ) x % of congener or DDT in bottom sediments found in suspended sediments \(^{(2)}\) (TCBP = 48 %, HCBP = 46 %, DDT = 15.3 %).

\(^{c}\) Rowan and Rasmussen (1992)

\(^{d}\) Berg et al. (1993)

\(^{e}\) Baumann and Whittle 1988

\(^{1}\) Oliver and Bourbonniere (1985)

\(^{2}\) Oliver and Charlton (1984)
Table 6. Parameters used in the steady-state model to determine the concentration and percent of zebra mussel body burden from contaminated algae.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_u$</th>
<th>$k_d$</th>
<th>$C_w$</th>
<th>$C_p$</th>
<th>$C_e$</th>
<th>% AE</th>
<th>Fr</th>
<th>% FE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mL g$^{-1}$ h$^{-1}$</td>
<td>h$^{-1}$</td>
<td>ng/L</td>
<td>ng/g</td>
<td>ng/L</td>
<td>mL/mg</td>
<td>wet wt/h</td>
<td></td>
</tr>
<tr>
<td>HCBP</td>
<td>895 $^a$</td>
<td>0.005 $^a$</td>
<td>0 - 0.5</td>
<td>25.3 $^b$</td>
<td>0.2 $^c$</td>
<td>68.6</td>
<td>1527 $^d$</td>
<td>100 $^d$</td>
</tr>
<tr>
<td>DDT</td>
<td>736</td>
<td>0.007</td>
<td>0 - 0.02</td>
<td>0.37 $^b$</td>
<td>&quot;</td>
<td>44.9</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>BaP</td>
<td>759</td>
<td>0.009</td>
<td>0 - 0.3</td>
<td>1.0 $^c$</td>
<td>&quot;</td>
<td>53.0</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>TCBP</td>
<td>423</td>
<td>0.016</td>
<td>0 - 0.5</td>
<td>29.4 $^b$</td>
<td>&quot;</td>
<td>77.6</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

$^a$ Chapter 1; Fisher et al. (1993)

$^b$ Estimated from sediment concentrations of total PCB or DDT ($^1$) x % of congener in total PCB ($^1$) (TCBP =25 and HCBP=12 %) x % of congener or DDT in bottom sediments found in suspended sediments ($^2$) (TCBP = 48 %, HCBP = 46 %, DDT = 15.3 %).

$^c$ Makarewicz et al. (1989)

$^d$ Berg et al. 1993

$^e$ Baumann and Whittle (1988)

$^1$ Oliver and Bourbonniere (1985)

$^2$ Oliver and Charlton (1984)
environmental filtering rate (2952 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 5). Because field contaminant concentrations for phytoplankton in the Great Lakes were not available in the literature, the contaminant concentration of algae and the suspended sediment were assumed to be the same. In addition, phytoplankton concentrations of HCBP and TCBP were estimated from regressions of phytoplankton bioconcentration factors (BCFs) vs log $K_{ow}$ and water concentrations from Green Bay, Lake Michigan (Swackhammer and Skolung 1993; Swackhammer and Armstrong 1987). 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. The contaminant concentrations were corrected to reflect HCBP and TCPB water concentrations in western Lake Erie.

Zebra mussel steady-state contaminant concentrations were then determined using the estimated algal contaminant concentrations.

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 each class of
compound. The percent of the total zebra mussel steady-state contaminant concentration contributed by algae, suspended sediment or by water-borne 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 were not modeled. Reported Lake Erie water concentrations for each class of compound fell within the range of water concentrations modeled.

The results from the simulation in which contaminant concentrations in algae and sediment were assumed to be identical are presented in Table 7. When considering only the dietary routes of exposure, (contaminant water concentrations are zero), the algal route was the primary contributor to mussel contaminant body burden (54-97 %) as predicted by the assimilation efficiencies. At low water concentrations (detection limits) the algal route of exposure continued to be the more important route of dietary exposure. In addition, the total dietary exposure for the two PCBs contributed > than 75 % of the zebra mussel contaminant body burden while the dietary routes of exposure contributed < 50 % of DDT or BaP to the total contaminant body burden. The low contaminant concentrations of DDT and BaP in the food sources accounted for the lesser importance of dietary exposure. Finally, at high water contaminant concentrations, water was the primary route of contaminant exposure for all compounds. This is not surprising, since zebra mussels have very high contaminant clearance constants (k_d) from water (Table 4).

The estimated field phytoplankton contaminant concentrations for HCBP and TCBP (HCBP = 165 ng/g dry wt; TCBP = 46.3 ng/g dry wt) were modeled and the
Table 7. Model estimates of zebra mussel contaminant concentrations from algal, suspended sediment and water routes of exposure for three water concentrations (ng/L) and the percent contribution to the mussel body burden from each route of exposure. The concentration of algae and suspended sediments are identical.

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<tbody>
<tr>
<td></td>
<td>µg/Kg wet wt</td>
<td>µg/Kg wet wt</td>
<td>µg/kg wet wt</td>
<td>ng/L</td>
</tr>
<tr>
<td>HCBP</td>
<td>11.9 41.8</td>
<td>16.5 56.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>11.9 31.8</td>
<td>16.5 44.3</td>
<td>8.95</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>11.9 10.1</td>
<td>16.5 14.0</td>
<td>89.50</td>
<td>75.9</td>
</tr>
<tr>
<td>DDT</td>
<td>0.096 46.0</td>
<td>0.113 54.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>0.096 23.0</td>
<td>0.113 26.9</td>
<td>0.21</td>
<td>50.1</td>
</tr>
<tr>
<td></td>
<td>0.096 4.2</td>
<td>0.113 4.9</td>
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<td>90.0</td>
</tr>
<tr>
<td>BaP</td>
<td>0.18 39.1</td>
<td>0.28 60.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.18 6.1</td>
<td>0.28 9.5</td>
<td>2.50</td>
<td>84.4</td>
</tr>
<tr>
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<td>0.18 0.7</td>
<td>0.28 1.1</td>
<td>25.30</td>
<td>98.2</td>
</tr>
<tr>
<td>TCBP</td>
<td>0.15 2.4</td>
<td>6.79 97.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.15 2.0</td>
<td>6.79 80.3</td>
<td>1.32</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>0.15 0.8</td>
<td>6.79 31.0</td>
<td>13.20</td>
<td>68.2</td>
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</table>

Zebra Mussel Contaminant Concentration

Route of Exposure

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</thead>
<tbody>
<tr>
<td></td>
<td>µg/Kg wet wt</td>
<td>µg/Kg wet wt</td>
<td>µg/kg wet wt</td>
<td>ng/L</td>
</tr>
<tr>
<td>HCBP</td>
<td>11.9 41.8</td>
<td>16.5 56.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>11.9 31.8</td>
<td>16.5 44.3</td>
<td>8.95</td>
<td>23.9</td>
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<td>11.9 10.1</td>
<td>16.5 14.0</td>
<td>89.50</td>
<td>75.9</td>
</tr>
<tr>
<td>DDT</td>
<td>0.096 46.0</td>
<td>0.113 54.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.096 23.0</td>
<td>0.113 26.9</td>
<td>0.21</td>
<td>50.1</td>
</tr>
<tr>
<td></td>
<td>0.096 4.2</td>
<td>0.113 4.9</td>
<td>2.10</td>
<td>90.0</td>
</tr>
<tr>
<td>BaP</td>
<td>0.18 39.1</td>
<td>0.28 60.9</td>
<td>0</td>
<td>0</td>
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<td>0.28 9.5</td>
<td>2.50</td>
<td>84.4</td>
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<td>0.18 0.7</td>
<td>0.28 1.1</td>
<td>25.30</td>
<td>98.2</td>
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<tr>
<td>TCBP</td>
<td>0.15 2.4</td>
<td>6.79 97.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.15 2.0</td>
<td>6.79 80.3</td>
<td>1.32</td>
<td>17.7</td>
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<tr>
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<td>0.15 0.8</td>
<td>6.79 31.0</td>
<td>13.20</td>
<td>68.2</td>
</tr>
</tbody>
</table>
results are presented in Table 8. Because the contaminant concentrations in the algae were higher than those of the suspended sediments, the contribution of the algae to the dietary exposure increased for the two PCBs particularly for HCBP (56 vs 90 %). At low water concentrations, algae remained the primary route of exposure for the two PCB congeners (84 and 88 % for HCBP and TCBP respectively) while sediment and water contributed little to the total contaminant body burden. At high contaminant concentrations, water and algae were about equal in importance while contaminated suspended sediment contributed less than 6 % to the total body burden.

Steady-state contaminant body burdens determined by the model were compared to those reported for a nearshore, western Lake Erie mussel population (Kries et al. 1991). Contaminant concentrations in the field mussels were reported as total PCB (520 µg/Kg) and sum of DDT analogs (44 µg/Kg). Thus, the amount of DDT, HCBP and TCBP in the field mussels had to be estimated from the percent of the total each compound represented (HCBP = 12 %, TCBP = 25 % and DDT = 20 %). The contaminant concentrations of the in situ zebra mussels (DDT, 4.4 µg/Kg; TCBP, 125 µg/Kg), fell below the range of model estimates for mussel concentrations of DDT (0.21-2.3 µg/Kg) and TCBP (6.15-24.0) while the model value for mussel concentrations of HCBP (28-209 µg/Kg) was greater than that reported for the field mussels (HCBP, 62.4 µg/Kg). This may be because actual algal concentrations of HCBP in western Lake Erie are lower than the algae concentrations estimated from the Green Bay phytoplankton. Over all, our model generated a conservative estimate of contaminant steady-state concentrations in western Lake Erie mussels.
Table 8. Model estimates of zebra mussel contaminant concentrations from algal, suspended sediment and water routes of exposure for three water concentrations (ng/L) and the percent contribution to the mussel body burden from each route of exposure. The estimated, field contaminant concentration for algae was used.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sediment</th>
<th>Algae</th>
<th>Water</th>
</tr>
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<tr>
<td></td>
<td>Conc.</td>
<td>Conc.</td>
<td>Conc.</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>HCBP</td>
<td>11.9</td>
<td>107.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>9.9</td>
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<td>51.5</td>
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<td>TCBP</td>
<td>0.15</td>
<td>10.7</td>
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<td>1.4</td>
<td>98.6</td>
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<td></td>
<td>1.2</td>
<td>88.0</td>
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<td>0.15</td>
<td>10.7</td>
<td>0.05</td>
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<tr>
<td></td>
<td>0.6</td>
<td>44.5</td>
<td>0.50</td>
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</table>
The model results indicated that contaminant assimilation efficiencies can predict the primary route of dietary exposure when water-borne contaminant concentrations are negligible or near the compound detection limit. However, at high levels of water-borne contaminants the predictive ability was weakened. In addition, the predictive strength of the assimilation efficiencies was stronger at higher algae contaminant concentrations. Thus, the results of our model may be modified by variations in contaminant levels in the food or water source, as well as changes in food availability. 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; Berg et al. 1993), and contaminant assimilation efficiencies may change with fluctuations in food concentration or with the contaminant concentration of the ingested material. Hence, to make the model more representative of the western Lake Erie ecosystem, factors like those listed above must be incorporated into the model.

In sum, zebra mussel assimilation efficiency from algae was greater than from suspended sediments. Excluding HCBP, the contaminant lipid solubility (log \( K_{ow} \)) was predictive of assimilation efficiency from spiked algae but not of contaminant assimilation from suspended sediments. Predictions of the relative importance of the algal or suspended sediment route of dietary exposure using only contaminant assimilation efficiencies were correct when water-borne contaminant levels were near or below the compound detection limit. At low contaminant water concentrations, ingested material was the most important contaminant exposure route for western
Basin, Lake Erie mussel but water became the primary route of contaminant exposure when contaminant levels in this medium were high (ten times detection limit). Of the ingested material, contaminated algae is the primary dietary contributor to Lake Erie zebra mussels' steady-state contaminant body burden at all contaminant water concentrations. Finally, to improve predictions of western Lake Erie mussel contaminant accumulation, physiological and environmental variations in the model parameters must be incorporated into the model.
CHAPTER III
TROPHIC TRANSFER OF PCBS FROM ZEBRA MUSSEL FECES TO THE
BENTHIC INVERTEBRATE, GAMMARUS FASCIATUS.

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 in the western basin of Lake Erie (Hebert and Mackie 1989; Sea Grant 1991). Filtration of water, algae and suspended material contaminated with lipophilic compounds such as PCBs will increase the zebra mussels’ contact with lipophilic contaminants, particularly in littoral areas that have high contaminant loads. Further, the biodeposition of contaminated material in the form of pseudofeces and feces will increase the risk of foodchain transfer to benthic organisms associated with the zebra mussel colonies.

Studies of the effect of bivalve biodeposition on contaminant sedimentation are few, particularly for freshwater systems. Based on research of biodeposition by oysters and marine invertebrates, it is believed that filtration of fine particles from the water column may significantly influence the movement of contaminants associated
with this particle fraction (Reynoldson 1987). In addition, the fecal pellets and pseudofeces of four estuarine bivalve species contained metal concentrations higher than surface sediment concentrations (Brown 1986). These results suggest that zebra mussel biodeposition of contaminated material will cause enrichment of that material in the sediments. The movement of contaminated material to the benthos will be especially important in areas where zebra mussel populations are large (Negus 1966; Reynoldson 1987). Benthic detritivores that ingest or contact contaminated feces or pseudofeces will be at a higher than normal risk of contaminant exposure (Pinkney et al. 1985).

*Gammarus fasciatus*, a benthic amphipod, has been found in increasing numbers in zebra mussels colonies purportedly in response to the structural 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 body burdens compared to gammarids not living in zebra mussel colonies. In addition, compounds desorbed from contaminated detritus to the surrounding water may be absorbed across the gill surfaces of foraging gammarids. 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.

To assess the hazard that zebra mussel contaminant sedimentation may pose for *Gammarus fasciatus*, gammarid contaminant assimilation efficiencies from PCB
spiked zebra mussels feces were measured. In addition, gammarid bioconcentration of water-borne PCBs was determined using a kinetic mass-balance model. The experimental data were then coupled with physiological and environmental parameters to assess the relative contribution of water and food routes of exposure. The model also was used to measure contaminant transfer from algal detritus to the gammarid. Finally, the relative amounts of contaminants transferred to the gammarid from zebra mussel feces and from algal detritus were compared.

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'-hexachlorobiphenyl (HCBP, 20 mCi/mmol); 2,2',4,4'-tetrachlorobiphenyl (TCBP, 13.8 mCi/mmol). All chemicals were greater than 97% pure as determined by thin layer chromatography and radiometric analysis (Leversee et al. 1982).

Organisms

Gammarid: 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. Cultures were maintained in 55 L tanks filled with hard standard reference water (SRW) (USEPA 1975) at $17 \pm 2 ^{\circ}C$ 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 3 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.

Zebra mussels: Mussels were collected from littoral zones of Lake Erie by SCUBA divers off-shore of Kelly’s Island, Ohio. Mussels recovered from the lake were transported in moist air to holding facilities at the Ohio State University, Columbus, Ohio. Cultures were maintained as described in Chapter 1. Adult mussels (25-30 mm) were used for production of spiked feces.

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

Water: Hard (hardness 160-180 mg/L as CaCO₃, and alkalinity 110-120 mg/L as CaCO₃), buffered, standard reference water (SRW) adjusted to pH 8 (USEPA 1975) was used in all gammarid experiments. Because potassium-containing compounds can be toxic to zebra mussels (Fisher *et al.* 1991), Na₂HPO₄ was substituted for the recommended K₂HPO₄ buffer when preparing SRW used with zebra mussels.
Preparation of Contaminated Zebra Mussel Feces

Three to four days before an experiment, algae (approximately $0.3 \times 10^7$ cell/mL) suspended in 90 mL SRW were spiked with a radiolabeled chemical and gently agitated for 3 h. The algal cells were then centrifuged (4000 rpm), the supernatant removed and the algal pellet rinsed with clean SRW. The algal pellet was centrifuged and rinsed three times. The pellet was resuspended in 90 mL of clean SRW and equal volumes (30 mL) of the algae solution were added to each of 3, 4 L aquarium filled with 30 zebra mussels (25-30 mm) so that algae concentrations were approximately $0.3 \times 10^5$. No pseudofeces were produced at this low algal cell concentration. Zebra mussel feces were removed by pippette from the aquaria 2 to 3 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.

Preparation of Spiked Water for Bioconcentration Experiments

One day prior to initiation of an experiment, hard SRW (15 L) was spiked with radiolabeled compound dissolved in 0.04-6.5 mL of acetone and stirred for 1 h. After equilibration (4-5 h), the spiked water was divided among the 1-L, glass test containers (250 mL/container) and the foil-covered containers were placed overnight in a darkened environmental chamber (Forma Scientific N37422, Marietta, Ohio) at
20 °C. On the test day, the initial toxicant concentration was determined by taking 3-1 mL water samples for each container before placing gammarids into the test containers.

Contaminant Assimilation Efficiencies

Contaminant assimilation efficiencies (% AE) were determined using a pulse-chase experimental protocol developed from methods of Luoma et al. (1992). 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 ≥ 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 3 replicate, 2-L glass beakers filled with 1 L of hard SRW to which 0.04-0.05 g spiked zebra mussel feces had been added. A fourth beaker filled with 1 L of SRW and spiked feces but lacking gammarids was used as a control. Additional aliquots of the spiked feces were used to determined zebra mussel feces contaminant concentration and feces dry/wet ratio. After a 4-6 h exposure, 5 gammarids from each beaker were processed for liquid scintillation counting (LSC) to determine the contaminant concentration in the gammarid (tissue and gut), C₀. 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 (1000 mL dioxane, 500 g naphthalene, 5 g diphenyloxazole). The gammarid and water samples were analyzed by LSC using a Beckman LS 6000IC liquid scintillation system (14C 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 freely 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 clean 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 the 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 corrected for elimination of assimilated contaminant by the equation;

\[ \ln C_{24} = \ln C_{E} - k_{d}t. \]  

(1)

where \( C_{24} \) = contaminant concentration in gammarids at \( T_{24} \) (µg/Kg wet wt)

\( C_{E} \) = contaminant concentration assimilated by gammarids (µg/Kg wet wt)

\( k_{d} \) = contaminant elimination rate constant (h\(^{-1}\))
\[ t = \text{duration of the "chase period" (h)} \]

The amount of water-borne contaminant taken up by the experimental gammarids 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 2 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 \( C_w \).

Percent assimilation efficiencies were determined using the following equation:

% Contaminant Assimilation Efficiency \( \% \ AE \) =

\[
\left[ \frac{(C_E - C_w)}{(C_0 - C_w)} \right] \times 100 \tag{2}
\]

where \( C_E \) = contaminant concentration assimilated into gammarid body (\( \mu g/Kg \) wet wt)

\( C_0 \) = total contaminant concentration in gammarid (\( \mu g/Kg \) wet wt)

\( C_w \) = contaminant concentration from water-born compound (\( \mu g/Kg \) wet wt).

In this calculation, \( C_0 \) is assumed to be the total gammarid contaminant exposure received during the "pulse" period of the experiment.

Analysis of variance (ANOVA, \( p < 0.05 \)) was performed on assimilation efficiencies using the NCSS statistical program (NCSS 1991).

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 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 determined 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) =

\[
\frac{(T_0 \text{ mg dry wt mussel feces} - T_4 \text{ mg dry wt mussel feces})}{\text{g wet wt gammarid/4 h}}. \quad (3)
\]

Uptake Clearance Rates

Individual adult, male gammarids were exposed to contaminants in 1-L glass beakers containing 250 mL of spiked water. After 0.5, 1, 1.5, 2, 4, or 6 h of exposure, 5 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 sample of the acetone rinse (1 mL) was analyzed for radioactivity.
Elimination Experiment

50 adult, male gammarids were exposed for 6 h in 5 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 5 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 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 3-1 mL water samples at each of the sampling periods.

Kinetic Model

A mass-based model was used to estimate uptake contaminant clearance, \( k_u \) (Fisher et al. 1993). \( k_u \) describes the volume of water cleared of contaminant per mass of organism per time and has units of mL g\(^{-1}\) h\(^{-1}\). The uptake clearance constant is conditional with respect to the experimental parameters under which the \( k_u \) is measured. In this calculation, the organism is defined as wet tissue only. Contaminants are assumed to partition between the organism and the water such that;

\[
dQ_A/dt = k_w Q_w - k_s Q_s. \tag{4}
\]

Assuming mass balance in the system:

\[
A = Q_w + Q_s \tag{5}
\]
where \(Q_a\) = quantity of contaminant in the animal (\(\mu g\))

\[k_1 = \text{conditional uptake rate constant (h}^{-1}\text{)}\]

\(Q_w\) = quantity of contamination in the water (\(\mu g\))

\[k_d = \text{the conditional elimination rate constant (h}^{-1}\text{)}\]

\(A\) = total amount of compound in the system (\(\mu g\)).

The basic assumptions of the model are that the mass of compound does not change in the system and that 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 from the equation. The following simplified integrated initial rates equation resulted:

\[k_1 = (-\ln(1-Q_a/A))/t\]  \hspace{1cm} (6)

This conditional rate constant is a system dependent value and must be converted to a system independent clearance (\(k_a\)) by the following equation;

\[k_a = k_1 \text{ (volume of water/wet mass of tissue).} \]  \hspace{1cm} (7)

The elimination data were fit to a first order elimination model:

\[
\frac{dC_a}{dt} = k_0 C_a
\]  \hspace{1cm} (8)

Integration of equation 5 yields:

\[
\ln C_a = \ln C_{ao} - k_d t
\]  \hspace{1cm} (9)

where 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:

\[ \text{BCF} = \frac{k_a}{k_d}. \]  

(10)

Log transformed \( k_a \) 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 \( k_a \) 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

Assimilation Efficiencies

Gammarid gut clearance of TCBP and HCBP after exposure to contaminated zebra mussel feces in the "pulse" period of the experiment is illustrated in Figure 8. After 24 h, fecal elimination of HCBP and TCBP was slow and elimination of HCBP had reached its highest level. Based 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 assimilation efficiencies determined in the pulse-chase experiment are presented in Table 9. Assimilation efficiency of HCBP (89.4) was greater than for TCBP (79.0), however, the two values were not statistically different.
Figure 8. Cumulative compound in gammarid feces during the "chase" period of the assimilation experiment.
Table 9. Contaminant assimilation efficiencies (% AE) and kinetic parameters for uptake and elimination of compounds by *Gammarus fasciatus*. Numbers in parenthesis are standard errors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% AE</th>
<th>$k_u$</th>
<th>$k_d$</th>
<th>BCF</th>
<th>Log * $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCBP</td>
<td>89.4</td>
<td>779</td>
<td>0.004</td>
<td>194,848</td>
<td>6.90 1</td>
</tr>
<tr>
<td></td>
<td>(7.2)</td>
<td>(58)</td>
<td>(0.001)</td>
<td>(14,500)</td>
<td></td>
</tr>
<tr>
<td>TCBP</td>
<td>79.0</td>
<td>432</td>
<td>0.004</td>
<td>95,736</td>
<td>5.90 1</td>
</tr>
<tr>
<td></td>
<td>(14.0)</td>
<td>(64)</td>
<td>(0.001)</td>
<td>(16,777)</td>
<td></td>
</tr>
</tbody>
</table>

* Within a column, values with the same letter are not significantly different.
1 Shui and MacKay (1986)
Kinetic Experiments

Kinetic parameters determined for gammarids are presented in Table 9. Uptake clearance constants were significantly higher for HCBP (779 mL g\(^{-1}\) h\(^{-1}\)), the more lipophilic compound (log \(K_{ow}\) 6.9), than for TCBP (432 mL g\(^{-1}\) h\(^{-1}\))(log \(K_{ow}\) 5.9). Elimination rate constants, \((k_d)\) were identical for the two compounds while BCF values were significantly greater for HCBP (194,848 mL g\(^{-1}\)) than for TCBP (95,736 mL g\(^{-1}\)).

Feeding Experiment

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. Reported *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 population of *Gammarus pulex* (0.0042-0.0104 mg/mg animal/h) (Crane and Maltby 1991).

DISCUSSION

Assimilation Efficiencies.

Contaminant assimilation from food has been reported for a number of aquatic organisms, including fish, bivalves, and benthic invertebrates. Contaminant assimilation is affected by food digestibility, gut retention time size and feeding behavior of the aquatic organisms (Jahan-Parwar *et al.* 1990; Ekelund 1989). The
percent assimilation of PCBs from highly digestible food was greater than 50 % for the guppy (51-61 %) (Gobas et al. 1988) and the gastropod *Aplysia californica* (84-94 %) (Jahan-Parwar et al. 1990) and *Dreissena polymorpha* (68.8-77 %) (Chapter 3). PCB assimilation from less digestible sediment particles is considerably lower; mysids (53 %), the bivalve *Malcomna nasuta* (38 %) and freshwater oligochaetes (36-15 %) (Klump et al. 1987; Lee et al. 1990; Klump et al. 1991). The gammarid PCB assimilation efficiencies determined in this study (79 and 89.4 %) fall in the range of PCB assimilation efficiencies from highly digestible food listed above. PCB assimilation efficiencies for other gammarid species were not available for comparison. However, PCBs were taken up from contaminated food (fungus) by the marine *Gammarus tigrinus* and after 24 h of elimination in clean water, 64.3 % of the PCBs were retained in the gammarids (Pinkney et al. 1985). Thus, for gammarids living in zebra mussel colonies, ingestion of contaminated zebra mussel feces can be a significant source of PCB exposure.

The negative correlation of assimilation efficiencies with compound lipid solubility (octanol/water partition coefficient, $K_{ow}$) can be predictive of compound assimilation in fish and mussels (Means et al. 1980; Muir and Yarechewski 1988; Janhan-Parwar et al. 1990; Chapter 2). Gammarid assimilation efficiencies of TCBP and HCBP determined in this study 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 with log $K_{ow}$ and assimilation efficiencies can be determined.
The kinetics of gammarid uptake from water were characteristic of nonpolar, nonmetabolized, lipophilic compounds in invertebrates; compound uptake is rapid and elimination is slow resulting in significant bioconcentration factors (Spacie and Hamelink 1985). A direct comparison of this study’s BCF values to BCFs reported in the literature was not possible because kinetic methodologies used were different. However, the freshwater *Gammarus psuedolimnaeus* exposed 60 d to water-borne PCBs had significant bioconcentration factors 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 bioconcentration factors of 157,778 and 463,333, again depending on the water concentration (Lynch and Johnson 1982). Thus, our results support the finding that water-borne PCBS are readily bioconcentrated by freshwater amphipods such as *Gammarus fasciatus*.

A positive correlation between compound lipid solubility and BCF values has been demonstrated for a variety of nonpolar, lipophilic compounds including PCBs (Geyer *et al.* 1982,1985; Veith *et al.* 1980). Hence, the greater lipid solubility of HCBP over that of TCBP is the most likely cause for the significant greater HCBP uptake clearance constant and BCF value. Lipid solubility also has been negatively correlated with elimination rate constants (Fisher *et al.* 1993; Bruner *et al.* 1993). In this study, elimination of HCBP and TCBP was statistically identical although HCBP is more lipid soluble than TCBP. The elimination period used in this study could not be extended beyond 1 week due to the high incidence of gammarid molting and
mortality. Thus a longer elimination period may be necessary to illicite a lipid solubility effect on compound elimination.

Based on the kinetic data and assimilation efficiencies, gammarids can readily assimilate PCBs from contaminated food and absorb them from water. To determine the relative importance of each route of exposure, a steady-state model was constructed to measure gammarid steady-state contaminant concentrations for each of the exposure routes (equation 11-13).

\[
dC_a/dt = 0 = (k_u \times C_w) + (Fr \times \% AE \times C_f) - (k_d \times C_a) \\
k_u \times C_a = (k_u \times C_w) + (Fr \times \% AE \times C_f) \\
C_a = (k_u \times C_w) + (Fr \times \% AE \times C_f)
\]

where \( C_a \) = contaminant concentration in the animal (\( \mu g/kg \) wet wt)

\( k_u \) = uptake clearance rate (mL g\(^{-1}\) h\(^{-1}\))

\( C_w \) = contaminant concentration in water (ng L\(^{-1}\))

\( Fr \) = feeding rate (ng mg\(^{-1}\) wet wt h\(^{-1}\))

\( AE \) = compound assimilation efficiency (%)

\( C_f \) = contaminant concentration of food (mg g\(^{-1}\) dry wt)

\( k_d \) = elimination rate (h\(^{-1}\))

The basic assumptions of the model are; 1) there is a steady-state condition in which contaminant flux into the organism is equal to the contaminant flux our of the organism, 2) contaminant uptake is only from water and feces, 3) contaminant uptake and elimination rates will not vary and, 4) feeding rates and toxicant assimilation
efficiencies will not change with concentration or environmental conditions.

The model parameters are listed in Table 10. The gammarid feeding rate, PCB congener assimilation efficiencies, uptake clearance constants and elimination rate constants used in the model were determined in this study (Table 10). 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). Reported levels of PCBs in Lake Erie water samples were difficult to interpret because it was not always evident that suspended material had been removed from the water samples before analysis. In addition, water concentrations of individual PCB congeners were not available. Therefore, we varied the water concentrations of HCBP and TCBP from zero to ten times the detection limit for PCBs and then determined 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. The reported water concentrations for total PCBs in Lake Erie fell within the range of water concentrations modeled.

Gammarid steady-state contaminant concentrations from exposure to PCB contaminated water and zebra mussel feces are listed in Table 11. Gammarid steady-state HCBP concentrations were higher than those of TCBP. 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
Table 10. Parameters for the steady-state model used to determine gammarid contaminant concentration from zebra mussel feces route of exposure.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_u$ (mL g$^{-1}$ h$^{-1}$)</th>
<th>$k_d$ (h$^{-1}$)</th>
<th>$C_w$ (ng/L)</th>
<th>$C_f$ (mg/kg dry wt)</th>
<th>% AE</th>
<th>FR (mg dry wt/mg wet wt/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCBP</td>
<td>779</td>
<td>0.004</td>
<td>0 - 0.5</td>
<td>0.877</td>
<td>79.0</td>
<td>0.0026</td>
</tr>
<tr>
<td>TCBP</td>
<td>423</td>
<td>0.004</td>
<td>0 - 0.5</td>
<td>0.076</td>
<td>89.4</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Table 11. Model estimates of gammarid steady-state contaminant concentrations from fecal and water routes of exposure for two water concentrations (ng/L) and the percent contribution to the total gammarid body burden from each route of exposure.

<table>
<thead>
<tr>
<th>Gammarid Steady-State Contaminant Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>HCBP</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>TCBP</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
water concentrations, ingestion of contaminated zebra mussel feces contributed the greatest percentage of the total contaminant load. At high water concentrations (0.5 ng/L), HCBP contaminated feces was the primary source of HCBP in gammarids while TCBP spiked feces contributed only 42% to the total body burden.

The model also was used to generate gammarid steady-state concentrations from feeding on an algal detritus. 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)(Chapter 2). The gammarid steady-state concentration of HCBP and TCBP from these two sources are presented in Table 12. The steady-state concentrations of zebra mussel feces and the algal detritus are listed also.

Gammarid feeding on zebra mussel feces contaminated with either HCBP or TCBP would have larger steady-state concentrations than when gammarids fed on algal detritus. This was because of the higher contaminant concentration in the zebra mussel feces than in the algal detritus. When the gammarid PCB concentrations acquired from mussel feces or algal detritus were compared to the PCB concentrations in the two different food sources, gammarid PCB concentrations were 3-4 times greater than in the algal detritus (0.023-0.10 vs 0.007-0.03 mg/Kg wet wt) and 5 times greater than the PCB concentration in the zebra mussel feces (0.04-0.51 vs 0.008-0.09 mg/Kg wet wt).

Biomagnification is the process in which tissue contaminant concentrations increase systematically as the chemical moves through 2 or more trophic levels (Rand
Table 12. Steady-state concentrations of HCBP and TCBP in gammarids feeding on contaminated feces, for gammarids feeding on contaminated algae, for zebra mussel feces, and for algae detritus.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Steady State Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gammarid Feeding on Feces</td>
</tr>
<tr>
<td></td>
<td>mg/Kg wet wt</td>
</tr>
<tr>
<td>HCBP</td>
<td>0.51</td>
</tr>
<tr>
<td>TCBP</td>
<td>0.04</td>
</tr>
</tbody>
</table>
and Petrocelli 1985). The systematic increase in contaminants at each trophic level has been expressed by multiples of whole numbers ie 2x, 5x, 10x (Macek et al. 1979). Thus, the five fold increase of gammarid PCB levels over that of its food source suggests that PCB biomagnification is possible in the zebra mussel feces-gammarid-fish food chain. PCB biomagnification in a detrital-based food chain has not been examined. However, in the pelagic food chains of several Canadian lakes and in Lake Ontario, total PCBs and tetra and hexachlorobiphenyls were biomagnified (Oliver and Niimi 1988; Rasmussen et al. 1990) with the magnification process increasing with as the food chain lengthened (Rasmussen et al. 1990). Thus, PCB biomagnification in the detrital based food chain is possible. However, to conclusively determine if there is PCB biomagnification in detrital food chains, additional contaminant trophic transfers must be examined.

In sum, gammarid's contaminant assimilation efficiencies of both HCBP and TCBP were high (79 and 89.4 % respectively). There was no statistically difference between compound assimilation efficiencies and hence no significant correlation to compound lipid solubility. BCF values determined for both congeners were high and gammarid bioconcentration of HCBP from water was greater than for TCBP as measured by BCF values. Based on the results of the steady-state model, at low contaminant water concentrations (at compound detection limit) the dietary route of exposure was the primary source of PCB congeners. At high water contaminant concentrations (10 times the compound detection limit) dietary exposure contributed a smaller percent of the total body burden of TCBP than HCBP. Dietary exposure still
remained the primary contaminant source for HCBP. Gammarid steady-state body burdens were 5 times greater than contaminant levels in the zebra mussel feces suggesting a potential for PCB biomagnification in the zebra mussel detritus-gammarid-fish food chain.
CHAPTER IV
SUMMARY

The zebra mussel population in western Lake Erie continues to grow and expand into areas purportedly not suitable for mussel colonization (soft substrate). Fish and bird predation has not checked the mussel population growth (French and Bur 1993) and even if algal populations are reduced by mussel grazing, the continued stirring of the rich bottom sediments by frequent storms will provide enough food to maintain mussel populations (Garton pers. com.) Thus, the zebra mussel is destined to become an established and important species in the western Lake Erie ecosystem.

The zebra mussel’s impact on contaminant cycling in western Lake Erie will increase as mussel populations spread over more areas of the lake. Recently, there have been reports of reproductive failure in bald eagles feeding on Lake Erie fish and incidences of high levels of PCBs and reproductive failure in area herring gulls. These events have caused researchers to suspect PCB trophic transfer through zebra mussel food chains although substantive evidence for this accusation has not been produced. Therefore, any knowledge that will increase our understanding of zebra mussel contaminant accumulation and trophic transfer is extremely valuable. It is hoped that the results of this study, summarized below, will contribute to that data base.
CONTAMINANT ACCUMULATION

Bioconcentration

From this study's results, we now know that, regardless of size or reproductive status (lipid content), zebra mussels bioconcentrated lipophilic compounds such as PCBs and PAHs to extremely high levels. In addition, we can reasonable assume there will be seasonal and size variations in mussel bioconcentration for some compounds although the evidence for size-dependent bioconcentration could be strengthened by measuring bioconcentration in a more extended range of mussel size classes. Results from this study also indicated that short-term exposure methodology is an effective means of measuring zebra mussel contaminant bioconcentration kinetics. However, validation of this study's bioconcentration results in long term laboratory and field experiments is still needed.

Contaminant Assimilation from Dietary Sources

Zebra mussel assimilation of lipophilic contaminants from food varied with the compound and the food source. Zebra mussel contaminant assimilation efficiency (% AE) from a highly digestible food source was significantly higher than when these same compounds are sorbed to a less digestible suspended sediment. What has yet to be determined is how mussel contaminant assimilation will be affected by variations in food contaminant levels or food availability; two variables that will vary geographically or temporal within the western basin of Lake Erie.
Predictive Correlations

Based on results of this study, correlations between compound lipid solubility (log $K_{ow}$) can a useful predictor of contaminant accumulation in zebra mussels with some limitations. Correlation of compound log $K_{ow}$ with BCF values had some predictive value for zebra mussel bioconcentration of lipophilic compounds while regressions of log % AE with compound log $K_{ow}$ may be useful in predicting mussel assimilation of lipophilic compounds from highly palatable food sources. However, this latter correlation will not always be reliable as evidenced by the effect of HCBP assimilation on this relationship. More compounds must be tested to strengthen the predictive value of any the above correlations.

Relative Importance of Contaminant Exposure Routes

The relative importance of dietary and water-borne contaminant exposure to zebra mussel contaminant levels was successfully determined using a simple steady-state model. Dietary contaminant exposure was the primary contaminant source for mussels when water concentrations were below or at a compound’s detection limit. At this low contaminant water level, predictions of mussel contaminant accumulation based solely on assimilation efficiencies was valid. At high contaminant water concentrations, the water-borne contaminant exposure was the most important source of mussel contaminant exposure. Thus, both water or dietary exposure can contribute to mussel steady-state contaminant levels depending on contaminant concentrations in the environment.
Generally, the model underestimated mussel’s steady-state contaminant concentrations when compared to contaminant concentrations in field collected zebra mussels. Errors in estimating contaminant concentrations in environmental media may have reduced the model’s accuracy. In addition, zebra mussel contaminant assimilation efficiencies, feeding rates and efficiencies as well as kinetic parameters used in the model were estimated for a limited mussel size class (21 ± 1 mm) and could not possibly represent the actual accumulation processes for an entire mussel population. Thus, the model’s accuracy will be improved as additional information about zebra mussel contaminant accumulation becomes available and as the data base on environmental contaminant levels improves.

TROPHIC TRANSFER

Gammarids had significant bioconcentration of HCPB and TCBP and high assimilation efficiencies of these compounds from contaminated zebra mussel feces. Incorporation of this information into a steady-state model, produced estimates of gammarid steady-state PCB concentrations.

Figure 9 depicts the movement of HCBP and TCBP through a simplified lake ecosystem. The environmental PCB concentrations in the water, phytoplankton, suspended sediments and zebra mussel feces were taken from the steady-state models (Chapter 2 and 3) and except for the water concentrations, converted to mg of contaminant per Kg wet wt of the media. Steady-state PCB concentrations in gammarids and zebra mussels were estimated from the models and are expressed as
organisms wet wt (Chapter 2 and 3). I will use this figure to illustrate the trophic transfer results.

When gammarid steady-state PCB concentrations were compared to contaminant levels in the food source (zebra mussel feces), gammarid PCB concentrations were 4-5 times higher, implying biomagnification may take place in the feces-gammarid-fish-bird food chain (Figure 9). Comparison of the gammarid concentration of HCBP and TCBP to those in the zebra mussel, indicated that gammarids had 3-4 times more PCBs than the zebra mussel. Consequently, if a fish would ingest 1 g of gammarids it will be exposed to 3-4 times more PCBs than if it ingested 1 g of zebra mussel tissue. 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 zebra mussel detritus-gammarid-fish-bird food chain is expected to funnel more contaminants to higher trophic levels than the mussel-fish-bird food chain. Finally, the model estimate of the gammarid steady-state PCB concentrations is probably a conservative estimate since it did not include PCBs associated with mussel pseudofeces and dead mussel tissue. Once the contribution of these sources to gammarid PCB levels are determined, the biomagnification process in the detrital food chain may be enhanced.
Figure 9. HCBP (A) and TCBP (B) concentrations in various compartments of a lake ecosystem. PCB concentrations were estimated from environmental data and from steady-state models.
APPENDIX A

Data and Protocols Relative to Chapter I
Table 13. Regression equations and 95% confidence intervals calculated for comparison of elimination rates ($k_d$).

<table>
<thead>
<tr>
<th>Lipid Level</th>
<th>Size</th>
<th>Compound</th>
<th>Regression Equation</th>
<th>$r^2$</th>
<th>SE</th>
<th>95% Confidence Intervals</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>21</td>
<td>HCBP</td>
<td>$y = -0.005x + 5.6$</td>
<td>0.78</td>
<td>0.001</td>
<td>0.003-0.007</td>
<td>9</td>
</tr>
<tr>
<td>High</td>
<td>21</td>
<td>B(a)P</td>
<td>$y = -0.009x + 6.6$</td>
<td>0.70</td>
<td>0.001</td>
<td>0.007-0.011</td>
<td>9</td>
</tr>
<tr>
<td>High</td>
<td>21</td>
<td>TCBP</td>
<td>$y = -0.016x + 6.6$</td>
<td>0.92</td>
<td>0.002</td>
<td>0.012-0.020</td>
<td>10</td>
</tr>
<tr>
<td>High</td>
<td>21</td>
<td>Pyrene</td>
<td>$y = -0.021x + 5.2$</td>
<td>0.87</td>
<td>0.003</td>
<td>0.016-0.027</td>
<td>10</td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>HCBP</td>
<td>$y = -0.007x + 7.3$</td>
<td>0.87</td>
<td>0.001</td>
<td>0.005-0.009</td>
<td>9</td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>BaP</td>
<td>$y = -0.017x + 6.6$</td>
<td>0.94</td>
<td>0.002</td>
<td>0.014-0.021</td>
<td>9</td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>TCBP</td>
<td>$y = -0.014x + 5.8$</td>
<td>0.81</td>
<td>0.002</td>
<td>0.010-0.019</td>
<td>10</td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>Pyrene</td>
<td>$y = -0.021x + 4.8$</td>
<td>0.82</td>
<td>0.004</td>
<td>0.014-0.028</td>
<td>10</td>
</tr>
<tr>
<td>Low</td>
<td>21</td>
<td>HCBP</td>
<td>$y = -0.006x + 5.7$</td>
<td>0.76</td>
<td>0.001</td>
<td>0.004-0.008</td>
<td>12</td>
</tr>
<tr>
<td>Low</td>
<td>21</td>
<td>BaP</td>
<td>$y = -0.008x + 6.8$</td>
<td>0.88</td>
<td>0.001</td>
<td>0.006-0.010</td>
<td>12</td>
</tr>
<tr>
<td>Low</td>
<td>21</td>
<td>TCBP</td>
<td>$y = -0.021x + 5.1$</td>
<td>0.99</td>
<td>0.001</td>
<td>0.019-0.023</td>
<td>7</td>
</tr>
<tr>
<td>Low</td>
<td>21</td>
<td>Pyrene</td>
<td>$y = -0.024x + 5.3$</td>
<td>0.98</td>
<td>0.002</td>
<td>0.021-0.027</td>
<td>7</td>
</tr>
</tbody>
</table>
Table 14. Percent lipid by dry weight, % lipid by dry weight corrected for lipid and % lipid wet wt corrected for lipid for all zebra mussels used in the kinetic experiments.

<table>
<thead>
<tr>
<th>Size mm</th>
<th>Compound</th>
<th>Lipid Level</th>
<th>% Lipid Dry</th>
<th>% Lipid Dry Corrected</th>
<th>% Lipid Wet Corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>HCBP</td>
<td>High</td>
<td>13.6</td>
<td>15.9</td>
<td>1.8</td>
</tr>
<tr>
<td>21</td>
<td>BaP</td>
<td>High</td>
<td>13.6</td>
<td>15.9</td>
<td>1.8</td>
</tr>
<tr>
<td>21</td>
<td>TCBP</td>
<td>High</td>
<td>13.3</td>
<td>15.5</td>
<td>2.0</td>
</tr>
<tr>
<td>21</td>
<td>Pyrene</td>
<td>High</td>
<td>13.3</td>
<td>15.5</td>
<td>2.0</td>
</tr>
<tr>
<td>21</td>
<td>HCBP</td>
<td>Low</td>
<td>6.6</td>
<td>7.0</td>
<td>0.9</td>
</tr>
<tr>
<td>21</td>
<td>BaP</td>
<td>Low</td>
<td>6.6</td>
<td>7.0</td>
<td>0.9</td>
</tr>
<tr>
<td>21</td>
<td>TCBP</td>
<td>Low</td>
<td>5.1</td>
<td>5.4</td>
<td>0.73</td>
</tr>
<tr>
<td>21</td>
<td>Pyrene</td>
<td>Low</td>
<td>5.1</td>
<td>5.4</td>
<td>0.73</td>
</tr>
<tr>
<td>15</td>
<td>HCBP</td>
<td>High</td>
<td>11.7</td>
<td>12.2</td>
<td>1.7</td>
</tr>
<tr>
<td>15</td>
<td>BaP</td>
<td>High</td>
<td>11.7</td>
<td>12.2</td>
<td>1.7</td>
</tr>
<tr>
<td>15</td>
<td>TCBP</td>
<td>High</td>
<td>19.4</td>
<td>24.3</td>
<td>3.2</td>
</tr>
<tr>
<td>15</td>
<td>Pyrene</td>
<td>High</td>
<td>19.4</td>
<td>24.3</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Table 15. Comparison of kinetic parameters between two lipid levels for four compounds.

<table>
<thead>
<tr>
<th>LIPID LEVEL</th>
<th>COMPOUND</th>
<th>$k_u$</th>
<th>$k_d$</th>
<th>BCF</th>
<th>$K_{ow}$ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIGH</td>
<td>HCBP</td>
<td>895 a</td>
<td>0.005 a</td>
<td>173,370 a **</td>
<td>6.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(111)</td>
<td>(0.001)</td>
<td>(21,539)</td>
<td></td>
</tr>
<tr>
<td>LOW</td>
<td>HCBP</td>
<td>380 b</td>
<td>0.006 a</td>
<td>63,304 b</td>
<td>6.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(69)</td>
<td>(0.001)</td>
<td>(11,551)</td>
<td></td>
</tr>
<tr>
<td>HIGH</td>
<td>B(a)P</td>
<td>759 a</td>
<td>0.009 a</td>
<td>51,600 a</td>
<td>5.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(91)</td>
<td>(0.0006)</td>
<td>(6,287)</td>
<td></td>
</tr>
<tr>
<td>LOW</td>
<td>B(a)P</td>
<td>331 b</td>
<td>0.008 a</td>
<td>41,239 a</td>
<td>5.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(68)</td>
<td>(0.001)</td>
<td>(8,485)</td>
<td></td>
</tr>
<tr>
<td>HIGH</td>
<td>TCBP</td>
<td>423 a</td>
<td>0.016 a</td>
<td>30,141 a</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(43)</td>
<td>(0.002)</td>
<td>(16,728)</td>
<td></td>
</tr>
<tr>
<td>LOW</td>
<td>TCBP</td>
<td>443 a</td>
<td>0.021 a</td>
<td>20,265 a</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(52)</td>
<td>(0.001)</td>
<td>(10,367)</td>
<td></td>
</tr>
<tr>
<td>HIGH</td>
<td>PYRENE</td>
<td>351 a</td>
<td>0.021 a</td>
<td>16,028 a</td>
<td>5.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(34)</td>
<td>(0.003)</td>
<td>(1,561)</td>
<td></td>
</tr>
<tr>
<td>LOW</td>
<td>PYRENE</td>
<td>323 a</td>
<td>0.024 a</td>
<td>12,956 a</td>
<td>5.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(34)</td>
<td>(0.002)</td>
<td>(1,307)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parenthesis are standard errors.

** In each pair, values with the same letter are not significantly different, p < 0.05.
Table 16. Comparison of kinetic parameters for two size classes of high lipid zebra mussels.

<table>
<thead>
<tr>
<th>SIZE mm</th>
<th>COMPOUND</th>
<th>$k_u$ mL/g/h</th>
<th>$k_d$ h⁻¹</th>
<th>BCF mL/g</th>
<th>$K_{ow}$ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>HCBP</td>
<td>895 * (111)</td>
<td>0.005 * (0.001)</td>
<td>174,620 * ** (21,208)</td>
<td>6.93</td>
</tr>
<tr>
<td>15</td>
<td>HCBP</td>
<td>1,608 b (210)</td>
<td>0.007 * (0.001)</td>
<td>216,101 * b (31,091)</td>
<td>6.93</td>
</tr>
<tr>
<td>21</td>
<td>B(a)P</td>
<td>735 * (89)</td>
<td>0.009 * (0.001)</td>
<td>51,678 * (6,269)</td>
<td>5.98</td>
</tr>
<tr>
<td>15</td>
<td>B(a)P</td>
<td>1,259 b (185)</td>
<td>0.017 b (0.002)</td>
<td>84,490 b (12,393)</td>
<td>5.98</td>
</tr>
<tr>
<td>21</td>
<td>TCBP</td>
<td>423 * (83)</td>
<td>0.016 * (0.002)</td>
<td>30,141 * (16,728)</td>
<td>5.95</td>
</tr>
<tr>
<td>15</td>
<td>TCBP</td>
<td>1,070 b (123)</td>
<td>0.014 * (0.002)</td>
<td>74,720 b (9,182)</td>
<td>5.95</td>
</tr>
<tr>
<td>21</td>
<td>PYRENE</td>
<td>350 * (34)</td>
<td>0.021 * (0.003)</td>
<td>16,028 * (1,561)</td>
<td>5.20</td>
</tr>
<tr>
<td>15</td>
<td>PYRENE</td>
<td>659 b (72)</td>
<td>0.021 * (0.004)</td>
<td>32,024 b (3,514)</td>
<td>5.20</td>
</tr>
</tbody>
</table>

* Numbers in parenthesis are standard errors.

** In each pair, values with the same letter are not significantly different, p < 0.05.
Table 17. $k_u$, $k_d$, BCF and Log $k_u$, $k_d$ and BCF values used in regression analysis.

<table>
<thead>
<tr>
<th>Size Comp.</th>
<th>$k_u$</th>
<th>$k_d$</th>
<th>BCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td>high</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>21 HCBP</td>
<td>895</td>
<td>380</td>
<td>2.58</td>
</tr>
<tr>
<td>BaP</td>
<td>759</td>
<td>331</td>
<td>2.52</td>
</tr>
<tr>
<td>TCBP</td>
<td>423</td>
<td>443</td>
<td>2.65</td>
</tr>
<tr>
<td>PYRENE</td>
<td>352</td>
<td>323</td>
<td>2.51</td>
</tr>
<tr>
<td>15 HCBP</td>
<td>1683</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>BaP</td>
<td>1305</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TCBP</td>
<td>989</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>PYRENE</td>
<td>640</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 18. Results of regression analysis of Log $K_{ow}$ vs $k_u$, $k_d$, or BCF for two lipid levels and two size classes of mussels.

<table>
<thead>
<tr>
<th>Lipid Level</th>
<th>Size mm</th>
<th>Kinetic Parameters</th>
<th>Regression Equation</th>
<th>$r^2$</th>
<th>SE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>High 21</td>
<td>$k_u$</td>
<td>$y = 327.7x - 1357$</td>
<td>0.77</td>
<td>128</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_d$</td>
<td>$y = -.010x + 0.70$</td>
<td>0.86</td>
<td>0.003</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>$y = 96383.2x - 510245$</td>
<td>0.87</td>
<td>26775</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Low 21</td>
<td>$k_u$</td>
<td>$y = 27.4x + 204.7$</td>
<td>0.12</td>
<td>53</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_d$</td>
<td>$y = -0.0107x + 0.77$</td>
<td>0.44</td>
<td>0.0086</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>$y = 30371x - 147170$</td>
<td>0.88</td>
<td>7965</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>High 15</td>
<td>$k_u$</td>
<td>$y = 616.9x - 2543.8$</td>
<td>0.94</td>
<td>115</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_d$</td>
<td>$y = -0.0081x + 0.64$</td>
<td>0.93</td>
<td>0.0016</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BCF</td>
<td>$y = 124425.4x - 640079$</td>
<td>0.90</td>
<td>29480</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
Table 19. Results of regression analysis of Log $K_{ow}$ vs Log $k_u,k_d$, or BCF for two lipid levels and two size classes of mussels.

<table>
<thead>
<tr>
<th>Lipid Level</th>
<th>Size</th>
<th>Kinetic Parameters</th>
<th>Regression Equation</th>
<th>$r^2$</th>
<th>SE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>21</td>
<td>$k_u$</td>
<td>$y = 0.0239x - 1.31$</td>
<td>0.75</td>
<td>0.973</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$k_d$</td>
<td>$y = -0.372x + 0.26$</td>
<td>0.85</td>
<td>0.111</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCF</td>
<td>$y = 0.618x + 0.95$</td>
<td>0.94</td>
<td>0.108</td>
<td>4</td>
</tr>
<tr>
<td>Low</td>
<td>21</td>
<td>$k_u$</td>
<td>$y = 0.034x + 2.36$</td>
<td>0.13</td>
<td>0.061</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$k_d$</td>
<td>$y = -0.363x + 0.27$</td>
<td>0.72</td>
<td>0.162</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCF</td>
<td>$y = 0.408x + 2.02$</td>
<td>0.85</td>
<td>0.021</td>
<td>4</td>
</tr>
<tr>
<td>High</td>
<td>15</td>
<td>$k_u$</td>
<td>$y = 0.245x + 1.57$</td>
<td>0.90</td>
<td>0.571</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$k_d$</td>
<td>$y = -0.285x - 0.15$</td>
<td>0.91</td>
<td>0.063</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCF</td>
<td>$y = 0.491x + 1.97$</td>
<td>0.99</td>
<td>0.024</td>
<td>4</td>
</tr>
</tbody>
</table>
LIPID ANALYSIS - PROTOCOL

One day before analysis

1. Sort zebra mussels into size classes and record length. Remove tissue from mussel, blot dry and determine weight wet for 40 mussels in each size class.

2. Place the viscera from 20 mussels of a size class in separate test tubes, squash tissue with hand held homogenizer and extract in 6 ml (large zebra mussels) or 3 ml (small zebra mussels) of chloroform/methanol 2/1.

3. Cover the tubes with aluminum foil (wrap tightly to avoid evaporation of the extract solvent) and refrigerate overnight or until tissues float to the top of the test tubes.

4. The 20 remaining mussel viscera should be placed in a oven (50-60 °C) for 24 hr and then desiccate for 24 h. The dry weight should be recorded and a dry to wet ratio determined.

5. After extraction overnight, a 0.5 ml sample of each mussel extract should be analyzed for total lipid using the colorometric method of Handel (1985).

CALCULATIONS

Determine a regression equation from the standard curve. Solve regression equation from standard curve for "x" by substituting the absorbance value from each mussel for "y". Multiply this result by 12 (large zebra mussels extracted in 6 ml of solvent) or 6 (small zebra mussels extracted in 3 ml of solvent) to arrive at total μg of lipid per mussel.

% lipid dry wt = (μg lipids/ wet wt of mussel x dry/wet ratio) x 100

One Week Before Experiment

1. Place glass slides and petri dishes in aquaria to acquire bacterial "slime".
2. Determine lipid level of mussels to be tested.
3. Make buffer solution to be used with SRW (1 M Na₂HPO₄).

Four Days Before Experiment

1. Plate mussels, dorsal side down, on slides and petri dishes (2 mussels/slide) or 15 mussels per petri dish).
2. Use standard reference water adjusted to pH 8 and aerate aquaria.
3. Place aquaria with mussels in incubator at test conditions (correct temperature and no light).
4. Feed mussels daily (10 mL TetraMin solution/aquaria).
5. Prepare scintillation vials, make scintillation cocktail.
6. Prepare filter paper to absorb internal water (for 20 mm zebra mussels strip is 2.7 x 10.7 cm; for 15 mm mussels strip is 2.7 x 7.9 cm).
7. Prepare data sheets (weights and length for uptake and elimination tests, elimination record sheet) and filtration record sheets (for both uptake and elimination tests).

Two Days Before Experiment

1. Prepare standard reference water (15 L per uptake test, 30 L total) and place in incubator to bring water to test conditions. Aerate water.
One Day Before Experiment

1. Adjust pH of SRW (15 L per uptake test, 30 L total) and then spike water (compound in acetone carrier) and spin 1 h. Equilibrate 4-5 h and then pour into test containers. (500 mL/ beaker x 30 beakers; 3 L per aquaria x 5 aquaria). Place test containers covered with aluminum foil in the incubator over night.

2. Prepare 40 L of SRW, adjust pH and pour into depuration chambers. Place these containers in the incubator overnight.

Test day

Uptake Experiment

1. Take T<sub>0</sub> water samples from the uptake test beakers; check water temperature.

2. Check mussels in plating aquaria for filtering. Any mussels not filtering, discard. Remove slides with mussels attached from the plating aquaria. If necessary, remove extra mussels from each slide so that there is only one mussel per slide. Rinse the slide and mussel thoroughly and place on paper towel until enough mussels are recovered from the plating aquaria to fill all test beakers.

3. Gently place one slide in each beaker so that mussel is upright.

4. Record filtering behavior of the mussels after a 5 minute acclimation period, every 5 min for the first 15 min and every 0.5 h thereafter.

5. Remove 5 test beakers from the incubator after 0.5, 1.0, 1.5, 2.0, 4.0 and 6.0 h of filtering.

At each sampling time:

1. Take 1 mL water samples from each beaker.
2. Remove zebra mussels from the glass slide and rinse thoroughly in clean DI.

3. Record the total length (mm), total weight (g), collect internal water on folded filter paper strip and place strip in scintillation vial.

4. Remove viscera from the shell, rinse, blot dry and weigh. Rinse valves, blot dry and weigh. Place viscera and valves in separate scintillation vials.

5. Dump water from test beaker into waste container. Treat waste water with chlorine bleach and dump into hot sink after 24 h.

6. Rinse empty beaker with 30 mL of DI, then with 20 mL of acetone and take a 1 mL sample of the acetone wash.

7. Fill all vials with 5 mL of scintillation cocktail and cover with aluminum foil overnight. Count each sample for 5 min in Beckman LS6000IC liquid scintillation counter.

Elimination Experiment

1. Prepare water as for uptake experiments and pour 3 L into 5, 4 L aquaria.

2. Take T₀ water samples (1 mL) from the aquaria.

3. Observe filtering of the mussels attached to petri dishes in the plating aquaria and remove any mussels not filtering.

3. Rinse off zebra mussels attached to petri dishes and place in 5 replicate aquaria. Monitor filtering every 0.5 h.

4. After 6 h remove zebra mussels that have not filtered over the length of the experiment.

5. Take T₆ water samples (1 mL) from the uptake aquaria.
6. Take $T_0$ zebra mussels from each aquaria and then rinse the remaining zebra mussels and petri dishes thoroughly with DI and place into elimination chambers filled with clean SRW.

7. At each sampling time, take a 1 mL water sample, record the water temperature and pH, and remove 1 zebra mussel from each aquaria. Feed the remaining mussels 10 mL of TetraMin solution and change water 2 h later. Process zebra mussels the same way as in uptake experiments.

9. Discard the water into waste containers and treat with chlorine bleach.

10. Place 40 L of SRW into incubator and aerate for use the next day.

11. Continue taking daily samples up to 7 days.

Calculations

Water samples

$$\text{DPM to PPM} = \text{DPM} \times 1000 / \text{SA of compound}$$

$$\mu g = \text{PPB} \times \text{water volume in liters (0.5 or 3.0)}$$

Valves and Guts

$$\text{DPM to PPM} = \frac{\text{DPM}}{\text{weight (kg)}} / \text{SA of compound}$$

$$\mu g = \text{PPB} \times \text{weight (kg)}$$

Wash

$$\text{DPM to PPM} = \text{DPM} \times 1000 / \text{SA of compound}$$

$$\mu g = \text{PPB} \times \text{volume of acetone wash (0.02 L)}$$
**IH**

mL of IH = Total weight - (gut weight + valve weight)/ by temperature correction factor (for 20 °C this = 0.997187)

DPM to IH PPM = (DPM/mL IH) x 1000 / SA of compound

PPB = PPM x 1000

\( \mu g = \text{PPB} \times (\text{mL IH}/1000) \)

**Kinetic Parameter**

\( k_t = -\ln \left(1 - \frac{\text{quantity of compound (} \mu g \text{) in mussel}}{\text{total quantity compound (} \mu g \text{) in } T_0 \text{ water}} \right) / t \)

\( k_d = k_t \times \text{vol of H}_2\text{O/mass (} g \text{) of mussel tissue} \)

\( k_d = \text{slope of regression of } \ln \text{ contaminant concentration (ppb) of mussel tissue vs time (h).} \)

**BCF =** \( k_d / k_t \)

**Mass Balance Calculations**

\( \% \text{ change} = \left( \frac{\mu g \text{ start (} T_0 \mu g \text{ H}_2\text{O} + \mu g \text{ wash}) - \mu g \text{ guts} + \mu g \text{ H}_2\text{O} + \mu g \text{ valves} + \mu g \text{ IH}}{\mu g \text{ start}} \right) \times 100 \)

\( \% \text{ Valves} = \left( \frac{\mu g \text{ valves}}{\mu g \text{ start}} \right) \times 100 \)

\( \% \text{ IH} = \left( \frac{\mu g \text{ IH}}{\mu g \text{ start}} \right) \times 100 \)

\( \% \text{ Wash} = \left( \frac{\mu g \text{ wash}}{\mu g \text{ start}} \right) \times 100 \)

If \% change is > than 30 \% do not use this sample. Check average wash at each time interval to see if there is an increase in concentration with time. If there is a trend but \% wash is small, loss to glassware is not to be included in the model.
APPENDIX B

Data and Protocols Relative to Chapter II
Table 20. Media particle or cells concentration (particles/mL), by weight (μg particles or algae/mL), and water contaminant concentration (μg/mL) at T₀ and T₂ for algae and suspended sediment pulse-chase experiments. DPM's of feces at T₂ is also listed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cells/mL x 10⁵</th>
<th>T₀ μg/mL</th>
<th>μg/L</th>
<th>T₂ μg/mL</th>
<th>μg/L</th>
<th>Feces DPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCBP</td>
<td>0.21 (0.01)</td>
<td>0.76 (0.07)</td>
<td>0.10 (0.005)</td>
<td>0.09 (0.04)</td>
<td>0.32 (0.07)</td>
<td>0.04 (0.002)</td>
</tr>
<tr>
<td>DDT</td>
<td>0.10 (0.004)</td>
<td>0.38 (0.01)</td>
<td>0.22 (0.01)</td>
<td>0.01 (0.004)</td>
<td>0.10 (0.07)</td>
<td>0.07 (0.004)</td>
</tr>
<tr>
<td>BaP</td>
<td>0.18 (0.03)</td>
<td>0.68 (0.01)</td>
<td>0.05 (0.003)</td>
<td>0.02 (0.02)</td>
<td>0.10 (0.02)</td>
<td>0.01 (0.0001)</td>
</tr>
<tr>
<td>TCBP</td>
<td>0.12 (0.02)</td>
<td>0.44 (0.06)</td>
<td>0.18 (0.01)</td>
<td>0.02 (0.003)</td>
<td>0.07 (0.01)</td>
<td>0.05 (0.01)</td>
</tr>
<tr>
<td>Suspended Sediments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCBP</td>
<td>0.11 (0.03)</td>
<td>7.9 (1.7)</td>
<td>0.21 (0.01)</td>
<td>0.02 (0.01)</td>
<td>4.2 (2.02)</td>
<td>0.08 (0.02)</td>
</tr>
<tr>
<td>DDT</td>
<td>0.07 (0.01)</td>
<td>3.3 (0.4)</td>
<td>0.26 (0.01)</td>
<td>0.02 (0.01)</td>
<td>2.5 (0.01)</td>
<td>0.12 (0.02)</td>
</tr>
<tr>
<td>BaP</td>
<td>0.20 (0.04)</td>
<td>5.4 (1.5)</td>
<td>0.07 (0.002)</td>
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<td>0.26 (0.02)</td>
<td>0.01 (0.003)</td>
<td>1.9 (0.5)</td>
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Table 21. Compound mass-balance for pulse-chase experiments used to determine zebra mussel contaminant assimilation efficiencies from spiked algae and suspended sediments.

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<th>Compound</th>
<th>$T_0$ Water</th>
<th>$T_2$ Water</th>
<th>Tissue</th>
<th>Shell</th>
<th>Internal Water</th>
<th>Feces</th>
<th>Settled</th>
<th>% Mean</th>
<th>Total</th>
<th>% Total</th>
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Protocol for Pulse-Chase Experiment - Sediment and Algae

Two or 3 Days Before Experiment

1. Place similar sized zebra mussels in aquarium of soft SRW (pH 8) and feed a low concentration of suspended sediment particles. (See below for methods to make sediment suspension).

2. Dry 30, numbered filter papers (25 mm dia., 0.45 um cellulose nitrate filters) in oven for 24 h.

One day Before Experiment

1. Place numbered filter papers into desiccator to cool and then weigh.

2. (Sediment) Place approximately 10-15 g of fine sediment (< 45 um) into a 1000 mL graduated cylinder. Add 1 L of soft SRW, stir and allow to settle for 1 h. Remove 90 mL of the suspension from the top of the cylinder (< 20 um fraction) and place in a beaker. Spike sediment suspension with radiolabeled compound (400,000 DPM) and gently agitate the beaker in a darkened incubator overnight at experimental conditions.

Experimental Day

1. (Sediment) Place all of the spiked sediment suspension into 6, 15 mL centrifuge tubes and spin for 15 min. to separate sediment particles from the contaminated water. Pour off supernatant and rinse sediment with clean water. Repeat the spin and rinse cycle 3 times. Resuspend the spiked sediment particles in 80 mL of SRW and place 20 mL into each of four aquaria filled with 2 L of SRW (particle concentration should be 0.2-0.1 x 10^5 particles/mL). Stir the water and then take 3, 4
mL water samples to determine the $T_0$ total contaminant concentration in the aquaria. Remove 100 mL of the water from each aquarium to determine particle concentration (Count 3, 2 mL sample of the water in a Coulter Counter), mass of the sediment per volume of water (filter 80 mL of water onto pre-weighed filter paper), concentration of contaminant in the sediment (analyze dried sediment on filter papers by LSC) and the concentration of compound desorbed from particles that are now in the water (centrifuge 15 mL/aquaria for 0.5 h and count 3, 4 mL aliquots of the supernatant).

(Algae) Spin algae from culture (cell concentration = $6.7 \times 10^6$ cells/mL) and resuspend in 90 mL of soft, SRW. Spike with labeled compound and gently agitate in darkened environmental chamber for 3 h. After 3 h, place spiked algae into 6, 15 mL centrifuge tubes and spin for 30 min. Pour off supernatant and rinse algae with clean water. Repeat the spin and rinse cycle 3 times. Resuspend the rinsed algae in 90 mL of SRW and place 20 mL into each of four aquaria filled with 2 L of SRW. (cell concentration should be approximately 0.2 to 0.1 $\times 10^5$ cells/mL). Take $T_0$ samples as described above for suspended sediment.

2. Add 8 mussels attached to a petri dish into 3 of the four aquaria. The fourth aquarium is the control for settling and determining correction for water-borne contaminant. Place all aquaria into environmental chambers for 2 h. Aquaria are aerated and mussel filtering activity is monitored every 0.5 h during the exposure period.

3. After 2 h, take 3, 4 mL water samples from each of the aquaria to determine final total contaminant concentration in aquaria and then carefully remove 100 mL of water
from each aquaria to determine particle concentration, mass and water-borne contaminant concentration (identical procedure as described for sediment in #1).

Process half of the mussels from each aquarium by determining total length, total wet weight, and tissue and valve wet weight. Place individual tissue and valves into vials filled with scintillation cocktail to extract overnight. Rinse the remaining mussels thoroughly and place them in aquaria filled with clean SRW and return them to the environmental chamber.

4. Remove any feces from each test aquarium and filter onto preweighed filter papers to determine dry weight of feces. After weighing, analyze the dried feces for radiolabeled compound (LSC).

5. Remove approximately 800 mL of the control water (aquaria without mussels) and centrifuge for 0.5 h to remove particles from the water. After 0.5 h carefully pipette 250 mL of the supernatant into each of 2, 1-L beakers. Take 3, 4 mL water from each beaker and then expose 1 mussel per beaker for 1 h. At the end of the exposure period, take 3, 4 mL water samples from each beaker and process the mussels as described above.

Post Exposure

1. Place mussels in clean water and pulse with clean algae or sediment suspension (8 mL/aquaria of the clean algae or sediment suspension as described above) 2-3 times daily. Remove feces at each sampling point and filter onto pre-weighed filter paper. Determine dry weight and then count the filter papers to determine compound concentration in the feces. Take 3, 4 mL water samples from the aquaria to ensure
contaminant concentration is below background. At $T_{48}$, terminate the experiment and process all mussels as described above.

Calculations

Purged mussel tissue contaminant concentration was determined at time 48 h and corrected for elimination of assimilated contaminant, $C_E$ using the equation:

$$\ln C_{48} = \ln C_B - k_d t.$$  

where $k_d$ = compound elimination rate constant (h$^{-1}$)

$t$ = duration of "chase" period (48 h)

$C_{48}$ = zebra mussel contaminant concentration at end of "chase" period ($\mu g/Kg$)

The amount of water-borne contaminant taken up by the experimental mussels ($C_w$) was determined using the equation:

$$C_w = (C_c - C_b)t$$

where $C_c$ = contaminant concentration in control mussels

$C_b$ = background levels in unexposed mussels

$t$ = duration of "pulse" period (2 h).

Percent assimilation efficiencies were determined using the following equation:

$$\% \text{ Assimilation Efficiency} = \left( \frac{C_B - C_w}{C_0 - C_w} \right) \times 100$$

where $C_B$ = assimilated contaminant concentration in mussel tissue

$C_0$ = total contaminant concentration in mussel tissue.
Zebra Mussel Feeding Rate - Protocol

Three Days Before Experiment

1. Place zebra mussels in aquaria with soft SRW (pH 8) at 20 °C and feed mussels algae or sediment daily.

2. Preweigh 55 numbered, cellulose nitrate membrane filters (25 mm diameter, 0.45 um pore size).

Experimental Day

1. Place 15 g of sieved sediment in 1000 mL graduated cylinder filled with standard reference water (SRW). Allow to sit 1 hr and then remove top 80 mL of suspended sediment. Count particle numbers and add enough suspended sediment to 12 L of soft SRW adjusted to pH 8 so that particle counts are 0.9 x 10^4 particles/mL (12 mg dry wt/L). Stir for 0.5 h.

2. Take 200 mL of T₀ water to determine particle concentration and weight. (Filter 5, 30 mL aliquots of water).

3. Place 500 mL of water into each beaker and add 1 zebra mussel. Place beakers in environmental chamber at 20 °C.

4. Allow the mussels to filter for 3 h. Monitor filtering every hour.

5. After 3 h, carefully remove 100 mL from each beaker and reserve in clean beaker for determination of particle concentration and weight at T₀.

6. Remove zebra mussel and place in beaker with 500 mL clean SRW.

7. Remove pseudofeces from each beaker and place in clean 50 mL beaker.

Filter pseudofeces from each beaker individually onto preweighed filter paper.
8. At time 24 and 48 h, collect pseudofeces and add to filter paper so that pseudofeces are accumulated. Dry, cool in desiccator and determine dry weight of pseudofeces.

Ingestion rate = (amount cleared from water)/ wet wt mussel tissue/3 hr.

% Ingested = (amount cleared from water - pseudofeces)/amount cleared from water.
LIST OF REFERENCES


