2002

Defining Benthic Organism Exposure: Bioavailability and Effects of Non-Polar Organics

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DEFINING BENTHIC ORGANISM EXPOSURE:

BIOAVAILABILITY AND EFFECTS OF NON-POLAR

ORGANICS

A dissertation submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

By

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December 6, 2002

I HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER MY SUPERVISION BY Marc S. Greenberg ENTITLED Defining Benthic Organism Exposure: Bioavailability and Effects of Non-Polar Organics BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy.

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ABSTRACT

Greenberg, Marc Samuel. Ph.D., Department of Biological Sciences, Biomedical Sciences Ph.D. Program, Wright State University, 2002. Defining Benthic Organism Exposure: Bioavailability and Effects of Non-Polar Organics.

Laboratory and field tests were conducted to evaluate the hypothesis that factors such as the total organic carbon (TOC) contents and groundwater-surface water interactions (GSI) in the sediments can affect chemical desorption, bioavailability and benthic organism exposure. Laboratory studies were conducted with the polycyclic aromatic hydrocarbon fluoranthene (FLU) and the herbicide trifluralin (TF). Toxicokinetic parameters were determined for *Lumbriculus variegatus* and *Hyalella azteca* in water-only exposures to 0, 5, 20 and 50 g/L of the compounds and bioaccumulation was measured during exposures to 0, 100 and 200 mg/kg of FLU and TF spiked onto sediments from Lakes Erie and Huron. Mean uptake clearance rates ranged from 150-180 mL/g wet animal/h for FLU and 84-120 mL/g/h for TF, and elimination rates were 0.12-0.18 and 0.067-0.10/h for FLU and TF, respectively. The uptake clearances in sediments (k_s) ranged from 0.021 to 0.070 g dry sed/g wet animal/h for FLU and 0.013 to 0.041 g/g/h for TF. The desorption kinetics of FLU and TF from spiked sediments were measured over 34 d by extraction with Tenax®. The rapidly desorbing fraction for FLU and TF ranged from 31.3 to 54.9% of the initial concentrations and rates of the rapidly (k_{rap}), slowly (k_{slow}) and very slowly (k_{vs}) desorbing fractions were on

the order of 10^{-1}/h, $10^{-2—3}$/h and 10^{4}/h, respectively. The influence of GSI on contaminant bioavailability was demonstrated with *in situ* exposures of benthic invertebrates to river sediments that were contaminated primarily with chlorobenzenes (CBs). Hydrologic and chemistry data from nested mini-piezometers explained the exposure-effects relationships. Overall, downwelling conditions reduced the *in situ* exposure of organisms in surficial sediments, and hence, the toxicity and bioaccumulation of CBs. Data from these field and laboratory investigations were combined with literature values of contaminant partitioning (*i.e.,* K_{oc} values), and *L. variegatus* feeding rates and chemical assimilation efficiencies to parameterize a bioaccumulation model. Simulated tissue concentrations at sites containing contaminated sediments were compared to the body burdens measured in the field. The model predicted field observations within an order of magnitude and indicated that TOC, GSI and organism feeding behavior were sensitive parameters. The bioaccumulation model represents a useful tool that can reduce resource expenditures associated with site assessments and provide more accurate risk characterizations.

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ACKNOWLEDGMENTS

This research would not have been possible without funding support from The Institute for Environmental Quality and The Biomedical Sciences Ph.D. Program of Wright State University (WSU). Additional funding was obtained from the U.S. Environmental Protection Agency. The thoughts, reviews and constructive input from my Dissertation Committee are gratefully acknowledged. These members include: Drs. Wayne W. Carmichael, John M. Frazier, Robert D. Grubbs, Dan Krane, and Peter F. Landrum. I would like to sincerely thank my Dissertation Director, Dr. G. Allen Burton, Jr., for his generosity and his enthusiasm towards my ideas during all aspects of this research. I thank the numerous scientists with whom I have collaborated, from whom I have learned, or from whom I have received important comments and suggestions that improved my research. These individuals include: Drs. David W. Charters, P. Bruce Duncan, Jeffrey W. Fisher, Peter F. Landrum, Matti T. Leppänen, James T. Oris, and Prof. Jussi V. K. Kukkonen. The assistance of Mary Ann Layman is appreciated. The results reported in this dissertation would not have been possible without the help of numerous undergraduate and graduate students at WSU. The following individuals provided excellent technical assistance: John Brooker, E. Croisant, J. Gallagher, T. Hall, C. Irvine, J. Johnson, Daniel R. Lavoie, M. McElroy, L. Moore Eggert, Carolyn D. Rowland, Becky Schwab.

I would like to thank J. Coltrane, M. Davis, Duke, J. Garcia, Hundertwasser, W. Kandinsky, P. Metheny, I. Stravinsky, F. Zappa, R. Zimmerman and other musicians and artists who have inspired me throughout my personal and professional pursuits. Finally, I would not have been able to maintain the energy required to obtain this Ph.D. degree without the continued love and support of my family. I thank my parents, Dr. and Mrs. Leslie R. Greenberg and my in-laws, Mr. and Mrs. R. Brion Bauer. My loving wife, Chandra A. Greenberg is the poster-child for patience and my daughters Julia and Sydney now have their father home in time for dinner…most nights.

CHAPTER 1

General Introduction and Methods

INTRODUCTION

Contaminated sediments pose a major environmental hazard primarily because the sediments act as a major repository for the long-term storage of toxic chemicals discharged into surface waters (Burton, 1991; Landrum and Burton, unpublished manuscript). Sediments are comprised of heterogeneous mixtures of detritus, organic, and inorganic particles that settle at the bottom of a body of water (Power and Chapman, 1992). The inorganic particles include rock and shell fragments and mineral grains and the organic contents are usually a small fraction of the total sediment volume (Power and Chapman, 1992). However, organic matter is an important food source for benthic organisms and it has a major role in regulating the sorption and bioavailability of many contaminants (Reuber et al., 1987; Grathwohl, 1990). Pore (interstitial) water fills the spaces between sediment particles and the partitioning of contaminants between sediment organic matter and pore water is an important process responsible for the fate, transport and bioavailability of hydrophobic contaminants (Ankley et al., 1994; Harkey et al., 1995; Segstro et al., 1995; Kosian et al., 1999).

In aquatic environments, hydrophobic organic chemicals including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated benzenes (CBs) and some pesticides accumulate in sediments (Karickhoff et al., 1979; Karickhoff, 1981; Voice and Weber, 1983; Ingersoll et al., 1995). Through the various processes responsible for the transport of these stored toxicants and food chain accumulation and biomagnification (Norstrom et al., 1976; Thomann and Connolly, 1984; Gobas, 1992), sediment-associated contaminants may threaten ecosystems, including humans, for decades to come (USEPA, 1998a). These effects may include reductions in or changes to sediment-associated species that are a primary food source for other ecologically, recreationally or commercially sought out species such as fish, crabs, shrimp and waterfowl.

Methods for assessing the quality of sediments include laboratory and *in situ* (field) toxicity and bioaccumulation testing. Standard laboratory protocols for measuring the toxicity and bioaccumulation of sediment-associated contaminants exist (see citations in Ingersoll, 1995; USEPA, 2000a). Standards for the establishment of *in situ* protocols have recently been submitted to the American Society for Testing and Materials (ASTM) (Salazar and Salazar, *in press*; Burton et al., *in review*). Numerous *in situ* studies of sediment contaminant effects have appeared in the recent literature (Ireland et al., 1996; Chappie and Burton, 1997; Maltby, 1999; Greenberg et al., 2002) and these test procedures offer more environmentally realistic exposure conditions than those achieved in the

laboratory. Both laboratory and *in situ* tests were conducted in the research described in this thesis.

The study of chemical toxicokinetics in aquatic organisms is useful in predicting the accumulation of organic contaminants and in risk assessment when simple equilibrium partitioning (EqP) models are not applicable (Landrum et al., 1992a). Field conditions are typically dynamic and exposures can vary both temporally and spatially. Thus an assumption of equilibrium conditions is often inappropriate (Greenberg et al., 2002). In addition, sediment-associated organisms may accumulate organic contaminants from multiple exposure pathways (*e.g.,* uptake from surface water, diffusion from pore water, ingestion) (Forbes et al., 1998; Burton et al., 2000). Toxicokinetic models describe changes in body burdens that results from processes specific to the organisms such as absorption, distribution, metabolism, storage and elimination. Therefore, once kinetic parameters including the uptake rate coefficients of a chemical from water (k_u) or from sediments (k_s) and elimination (k_e) are known for organic pollutants, predictions of toxicant accumulation under field conditions and exposure and effects characterizations can be improved.

Numerous organism, sediment and chemical characteristics are known to affect the bioavailability of sediment-bound organic contaminants. Characteristics of organisms that play a role in bioavailability include the size (surface area to volume ratio), general behavior and movement of a species within the sediments, and modes and rates of feeding (Karickhoff and Morris, 1985a; Knezovich et al., 1987; Keilty et al., 1988a,b; Boese et al., 1990;

Leppänen and Kukkonen, 1998a,b; Hendriks et al., 2001). Sediment and pore water characteristics that are important determinants of bioavailability include the particle size distribution, clay type, the amount and quality of sediment organic carbon, dissolved organic carbon, and the partitioning between sediment particles and pore water (Neff, 1984; Rodgers et al., 1987; Landrum and Robbins, 1990; Power and Chapman, 1992). Finally, compound characteristics including the hydrophobicity and polarity of the compounds also affect bioavailability in sediments through sorption processes (Karickhoff and Morris, 1985b; Gobas et al., 1989; Cornelissen, 1999).

Desorption of organic contaminants from sediments has been a recent focus of investigation due to its role in bioavailability (Kraaij et al., 2002) and because bulk sediment concentrations of contaminants rarely serve as good predictors of exposure and effects (Burton, 1991). In general, sorption processes are not well understood (Pignatello and Xing, 1996). However, recent techniques using Tenax-TA® resins as infinite sinks in sediment-water systems have yielded reasonable estimates of rapidly reversible and strongly sorbed phases of organic contaminants (Pignatello, 1990; ten Hulscher et al., 1999; Kan et al., 2000; Cornelissen et al., 2001). Currently it is believed that the fraction of contaminants that rapidly desorbs from the sediment organic matter is bioavailable to organisms. Therefore, studies of the desorption kinetics of contaminants should be investigated in conjunction with effects and accumulation testing of sediment in both the laboratory and in the field.

The role of groundwater-surface water interactions (GSI) in determining the bioavailability of sediment-associated contaminants is an emerging issue. Studies have shown that ground water/surface water exchange can either transport contaminants through the sediments into groundwater by downwelling (Schwarzenbach and Westall, 1981; Schwarzenbach *et al.*, 1983) or into surface water by upwelling (Brick and Moore, 1996; Burgess *et al.*, 1996). The freely-dissolved and colloidal-bound fraction of contaminant in the pore water are the most easily mobilized during such flows (Burgess *et al.*, 1996b). Groundwater-surface water transition zones often occur at hazardous waste sites yet have not been addressed by the U.S. Environmental Protection Agency (USEPA). Upwelling and downwelling can cause chemical disequilibria within sediments, changing chemical partitioning and influencing transport, and thus must be investigated more thoroughly during *in situ* toxicity and bioaccumulation tests.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that are distributed widely in aquatic environments as a result of human activity (Laflamme and Hites, 1978; Neff, 1979). The kinetics and toxicity of the PAH fluoranthene (FLU; Figure 1.1A) to benthic invertebrates is fairly well characterized for a number of species including epibenthic marine and freshwater amphipods (DeWitt *et al.*, 1992; Suedel *et al.*, 1992; Kane Driscoll *et al.*, 1997a,b; Kane Driscoll and Landrum, 1997) and infaunal oligochaetes (Ankley *et al.*, 1995; Sheedy *et al.*, 1998). The acute toxicity of waterborne FLU ranged from 92.2 to >250 µg/L (48-h LC_{50}) and 30.3 to 103 µg/L (10-d LC_{50}) in *Daphnia magna, Hyalella azteca* and *Chironomus tentans* (Suedel and Rodgers,

1996). A 16-d LC_{50} value (719 µg/g dry sediment) for sediment-associated fluoranthene in *H. azteca* was recently reported (Kane Driscoll and Landrum, 1997). FLU is a commonly occurring PAH that has been implicated as an ecosystem stressor in numerous studies (ATSDR, 1990; USEPA, 1991). Therefore, it serves as a useful model compound for investigating the bioaccumulation and desorption of sediment-associated nonpolar organics.

Pesticide use in recent decades had led to the presence and detection of these compounds in nearly all sampled rivers and streams in the U.S. (USGS, 1999). The dinitroaniline herbicide trifluralin (TF; Figure 1.1B) is widely used in North America to control broadleaf weeds in numerous crops including soybeans, cotton, sunflower, tomatoes and barley (Nowell et al., 1999). Approximately 19 millions pounds of TF were applied in the U.S. in 1992 (Nowell et al., 1999). The acute toxicity of TF in aquatic invertebrates varies, with LC_{50} values in aquatic invertebrates ranging from 7.2 to 8700 µg/L (Mayer and Ellersieck, 1986). To date, only one study has investigated the bioaccumulation of sediment-associated TF (Yockim et al., 1980). Toxicokinetic parameters for this herbicide in benthic invertebrates have not been reported. Therefore, TF was also selected as a model compound for use in toxicokinetic and desorption studies.

Benthic invertebrates are important members of aquatic environments and toxic effects to their populations from exposure to contaminated sediments can cause perturbations in basic ecosystem functions (Snelgrove et al., 1997; Adams and Greeley, 2000; Brooks et al., 2002). Due to their trophic position, benthic invertebrates are responsible for processing detritus and organic matter and they

serve as food items for upper trophic level predators (Merritt and Cummins, 1996). Therefore, benthic macroinvertebrates may act as early sentinels of declining quality in aquatic systems through their use in biomonitoring studies (Colombo et al., 1995; Baumard et al., 1998; Labrot et al., 1999). Numerous freshwater and marine benthic macroinvertebrates are routinely used in toxicity and bioaccumulation studies of contaminated sediments (Ingersoll, 1995).

The amphipod, *Hyalella azteca* (Crustacea), and the oligochaete worm, *Lumbriculus variegatus,* were used in the research described here. *H. azteca* are epibenthic detritivores that inhabit the uppermost layers of sediments (USEPA, 2000a). *H. azteca* are found throughout the Americas in lakes, ponds and streams and their densities can reach levels of >10,000 individuals/m^2 (de March, 1981; Pennak, 1989). These amphipods feed by ingesting bacteria and grazing on algae (Hargrave, 1970). *H. azteca* can tolerate wide ranges of temperatures (0 to 33 ºC), dissolved oxygen concentrations (=0.3 mg/L), substrate types (clay, silt, sand) and salinity up to approximately 29‰ (Sprague, 1963; Nebeker et al., 1992; Ingersoll et al., 1992, 1996). *H. azteca* reproduce sexually and they are easy to maintain in the laboratory (USEPA, 2000a).

The oligochaete worm, *Lumbriculus variegatus*, is an infaunal species that selectively ingests sediment particles and has been used extensively in sediment toxicity and bioaccumulation testing (Leppanen, 1999; ASTM, 1995a; USEPA, 2000a). *L. variegatus* are distributed widely throughout North America and Europe and they can reach high densities in sediments (Brinkhurst and Jamieson, 1971). They typically reproduce by architomy (splitting) and they are

easy to culture in the laboratory with a doubling time of 10-14 days (USEPA, 2000a). The behavior, feeding habits and ease of handling and maintaining *H. azteca* and *L. variegatus* in the laboratory make them good test organisms for use in comparative tests of the accumulation of sediment-associated contaminants.

Outline of this thesis

The research described in this thesis was designed to yield information on the factors controlling the bioavailability of sediment-bound contaminants to benthic invertebrates. It was hypothesized that factors such as the total organic carbon contents and interactions between groundwater and surface water in the sediments can affect chemical desorption, bioavailability and organism exposure in freshwater stream systems. This hypothesis was addressed through investigations of the:

1) toxicokinetics of FLU and TF in benthic invertebrates exposed to sediments that were spiked with the test chemicals,
2) toxicokinetics of the compounds in water only exposures,
3) desorption kinetics of TF and FLU from sediments, and
4) *in situ* toxicity and bioaccumulation of organic contaminants in a stream system where GSI occurred and was measured.

Then, the data collected in items 1-4 above were used to develop and validate a mathematical model of bioaccumulation that was capable of predicting body burdens in organisms exposed to either laboratory-spiked or field-contaminated sediments.

GENERAL METHODS

Sediment collection, characterization and processing

Sediments from Lake Huron (Michigan, USA) and Lake Erie (Ohio, USA) were used in this research. Bottom surface sediments were collected on August 15, 2000 from Lake Huron Station 54 with a Ponar grab. The GPS coordinates for this sampling station were 45° 31' 0" (latitude) and 83° 25' 0" (longitude). Sediments were collected on Aug 29, 2000 from Lake Erie with a Birge-Ekman dredge. The collection site was in the western basin of the lake near South Bass Island and the GPS coordinates of the location were 49° 39' 49" (latitude) and 82° 49' 46" (longitude). Collected sediments were placed in 114-L plastic bags contained within insulated coolers and transported to the laboratory for storage at 4 °C until use. The sediments were wet sieved on May 3, 2001 by pressing the bulk sediments through an American Society for Testing and materials (ASTM)-approved U.S. standard #18 sieve (1.0 mm) and the ≤1.0 mm particles were retained and used in all sediment experiments.

Sediment wet:dry weight ratio and percent water were determined for the sieved sediments (n=5 per sediment) by weighing a wet sediment sample (12-20 g) into a pre-weighed foil pan and then drying at 60 °C to constant weight. The wet:dry ratios were 5.80 ± 0.06 and 4.42 ± 0.05 for the sieved Lakes Erie and Huron sediments, respectively and these values were used to calculate the wet masses of sediments required for spiking.

Sediment total organic carbon (TOC) and total nitrogen (TN) contents as a percent (± 1SD) of total dry sediment weight were determined by elemental

analysis after acidification to remove carbonates by the following protocol (Kane Driscoll and Landrum, 1997). Samples of 100 mg of dried sediments were weighed (Mettler AE 240 analytical balance, Mettler Instrument Corp., Hightstown, NJ, USA) into tared glass vials and 2 ml of 1 N HCl each were added to the vials which were then placed on a shaker table at 200 rpm for 24 h. Then the vials were placed into a drying oven at 60 °C until dry, capped tightly and placed in a dessicator until TOC analysis. Prior to TOC and TN analyses, a 10-20 mg subsample of the sediments from each vial was weighed into a tared foil thimble using a Mettler AT250 analytical balance and then the thimble was sealed by crimping. The samples were then analyzed on a model EA 1110 CHN Elemental Analyzer (CE Instruments, Milan, Italy). The organic carbon contents (as percent of dry weight) of the sediments prior to spiking were 1.82 ± 0.04% and 3.18 ± 0.13% for Lakes Erie and Huron, respectively.

Particle size distribution of the sieved sediments was provided by Duane Gossiaux (Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration). The fractionation analysis was performed by wet sieving quadruplicate, 10-g samples of each sediment with filtered Lake Michigan water, drying the fractions to constant weight and then calculating the mean percent by mass (± 1 SD) for each size-class (Table 1.1). Sieve sizes (ASTM-approved) used in particle separation were: #40, 425 µm; #140, 106 µm; #230, 63 µm; #400 38 µm; and #635, 20 µm.

Culture water and water quality characterization

Culture water was prepared in accordance with procedures recommended by the U.S. EPA (2000) by diluting aged (>2 d) building-supplied well water with aged (>1 d) Milli-Q water (Millipore, Bedford, MA, USA). This standard culture water is uniform in quality with the following general parameters: temperature, 23-24 °C; pH, 7.9-8.5; DO, >7.0 mg/L; hardness, 160-180 mg/L as $CaCO_3$; alkalinity, 130-160 mg/L as $CaCO_3$; conductivity, 260-300 µS/cm; and ammonia, <0.3 mg/L. The physical-chemical characteristics of water were determined using standard methods (APHA, 1985). Dissolved oxygen was measured using a YSI Model 57 oxygen meter (Yellow Springs Instruments, Yellow Springs, OH, USA) and ammonia was measured using an Accumet® AP63 pH/mV/Ion Meter equipped with an Accumet® ammonia ion-selective electrode (Fisher Scientific, Pittsburgh, PA, USA). An Orion Research Model 940 expandable ion analyzer (Thermo Orion, Beverly , MA, USA) equipped with an Accumet® pH indicating electrode (Fisher Scientific, Pittsburgh, PA, USA) was used to measure pH. Conductivity was measured with a Horiba Model B-173 meter (Spectrum Technology, Plainfield, IL, USA).

Organisms and culture conditions

Culturing methods for *H. azteca* and *L. variegatus* followed protocols recommended by the U.S. EPA (2000) with modifications as outlined by Borgmann (1996) and Leppänen and Kukkonen (1998a). *H. azteca* were obtained from the established cultures of Wright State University and *L. variegatus* cultures originated from NOAA/Great Lakes Environmental Research

Laboratory, Ann Arbor MI, USA. Both organisms were reared at 24 °C on a 16:8 h light:dark cycle. Specific culture procedures for each species are described below.

H. azteca were reared en masse in a 5-L aquarium with aerated laboratory culture water that was enriched with concentrations (mM) of the following salts: KCl (0.01), NaBr (0.01), NaCl (0.7) and CaCl2 (1.0). Such ionic enrichment of culture water has been shown to optimize both survival and growth of *H. azteca* used in aquatic testing procedures (Borgmann, 1996). This stock culture was fed 0.2-0.3 g rabbit chow (Purina Mills, St Louis, MO, USA), 30-50 mL of an algae (*Selanastrum capricornutum*)-cerophyll mixture, and up to three algae-covered tiles weekly. Reproductively competent adults (>30 d old) at a density of 50-60 individuals were placed in 1-L beakers filled with 900 mL of ion-enriched culture water to encourage amplexus and production of neonates. A 7.5 x 7.5 cm piece of presoaked, unbleached paper toweling was added as substrate and the beakers were gently aerated. Amphipods in the amplex beakers were fed 0.1 g of finely pulverized rabbit chow three times per week. Water renewals on the stock culture and amplex beakers were conducted on Monday (50%), Wednesday (100%) and Friday (50%) of each week to maintain water quality. Neonates (0-7 d old) from the amplex beakers were removed and enumerated each Wednesday and set aside for use in tests.

L. variegatus were raised in 5-L plastic or glass aquaria (10-20 g worms/aquarium) containing laboratory culture water. Shredded, presoaked, unbleached paper towels were used as substrate and the oligochaetes were fed

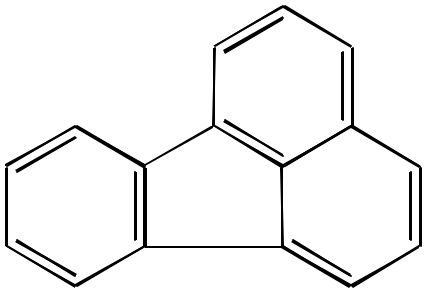
with 0.1-0.2 g of finely ground fish flakes (Tetramin®, TetraWerke, Melle, Germany) 4-5 times per week. A complete water renewal on each culture was carried out each week by passing the contents of the aquarium through a 425-µm sieve and gently rinsing with culture water. Cultures were split when the doubling time of the worms appeared to slow to >14 d or when ammonia levels in the cultures rose to =5 mg/L within a week.

Table 1.1. Particle size distribution of sediments used in experiments. Data are presented as mean percent by dry mass ± 1 SD.

	Lake Erie		Lake Huron	
Size Class (µm)	Mean % ± 1 SD	n	Mean % ± 1 SD	n
> 420	0.37 ± 0.14	4	0.67 ± 0.16	4
420-106	1.63 ± 0.06	4	8.45 ± 0.89	4
106-63	4.62 ± 4.16	4	3.27 ± 0.40	4
63-37	1.50 ± 0.14	4	7.94 ± 2.26	4
37-20	1.40 ± 0.25	4	10.58 ± 6.44	4
<20	90.57 ± 3.71	4	69.09 ± 9.13	4

Figure 1.1. Structure and chemical characteristics of the test compounds. **(A)** Fluoranthene (FLU). **(B)** Trifluralin (TF). MW = molecular weight; S_w = solubility in water; K_{ow} = octanol-water partition coefficient; K_{oc} = organic carbon partition coefficient; BCF = bioconcentration factor; LC_{50} = aqueous concentration causing mortality in 50% of the exposure population.

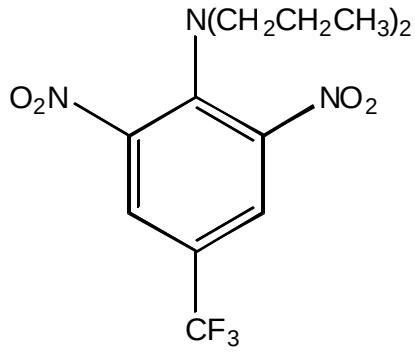
A. Fluoranthene



Formula:	$C_{16}H_{10}$
Class	PAH
MW:	202.26 g/mol
S_w:	0.20-0.26 mg/L
log Kow:	4.90-5.12
log Koc:	5.03
log BCF:	-0.92-3.24 (invertebrates, fish)

- Wide distribution; model non-polar organic compound
- Toxicity range (LC_{50}): 719 mg/kg sed dw
  112 µg/L

B. Trifluralin



Formula:	$C_{13}H_{16}F_3N_3O_4$
Class	Dinitroanaline
MW:	335.29 g/mol
S_w:	4 mg/L @ 27 ¡C
log Kow:	5.07-5.34
log Koc:	2.94-4.49
log BCF:	2.67-5.02 (some fish)

- Pre-emergece herbicide for controlling grasses and broad-leaved weeds
- Toxicity range (LC_{50}): 7.2 - 8700 µg/L

CHAPTER 2

Bioaccumulation and Toxicokinetics of Sediment-Associated Fluoranthene and Trifluralin in *Lumbriculus variegatus* and *Hyalella azteca*

INTRODUCTION

The toxicokinetics of a number of organic contaminants has been studied in *L. variegatus* and various amphipod species (Landrum et al., 1991; Kukkonen and Landrum, 1994; Leppänen and Kukkonen, 1998; Kane Driscoll et al., 1997a,b, 1998). Few studies have described the toxicokinetics of sediment-associated fluoranthene (FLU) in exposures of *L. variegatus* (Landrum et al., 2002) and none to date have provided estimates of the uptake and elimination of sediment-bound FLU by *H. azteca*. Trifluralin (TF) has received little attention in the aquatic toxicity literature, however it is persistent in sediments and therefore may pose a risk in aquatic habitats (Ying and Williams, 2000). The toxicokinetics of TF has been described in fish (Spacie, 1975; Spacie and Haemelink, 1979; Schultz and Hayton, 1993,1994,1999), but not in aquatic invertebrates. Since polycyclic aromatic hydrocarbons (PAHs) and pesticides often occur in complex mixtures within sediments where there is human activity (Burton, 1995; Nowell et al., 1999; USGS, 1999), the study of mixtures of chemicals from different classes is warranted.

The goals of this study were to measure the bioaccumulation and toxicokinetics of the PAH FLU and the dinitroaniline herbicide TF in exposures of *L. variegatus* and *H. azteca* exposed to equivalent-mass mixtures of these compoundsspiked onto uncontaminated sediments. The hypothesis was that: 1) the mixture of the compounds would not be toxic at the administered doses, 2) the conditional rates of uptake clearance and elimination of FLU and TF would increase with dose and 3) the kinetic rates of the compounds would not be different based on the similarity of their hydrophobicities (*i.e.,* log K_{ow} values).

MATERIALS AND METHODS

Experimental design

Lake Huron and Lake Erie sediments were spiked with FLU and TF. *H. azteca* and *L. variegatus* were exposed to these spiked sediments to determine the bioaccumulation and toxicokinetics of the compounds. Nominal sediment concentrations of FLU and TF for the exposures were 0, 100 and 200 mg/kg dry sediment (≈0.494, 0.989 µmol FLU/g dry weight; 0.298, 0.596 µmol TF/g dry weight). These levels of FLU were chosen because they are well below the most recently reported 16-d LC_{50} value (719 mg/kg dry sediment) for sediment-associated FLU in *H. azteca* (Kane Driscoll and Landrum, 1997) and these levels were not expected to be lethal to *L. variegatus.* Similar FLU levels have been measured at contaminated sites (Ireland et al., 1996) and therefore these exposure concentrations represent environmentally realistic levels. The levels of TF were chosen based on rangefinder studies conducted prior to this

definitive set of experiments (Greenberg, unpublished data). Four experiments were conducted and are described in Table 2.1. *L. variegatus* were exposed for 96 h and the uptake kinetics and mortality were determined by sampling organisms at 4, 8, 13, 24, 48 and 96 h. *H. azteca* were exposed for 48 h and sample times for toxicokinetics were 1, 3, 6, 12, 24 and 48 h. Test set up, initiation (*i.e.,* additions of animals), sampling and end dates are shown in Table 2.2. For both species, three beakers for each concentration were analyzed at each time point.

Chemicals

Radiolabeled [G-^{3}H]fluoranthene (FLU) (Lot No. CSL-95-564-92-28) was purchased from Chemsyn Science Laboratories (Lenexa, KS, USA) with a specific activity of 721 mCi/mmol. [Ring-UL-^{14}C]trifluralin (TF) (Lot No. 20K9401) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) with a specific activity of 16.8 mCi/mmol. The purity of the radiolabeled TF was determined to be >98% by the manufacturer (January, 2001) and was used without further purification. Because the [^{3}H]FLU was synthesized in 1996, its purity (from duplicate 2-µL samples) was periodically checked by thin-layer chromatography (TLC) followed by liquid scintillation counting (LSC; see Analytical methods) and was found to be >96% pure prior to spiking the sediments. Unlabeled FLU (Lot No. 39H3606, >98% purity) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and unlabeled TF (Lot No. 229-132B; >98% purity) was obtained from ChemService, Inc. (West Chester, PA, USA). Prior to the spiking of sediments, the radiolabeled chemicals were transferred to acetone (HPLC-

grade; Aldrich Chemical Co.) and their volumetric concentrations were checked on May 11, 2001 by liquid scintillation counting (LSC) of duplicate, 2-µL samples. The mean activities were 147.45 µCi/mL for [^{3}H]FLU and 3.71 µCi/mL for [^{14}C]TF. Acetone (HPLC-grade) was used to prepare spiking solutions.

All reagents used for extractions and analyses were of ACS-grade quality at a minimum. Chloroform, ethyl acetate and anhydrous ethyl ether were purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). Cyclohexane and hexane were obtained from EM Sciences (Darmstadt, Germany). Methanol was purchased from Burdick & Jackson (Muskegon, MI, USA) and anhydrous sodium sulfate was provided by Mallincrodt (St. Louis, MO, USA). The scintillation cocktail (Ultima Gold®) and solubilizer (Soluene®-350) used for radionuclide analysis were obtained from Packard BioScience, B.V. (Groningen, The Netherlands).

Sediment spiking

Sediments from Lakes Huron and Erie used for the toxicokinetics experiments were spiked with both FLU and TF at nominal concentrations for each chemical of 0 (control), 100 and 200 mg/kg dry weight sediment (≈0.494, 0.989 µmol FLU/g dry weight; 0.298, 0.596 µmol TF/g dry weight). These concentrations were expected to achieve pore water concentrations that were less than 50% of the aqueous solubility limits of FLU (260 µg/L; Karickhoff, 1981; Verschueren, 1983) and TF (4 mg/L; Mackay *et al.*, 1997; Montgomery, 1997). Lake Huron sediments were spiked on May 15, 2001 and Lake Erie sediments were spiked on May 18, 2001.

Stock spiking solutions (50 mL) of FLU and TF in acetone were prepared for each sediment concentration by combining [^{3}H]FLU and [^{14}C]TF and the appropriate amount of unlabeled compounds in acetone. Target activity levels of radioisotopes in the sediments were 15,000 disintegrations per minute (DPM) per gram of wet sediment for tritium and 7,500 DPM/g wet sediment of carbon-14. Separate 100-µL Gastight® syringes (Model 1710N, Hamilton Co., Reno, NV, USA) were used to dispense the radiolabeled chemicals and appropriate volumes of unlabeled FLU and TF from stock solutions (10 mg/mL each in acetone) were added to 50-mL volumetric flasks. Then acetone was added to bring the final stock spiking solution volumes up to 50 mL, a stir-bar was added and the solution sealed with a ground-glass stopper and gently mixed on a magnetic stir-plate (Thermix® Stirrer Model 120M, Fisher Scientific Co.). Duplicate 25-µL samples of each stock solution were placed into 12 mL of scintillation cocktail (Ultima Gold®, Packard BioScience, B.V., Groningen, The Netherlands) then analyzed, by LSC and the mean values were used to calculate the specific activities of the spiking solutions (µCi of radiolabeled compound/µmol of total nominal compound). Mean (± 1 SD) coefficients of variation (CV; %) for the duplicate samples of each solution were low (2.89 ± 1.63).

Sediments were spiked with FLU and TF using a modification of the standard rolling jar method (Ditsworth *et al.*, 1990; DeWitt *et al.*, 1992; Kane Driscoll *et al.*, 1997). Spiking was conducted at room temperature under constant yellow light (λ > 500 nm) to avoid potential photodegradation of FLU and TF. The stock solutions of FLU and TF in acetone were evaporated onto the

inside walls of 1-gal (3.785-L) glass jars. Sediments (0.77-2.22 kg wet wt) were weighed on a Mettler PM4000 balance (Mettler Instrument Corporation, Hightstown, NJ, USA) and along with 1.5 mL of culture water per 25 g wet sediment were added to the jars and the mixture was rolled for 3 h at room temperature, held overnight at 4 ºC, and rolled the next day for 5 h. The sediments were then stored at 4 ºC for >30 d to allow for dissolution and partitioning of the spiked compounds to occur (Northcott and Jones, 2000). Prior to the start of an experiment, spiked sediments were rolled again for 5-10 min to thoroughly mix the sediment particles with any water that had exuded from the sediments during storage. Three replicate sediment samples were taken from each concentration for LSC, wet to dry weight determination, and to determine the thoroughness of mixing. Triplicate sediment samples were taken from the 0 mg/kg (control) sediments for determination of organic carbon content.

After the experiments, the percent purity of the [^{3}H]FLU and [^{14}C]TF spiked onto sediments was determined by placing duplicate 2 g wet sediment samples into 15-mL borosilicate glass screw-cap test tubes, extracting, then analyzing the samples using TLC followed by LSC (see Analytical methods).

Test Organisms

Lumbriculus variegatus. Twelve days prior to the initiation of an experiment with *L. variegatus* (Table 2.2), approximately 2000 individuals from laboratory cultures were placed into a 38-L aquarium containing 3 cm of uncontaminated sediments and 10 cm of overlying culture water that was gently aerated. These sediments originated from Cedar Bog near Urbana, OH, USA

and were found to be free of organic contaminants and metals (Brookside Laboratories, New Knoxville, OH, USA) and were a rich source of organic carbon (13.21 ± 3.32 % by dry weight; n=3). The oligochaetes were placed into this clean sediment prior to their exposure to spiked sediments in order to encourage normal burrowing and feeding behavior and reproduction prior to use. Recent research with oligochaetes has shown that reproduction by architomy (fragmentation) followed by reduced feeding behavior during tests can lead to lower levels of contaminant accumulation, particularly if ingestion is an important route of uptake (Leppänen and Kukkonen, 1998b). Therefore, it has been suggested that the impacts of reproduction during accumulation testing should be minimized by carefully selecting smaller (<9 mg wet wt), feeding individuals (Van Hoof et al., 2001) who have completed regeneration of their heads and tails (Leppänen and Kukkonen, 1998c). *L. variegatus* for use in the present studies were then selected based on their size (1-2 cm length, 4.17 ± 0.35 mg wet wt/individual; n=8 measurements of 10 individuals each), the presence of a fully developed head and tail, and gut contents indicating active feeding. Prior to their introduction into test beakers, the animals were allowed to purge their guts for 3-5 hours. For ease of rapid addition of the *L. variegatus* to the test beakers, ten individuals each were gently added to 50-mL Falcon® centrifuge tubes (BD Biosciences, Boston, MA, USA) containing 20 mL of culture water.

Hyalella azteca. Neonates were harvested from WSU laboratory cultures of *H. azteca* on June 13, 2001 and June 20, 2001 for use in experiments 3 and 4 (Table 2.2), respectively. They were placed in 1-L beakers with 900 mL of gently

aerated culture water and maintained as previously described with the exception that they were not sieved weekly. This grow-out period of >30 d for the amphipods was necessary in order to obtain adequate tissue masses of *H. azteca* for residue analyses by LSC. Organisms from these batches were approximately 41-48 d old (experiment 3) and 48-55 d old (experiment 4) at test initiation, however they were not observed to be reproducing as they were kept under conditions that were not optimal for reproduction (U.S. EPA, 2000). Amphipods were randomly selected for testing and their mean individual wet weight was 0.19 ± 0.04 mg (n=5 measurements of 10 individuals each). For ease of rapid addition of the amphipods to the test beakers, twenty individuals each were gently added to 50-mL centrifuge tubes containing 20 mL of culture water.

Exposures and sampling

L. variegatus (experiments 1 and 2) and *H. azteca* (experiments 3 and 4) were exposed to FLU- and TF-spiked sediments for 96 and 48 h, respectively Table 2.1. Sediment (50 g wet wt, experiments 1 and 2; 30 g wet wt, experiments 3 and 4) was added to each 300-mL tall-form exposure beaker and 250 mL of culture water was carefully added with a squirt bottle. The sediment was allowed to settle for two (experiments 1 and 2) or four (experiments 3 and 4) days prior to the addition of test organisms. The experimental dates including test set-up, initiation and sample time points are given in Table 2.2.

Immediately prior to the addition of animals (Time =0 h), the beakers were randomly placed in a shallow (5 cm depth) water bath to maintain a constant

temperature and a water renewal of approximately one-half the volume of the overlying water within each beaker was performed using a Zumwalt splitter (Zumwalt et al., 1994; U.S. EPA, 2000). At this time, a pooled water sample (300 mL) was taken from 8 randomly sampled control sediment beakers for determination water quality characteristics including temperature (°C), pH, dissolved oxygen (DO; mg/L), hardness (mg/L as $CaCO_3$), alkalinity (mg/L as $CaCO_3$), conductivity (µS/cm) and ammonia (mg/L). Then, ten *L. variegatus* were added to each test beaker (experiments 1 and 2) or twenty *H. azteca* were added per beaker (experiments 3 and 4). Distribution of animals to the test beakers for each experiment took <10 min. Because of the time required to sample *H. azteca* during experiments 3 and 4 (ca. 2-3 h per time point), the addition of organisms to the 3 and 6 h time points was carried out approximately 26 and 33 h, respectively, after the start of the other time points (Table 2.2). This allowed for adequate time between *H. azteca* sample time points with no overlaps. Half (125 mL) of the overlying water in the test beakers was renewed daily during the *L. variegatus* exposures and only at 24h for the 48 h time point beakers in the *H. azteca* tests. Temperature and DO were measured daily in the control beakers and the full suite of water quality characteristics were determined at the end of each experiment. All experiments were run on a 16:8-h light:dark photoperiod at room temperature (22 ± 1 ºC) under yellow light (λ > 500 nm) to avoid photodegradation of the FLU or TF.

Food was not administered during any of the experiments as recommended for bioaccumulation testing with *L. variegatus* (U.S. EPA, 2000).

It was assumed that due to the short (48 h) duration of the *H. azteca* bioaccumulation assays in the present study, the survival of amphipods would be unaffected by the lack of food addition.

At each time point, triplicate beakers from each concentration were selected at random and were sieved and sampled and behavioral observations (*e.g.,* burrowing of *L. variegatus*, presence of *H. azteca* in the overlying water) were noted. Sediment samples were taken from each beaker for wet to dry weight determination (approximately 600 mg wet wt) and measurement of [^{3}H]FLU and [^{14}C]TF (approximately 100 mg wet wt) by LSC. Sediments from the controls (0 mg/kg of the test compounds) were sampled only at the first time point. Triplicate sediment samples were also taken from control beakers for total organic carbon (TOC) and total nitrogen (TN) determination. Mean percent survival and standard deviation was calculated based on the number of live organisms recovered from the sediments divided by the initial number added to each beaker. In experiments 1 and 2, the concentrations of [^{3}H]FLU and [^{14}C]TF were measured by LSC in all surviving *L. variegatus* from each beaker except at the 96 h time point where a pooled subsample from each the 100 and 200 mg/kg treatments (two individuals/beaker if possible) was frozen at –20 ºC in 15-mL borosilicate, screw-cap test tubes with Teflon®-lined caps until extraction and analysis of metabolites of the test compounds. In experiments 3 and 4, [^{3}H]FLU and [^{14}C]TF were measured in 10 of the surviving *H. azteca* from each beaker while the remaining surviving individuals were frozen as described above for metabolite analysis. Surviving control organisms from the beginning and end of

each experiment were stored frozen at –20 ºC in chloroform-rinsed (3 x 0.5 mL) 1-mL borosilicate tubes (Fisher Scientific Co.) prior to extraction and determination of lipid contents (see Analytical methods).

Indigenous worms (not *L. variegatus*) were observed during some of the sediment exposures and they were collected and analyzed for comparison of their body burdens to the organisms from cultures used for the experiments. The experiments, times and doses included the following: 1) experiment 1, 96 h, 100 mg/kg, 2) experiment 3, 24h, 100 mg/kg, and 3) experiment 4, 12 and 24 h in the 100 mg/kg treatment and 3 h in the 200 mg/kg treatment. These indigenous worms were sampled and their body burdens of FLU and TF were determined by LSC. It was assumed that these animals were at steady state because they were exposed to the test compounds from the time of spiking, through the equilibration period and up to the experimental time point from which they were sampled.

Analytical methods

Sediments. Sediment samples (approximately 100 mg wet wt) for radioisotope analysis were weighed (Mettler AE 240 analytical balance, Mettler Instrument Corp.) into tared 20-mL borosilicate glass scintillation vials (Kimble Glass Inc., Vineland, NJ, USA) and 1 mL of a solubilizing solution (Soluene®-350, Packard Instrument Co.) was added (Thomson, 1998). Then the samples were vortexed for 30 sec (Vortex Genie, Fisher Scientific Co.), tightly capped and allowed to sit for 24 h at room temperature in the dark. The solubilizer was added to digest organic matter, thus facilitating the extraction of the tritium- and [^{14}C]-labeled compounds from the sediment matrix, and has been used in similar

investigations (Leppänen and Kukkonen, 1998b; Lawrence et al., 2000). Following solubilization and extraction of the samples, 12 mL of scintillation cocktail (Ultima Gold®, Packard Instrument Co.) was added to each vial and the contents were vortexed for 10 sec. Then after subsidence of chemiluminescence (=48 h), radioactivity was quantified by LSC.

Tissues. Live animals collected at the various time intervals during the experiments were analyzed for: 1) measurement of the concentrations of [^{3}H]FLU and [^{14}C]TF equivalent activity in tissues, and 2) quantification of biotransformation products in *L. variegatus* sampled at 96 h. Due to the very low tissue masses in the *H. azteca* samples (<3 mg/sample) that were taken for metabolite analysis in experiments 3 and 4 (Table 2.2), biotransformation products were not measured for *H. azteca*.

L. variegatus sieved from the test beakers were removed to 40-mL glass petri dishes containing culture water and allowed to purge their guts for 6 h, as recommended by Mount et al. (1999). It was assumed that elimination of any accumulated FLU and TF over this 6 h purge time would not exceed 10% of the initial tissue concentrations at the time of sampling for compounds, such as FLU and TF, with log K_{ow} > 5 (Mount et al., 1999). Following the purging of their gut contents, the worms were blotted dry on paper towels, weighed to the nearest 0.01 mg (Cahn C-31 microbalance, Orion Research, Inc., Boston, MA, USA) and placed into 20-mL borosilicate glass scintillation vials with 1 mL of tissue solubilizer (Soluene®-350). After solubilizing for 24 h, scintillation cocktail (12 mL, Ultima Gold®) was added and each sample was vortexed for 10 sec.

The samples were stored in the dark at 24 ºC for 48 h to allow chemiluminescence to subside, and then radioactivity was measured by LSC.

H. azteca sieved from the test beakers were immediately rinsed in culture water, blotted dry on paper towels, weighed to the nearest 0.001 mg on a Cahn C-31 microbalance and placed into 7-mL borosilicate glass scintillation vials (Fisher Scientific Co.) with 500 µL of tissue solubilizer (Soluene®-350). After solubilizing for 24 h, scintillation cocktail (6 mL, Ultima Gold®) was added and each sample was vortexed for 10 sec. The samples were stored in the dark at room temperature for 48 h until chemiluminescence had subsided, and then radioactivity was measured by LSC.

Metabolites. Biotransformation of the test compounds by *L. variegatus* was evaluated using methods reported in the literature for the extraction and analysis of PAH metabolites (Harkey et al., 1994; Leppänen and Kukkonen, 2000; Millward et al., 2001). Parent FLU and TF and their metabolites were extracted by first thawing the frozen organisms stored in 15-mL test tubes to room temperature. Then the following extraction was performed twice. Five mL of ethyl acetate:acetone (4:1) was added to the test tubes which were then tightly capped. The samples were sonicated for 20 min in an ultrasonic water bath (Model FS30, Fisher Scientific Co.). To prevent the volatilization of the compounds due to excessive heat generated during sonication, ice was added to maintain a bath temperature that was approximately 20 °C. The sonicated samples were then centrifuged at 1500 x *g* for 2 min (Model CL International Clinical Centrifuge, International Equipment Co., Needham Heights, MA, USA).

The extracts were then transferred with a Pasteur pipet to solvent-rinsed test tubes by first passing the extract through anhydrous sodium sulfate (approximately 3 g) that was supported in a borosilicate glass funnel by a plug of glass wool. The Na_2SO_4 was used to dry the extract and it was rinsed with 3 x 1 mL of ethyl acetate:acetone (4:1) after its second use. The residual tissues were re-extracted twice with 5 mL of cyclohexane in the same fashion except that Na_2SO_4 filtration was not required. The combined extracts were reduced in volume to approximately 100 µL under a gentle stream of nitrogen (6-Port Mini-Vap, Supelco, Bellfonte, PA, USA) for TLC analysis.

The concentrated extracts were then introduced onto flexible-backed, silica gel plates (60 Å, 250 µm thickness) (Whatman, Ann Arbor, MI, USA). Each sample extract was spotted to a lane (3 cm width) on the TLC plate by careful dropwise addition with a Pasteur pipet such that the spot was <1 cm in diameter. The chromatographic origin was spotted 3 cm from the bottom of the plate. Small amounts of nonradiolabeled FLU and TF (1 drop each from 1 mg/mL solutions) were added over the sample spots, and the plate was developed with hexane:ethyl ether (9:1, v/v) solvent. After the run, the spots corresponding to FLU and TF were marked under UV light and the plate was analyzed for radioactivity by cutting the flexible plate into segments, placing them into 20-mL scintillation vials and counting the segments in 15 mL of scintillation cocktail (after a 48 h period to allow for the subsidence of chemiluminescence). The segmentation from the origin of the samples was as follows: 0-2, 2-4, 4-6, 6-7, 7-10, 10-12 cm. The 7-10 cm segment was required because the spots of

the visualized FLU and TF were not fully resolved (*i.e.,* they overlapped) and a 3-cm section was sufficient to contain both spots. The 10-12 cm segment was expected to have little or no associated radioactivity. However, it was included because in a few cases, spots on the outer edge lanes drawn on a 20 x 20 cm TLC plate traveled a few millimeters further than the inner lanes, and thus any radioactivity associated with these segments was assumed to be parent FLU or TF.

After the concentrated extracts were spotted and run on TLC plates, the glassware used in the extractions was rinsed to recover all residual radioactivity. Each extract evaporation test tube was rinsed with 3 x 1 mL of hexane and the rinses were combined in 20-mL scintillation vials to which 8 mL of scintillation cocktail was added for LSC. The tissue pellet was recovered from the sample test tubes by rinsing each with 3 x 2 mL of acetone. The rinses were combined to 20-mL scintillation vials. The acetone was evaporated to dryness under a stream of nitrogen and 1 mL of solubilizer (Soluene®-350) was added to the remaining tissue pellets. After 24 h of digestion, 12 mL of scintillation cocktail was added and the radioactivity was quantified by LSC. The unextractable radioactivity associated with the tissue pellet was assumed to be metabolites of FLU and TF that were covalently bound to cellular macromolecules (Kane Driscoll et al, 1997b). Preliminary analysis of tissues (n=3) spiked with known activities of [^{3}H]FLU and [^{14}C]TF determined that the extraction efficiencies of the radiolabeled compounds were 89.41 ± 1.22% and 82.29 ± 3.58% for FLU and TF, respectively.

The spiked sediment samples taken for determination of degradation products (not metabolites) in the sediments were also extracted and run on TLC plates by following the methods described above.

Liquid scintillation counting. All samples prepared for LSC were analyzed on a Tri-Carb Liquid Scintillation Analyzer (LSA) (Model 2300 TR, Packard Instrument Co.). The LSA was run in dual counting mode utilizing the inclusion method for the determination of tritium and carbon-14 activities in the samples (L'Annunziata and Kessler, 1998). The counting regions of the radionuclides in the dual analysis were based on their b-particle energies (*e.g.,* E_{max} values, 18.6 keV for ^{3}H, 156 keV for ^{14}C), and spillup and spilldown of the ^{3}H and ^{14}C pulses in each region were accounted for in the calculation of their activities. However, the spiking protocol was designed to reduce the error introduced by these spillovers, by using approximately 2x more ^{3}H activity (DPMs) in the sediments than ^{14}C. The counting regions were 0.0-12.0 keV for ^{3}H and 12.0-156 keV for ^{14}C. Each sample was counted for 20 min, and the data were corrected for quench using the external standards ratio method after correcting for background (L'Annunziata and Kessler, 1998). Quenched LSC standards (known amounts of radioactivity with progressively higher amounts of the quenching agent nitromethane) prepared in PPO/Dimethyl POPOP/toluene (2,5-diphenyl-oxazole/1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene/toluene) were obtained from Packard Instrument Co., to establish quench correction curves for ^{3}H and ^{14}C. Luminescence correction and static control options were utilized for the analyses. A background sample containing scintillation cocktail

and several matrix blanks (*e.g.,* sediments, test species; n =2) were included in each run. The total amounts of FLU and TF equivalents (parent compound and metabolites or breakdown products on a molar basis) in each sample were calculated using the nominal specific activities based on the isotopic dilution from the prepared stock solutions.

Lipids. The lipid contents of control animals sampled at the beginning and end of experiments 1-4 were determined using a microgravimetric technique (Gardner et al., 1985; Parrish, 1999). The method involved the extraction of lipid from a small sample of organisms (1.1-5.2 mg wet wt, *H. azteca*; 36.6-55.2 mg wet wt, *L. variegatus*). The frozen samples in 1-mL test tubes were thawed and ground with a blunt spatula. Then, the sample was extracted in chloroform:methanol:water (2:1:0.75, v/v) by first dispensing 400 µL of chloroform:methanol (2:1, v/v). The volume of deionized water that was added to each sample was calculated in order to account for the water content of the organisms, by subtracting the body water content (mg) of the organisms from 100 µL (=100 mg). The body water content in each sample was estimated using the wet to dry weight ratio of *L. variegatus* (7.13 ± 0.46, n=3 measurements of 10 individuals each) and *H. azteca* (1.70 ± 0.07, n=3 measurements of 10 individuals each) determined from laboratory cultures (M. Greenberg, unpublished data). After adding the water, the samples were vortexed for 1 min followed by centrifugation at 1500 x *g* for 2 min. Then, using a 100-µL Gastight® syringe, the chloroform layer was gently removed to a clean, chloroform-rinsed (3 x 0.5 mL), 3-mL borosilicate tube and its exact volume was recorded. The

residual samples were re-extracted twice with same volume of chloroform and the extracts were combined and evaporated under nitrogen to approximately 100 µL. The concentrated chloroform/lipid samples were quantitatively transferred with 100-µL syringes to double-walled tin foil cups which had been heated (50 °C for >4 h), dessicated and tared. The samples were oven-heated at 50 °C until the chloroform had completely evaporated. The remaining lipid was dessicated, weighed to the nearest 0.001 mg (Cahn C-31 microbalance), and expressed as percentage lipid per tissue wet mass.

Two blank samples were included with every analysis and the mean of the blanks was subtracted from the sample lipid weights. Blank contamination was found to be minimal (=6 µg; =5%). Preliminary analysis of soybean oil as a standard (n=4, 30 µL each) determined that the extraction efficiency of lipid content was 100 ± 0.47%.

Tissue and sediment concentrations

Concentration values are reported as mean (± 1 SD) in units of µmol/g wet wt for *L. variegatus* and *H. azteca,* and µmol/g dry wt for sediments. The concentrations represent measured parent compound equivalents of [^{3}H]FLU and [^{14}C]TF in the samples.

Biota/sediment accumulation factors (BSAFs)

BSAFs were calculated for each replicate sampled at the end-of-exposure based upon the concentrations of FLU and lipids in the bodies of *L. variegatus* and *H. azteca*. The BSAF is a ratio of the lipid-normalized concentration of a

contaminant in tissues to its organic carbon-normalized concentration in sediments and is calculated by the following equation (Lake et al., 1987; Millward et al., 2001):

$$\mathrm{BSAF} = \frac{C_{tss}/f_{lipid}}{C_s/f_{OC}} \quad (2.1)$$

where BSAF is the biota/sediment accumulation factor (g carbon/g lipid), C_{tss} is the tissue concentration at steady state (µmol/g wet wt), f_{lipid} is the fractional lipid contents of the tissues (g/g wet wt), C_s is the contaminant concentration in the sediments (µmol/g dry wt) and fOC is the fractional organic carbon contents of the sediments (g/g dry wt). BSAF values were expressed as means ± 1 SD.

Modeling

Accumulation data for FLU and TF were fit to a two-compartment first-order kinetic model (Landrum *et al.*, 1992a; Boese *et al.*, 1997):

$$\frac{dC_a}{dt} = k_s C_s - k_e C_a, \quad (2.2)$$

where C_a is the concentration in the organism (µmol/g wet wt), k_s is the conditional uptake clearance rate of a compound from sediments and pore water (g dry sediment/g wet wt organism/h), C_s is the concentration in the sediment (µmol/g dry sediment), k_e is the conditional elimination rate constant (1/h), and t is time (h). To apply this model, it was assumed that there was no growth of the organisms, the bioavailable concentrations of FLU and TF remained constant, and biotransformation of the compounds was sufficiently slow over the time course of the experiments. If C_s is held constant throughout the exposure, Equation 2.2 can be integrated to yield:

$$C_a = \left[k_s C_s (1 - e^{-k_e t})\right] / k_e, \qquad (2.3).$$

The accumulation data was modeled by least squares (LS) nonlinear regression using *SYSTAT for Windows, Version 9* (SYSTAT, Evanston, IL, USA). The Gauss-Newton algorithm for LS fitting of the data was used because it is known to result in reliable estimates, and convergence is rapid and not strongly dependent on the initial values of the parameters to be estimated (Ratkowsky, 1983; Smyth, 2002). The exact sample times for the replicates with their corresponding measured C_a values and the mean measured values of C_s were used in the calculations. The nonlinear fit to Equation 2.3 yielded LS estimates for k_s and k_e and are reported as the estimated value (± asymptotic standard error, A.S.E.).

Statistical analysis

Significant differences between the means of: 1) the sediment concentrations of each chemical (FLU, TF) sampled from the exposure beakers, 2) the survival of each test species (*L. variegatus, H. azteca*) recorded throughout the exposures, and 3) the wet weights of each species measured in the experiments were tested with two-factor analysis of variance (ANOVA; Zar, 1999) followed by pairwise comparisons among treatments (Tukey's honestly significant difference test). The two factors included in the ANOVA model were time and dose (0, 100, 200 mg/kg). Differences due to the interaction of or the main effects of time and dose were considered significant if $p = \alpha = 0.05$.

Lipids and BSAFs were analyzed using one-way ANOVA (Zar, 1999). For lipids, hypothesis testing was first performed between the sample means in each

experiment with respect to time. If there were no significant differences identified by this first ANOVA, then for each species, testing for significantly different mean lipid contents between sediment exposures (Lake Huron vs. Lake Erie) was performed. For BSAFs, separate ANOVAs for each chemical in an experiment were carried out with respect to dose. BSAFs for a given dose were also compared between sediment sources. Due to the different time scales of the tests for *H. azteca* and *L. variegatus* and because of species-specific differences in their sediment habitat preferences, feeding and behavior, statistical comparisons were not conducted between species.

Prior to testing with ANOVA, data normality was verified using Kolmogorov-Smirnov and Sharpiro-Wilk tests of normality, and homogeneity of variances were tested with Levene's and Bartlett's tests. Statistical analysis was performed using *Statistica for Windows, Version 5* (STATSOFT, Tulsa, OK, USA).

Significant differences between estimated conditional rate constants (k_s, k_e) from the LS nonlinear fitting of the tissue-time course data by Equation 2.3 were tested with the unpaired Student's t-test (Fisk et al., 1998). Based on statistical considerations for adjusting the degrees of freedom for the number of parameters fitted by a model when comparing individual estimated parameter values (Motulsky, 1999; Ratkowsky, 1983), the following equation was used to calculate the observed value of t (t_{obs}):

$$t_{obs} = \frac{E_1 - E_2}{\sqrt{A.S.E._1^{\,2} + A.S.E._2^{\,2}}} \qquad (2.4)$$

where t_{obs} is the observed value of t, E_i is the i^{th} fitted rate constant and $A.S.E._i$ is the asymptotic standard error of the i^{th} fitted rate constant. The total degrees of freedom (df_T) are given by:

$$df_T = (n_1 - p_1) + (n_2 - p_2) \quad (2.5)$$

where df_T is the total degrees of freedom, n_i is the number of data points in the i^{th} data set and p_i is the number of parameters fitted to the i^{th} data set. The null hypothesis is rejected when $|t_{obs}| = t_{crit}$, where t_{crit} is $t_{\alpha(2),df_T}$ and $p = \alpha = 0.05$. The contrasts performed separately for each species using this procedure included: 1) testing between dose for each compound in each experiment, 2) testing between compounds at each dose in each experiment, and 3) testing between experiments for each compound at each dose. The calculations described in Equations 2.4 and 2.5 were conducted using spreadsheet programming in Microsoft Excel, Version 2001 for Macintosh (MICROSOFT, Redmond, WA, USA).

RESULTS

Water and sediment characterization

The physical-chemical characteristics of the water and sediments in experiments 1-4 are summarized in Table 2.3. Temperatures during the exposures were very stable and dissolved oxygen concentrations remained high (mean range 5.33-7.20 mg O_2/L). Ammonia, which can be a major stressor during sediment tests (Burton, 1999), remained low (<1.0 mg/L). Overall, the overlying water within test beakers was of high quality during all experiments.

Lake Huron sediments were higher in TOC (mean range, 3.64-3.66%) and TN (0.56-0.62%) than were the sediments from Lake Erie (TOC, 2.00-2.08%; TN, 0.33-0.35%) by factors of approximately 1.8 and 1.7, respectively.

Sediment concentrations of the test compounds

The sediment concentration-time profiles of FLU and TF are shown in Tables 2.4 and 2.5. In experiments 1 and 2 using *L. variegatus*, factorial ANOVA identified significant differences between the 100 mg/kg and 200 mg/kg doses of FLU and TF. In the case of TF in experiment 2, the null hypothesis was rejected by ANOVA (p =0.04) with respect to time, however post-hoc comparisons by Tukey's Honestly Significant Difference (HSD) test did not identify any significant differences between the time point sample means (p >0.05). Therefore, it was concluded that the sediment concentrations of FLU and TF in measured in experiments 1 and 2 exhibited no significant changes during the exposures.

Sediment concentrations of FLU and TF in the exposures of *H. azteca* (experiments 3 and 4) exhibited some temporal variability. Factorial ANOVA of the FLU concentrations in samples from both Lakes Huron and Erie sediments, and the TF concentrations in spiked Lake Erie sediments, identified significant interactions of dose with time (Table 2.5). Post hoc comparisons using Tukey's HSD test identified that in the Lake Huron 200 mg/kg dose, there were significant differences (p <0.05) between the 1 h sample concentration means for FLU compared to the 3, 6, 12 and 24 h means. A closer evaluation of the FLU sediment concentrations from this treatment by box-and-whisker plotting (Zolman, 1993) identified that one replicate at the 1 h time point was an extreme

outlier (*i.e.,* >3 times the inner quartile range) and likely was the reason for these differences. For the Lake Erie sediment concentrations of FLU and TF, means comparisons by Tukey's HSD test indicated that, in the 200 mg/kg dose, significant differences occurred between the 6 h and the 0, 1, 3, 12 and 24 h mean FLU concentrations; and for TF significant differences were identified between the 6 h and the 1, 3, 12 and 24 h sample means. Examination of box-and-whisker plots of the Lake Erie sediment concentration data indicated that for both FLU and TF, one sample taken at 6 h from the 200 mg/kg dose was identified as an extreme outlier and another as a suspect outlier.

The null hypothesis was rejected with respect to the main factors of dose (ANOVA, p <0.001) and time (ANOVA, p <0.001) for the mean concentrations of TF in Lake Huron sediments. The significant difference with time in this case was due to the slightly higher measured concentrations of TF in the samples taken at –96 h (set up) of experiment 3 compared to samples taken at 1, 3, 12 and 24 h after the start of the exposure (Tukey's HSD, p <0.05).

Overall, since relatively few samples were the cause of these statistical differences in experiments 3 and 4, and because data points identified as "outliers" were taken during the exposures and thus will each have a corresponding tissue accumulation datum, a decision was made to use all of the data to calculate the mean sediment concentrations for use in the toxicokinetic modeling. Furthermore, since the variation in sediment concentrations of FLU and TF in experiments 3 and 4 did not exhibit any specific pattern, such as a linear or exponential loss over time, an alternative to the model described by

Equation 2.2 that accounts for loss from the source compartment or a change in bioavailability could not be applied.

The measured sediment concentrations of FLU and TF equivalents were generally lower than the nominal calculated concentrations spiked to the sediments. This may have been due to incomplete sorption of the chemicals during the spiking process and loss to the glass walls of the spiking jars, which were not made of borosilicate glass. The purity of the test compounds in the sediments was measured on January 29, 2002. This date was 256 and 259 d from the spiking date of the Lakes Erie and Huron sediments, respectively, nearly seven months after the dates of experiment 1, and six months following the execution of experiment 4. FLU purity was >95% parent compound in Lake Huron sediments and >88% parent compound in the sediments from Lake Erie. However, TF was only 44 and 58% parent compound in Lakes Huron and Erie sediments, respectively. These results suggest that for FLU the dominant exposure was to parent compound while exposures to parent TF and its degradation products were nearly equal.

Since the degradation of TF in the sediments was extensive (*i.e.,* >10%) the purity of the compound at a given time after spiking the sediments can be calculated by the following relationship assuming first-order decay (Tippler, 1987):

$$\frac{P_t}{P_0} = e^{-\lambda t} \qquad (2.6)$$

where P_0 and P_t are the fractional purities at the time of spiking (0 d; >0.98≈1) and a given time, t (d), respectively, and λ is the first-order decay constant (1/d).

The decay constants were calculated from the purity measurements reported above and were 0.0032/d for Lake Huron and 0.0021/d for Lake Erie sediments. These λ values were then used to estimate the fractional purity of TF at the times of the experiments. The estimated percentages of parent TF in experiments 1 (*L. variegatus*, Lake Huron), 2 (*L. variegatus*, Lake Erie), 3 (*H. azteca*, Lake Huron), and 4 (*H. azteca*, Lake Erie) were 87, 90, 80 and 84%, respectively.

Observations on test organism behavior

During experiment 1, *L. variegatus* in controls burrowed into the sediments quickly after addition to the test beakers. Individuals in the 100 mg/kg treatment beakers burrowed by the first sampling time (4 h) whereas most *L. variegatus* in the 200 mg/kg treatment had not burrowed until the 24 h sample time. By 13 h, and for the remainder of the test, there were many fecal pellets on the surface of the control and 100 mg/kg sediments and the *L. variegatus* collected at each sampling time had relatively full guts. However, at 13 h very little fecal matter was observed in the 200 mg/kg beakers and the guts of the worms contained little-to-no sediment. By 48 h, the worms in this higher dose were lethargic and continued to exhibit little feeding activity throughout the study. By the end-of-exposure (96 h) the worms lacked the typical bright red color of healthy individuals.

The *L. variegatus* in experiment 2 displayed similar behavior to the observations made during experiment 1. Worms in the control and 100 mg/kg treatments were feeding and had burrowed into the sediments quickly after addition to the test beakers and appeared active and healthy throughout the test.

Individuals in the 200 mg/kg dose were slow-moving and lethargic beginning at 13 h, and some remained on the sediment surface up to the 48 h time point and fed less than the control and lower dose worms, as indicated by less sediment in their guts and less fecal pellet production.

In experiments 3 and 4, *H. azteca* in all treatments appeared to be active and healthy throughout the 48-h exposures. During experiment 3, the amphipods inhabited the surface of the sediments and few were observed to be swimming in the overlying water. However, in the exposure to Lake Erie sediments (experiment 4), the amphipods at 200 mg/kg of FLU and TF appeared to avoid the sediments during the exposure because most were repeatedly observed to be swimming in the water column.

Survival, wet weight and lipid contents of test organisms

Percent survival of *L. variegatus* and *H. azteca* exposed to sediments spiked with FLU and TF was recorded at each time point (Figures 2.1-2.4). Mean percent survival of *L. variegatus* in the exposures remained high (> 80%) in all treatments until 96 h in the 200 mg/kg exposure to Lake Huron sediments, and until 24 h in the high-dose sediments from Lake Erie. Separate ANOVAs on the time point mean percent survivals of *L. variegatus* exposed to spiked Lake Huron and Lake Erie sediments detected significant interactions between dose and time ($F_{10,36}$ =11.48; p <0.0001 for Lake Huron; $F_{10,36}$ =11.43; p <0.0001 for Lake Erie). Multiple comparisons of the survival means of the oligochaetes exposed to Lake Huron sediments identified the 96-h mean (± 1 SD) percent survival in the 200 mg/kg treatment (33.3 ± 32.2%) as significantly reduced

(p <0.05, Tukey's HSD test) compared to all other means (Figure 2.1). Similarly, in the Lake Erie sediment exposure of *L. variegatus* at 200 mg/kg, the mean (± 1 SD) percent survival at 48 h (23.3 ± 5.7%) and 96 h (30.0 ± 36.1%) were significantly lower (p <0.05) than all other means (Figure 2.2). Although the mean (± 1 SD) percent survival (76.7 ± 25.2%) in the 200 mg/kg treatment at 24-h was <80%, it was not a identified as significant. The time-response relationship was sufficient to allow calculation of a median lethal time (LT_{50}) of 55 h for the worms in the to 200 mg/kg exposure using a logistic regression model (logit) (Ellersieck and La Point, 1995).

The mean percent survival of *H. azteca* in exposures to spiked sediments remained high (>85%; Figures 2.3 and 2.4). Separate ANOVAs on the time point mean percent survival for *H. azteca* exposed to spiked sediments identified differences with time ($F_{5,36}$ =2.69; p =0.04; for Lake Huron) and significant interaction between dose and time ($F_{10,36}$ =2.16; p <0.04; for Lake Erie), but due to the high levels of survival (>80%; USEPA, 2000a), these statistical differences were judged to be biologically non-significant.

Organisms used in the exposures were wet weighed at each sample time point (Tables 2.6 and 2.7). In exposures of *L. variegatus* to spiked Lake Huron sediments, there were significant differences with respect to dose (ANOVA, $F_{2,35}$ =17.12; p <0.001) and post-hoc comparisons of means identified that the mean wet weights of the worms exposed at 200 mg/kg were significantly lower than either the control or 100 mg/kg treatment worms (Tukey's HSD test, p < 0.05). For the wet weights of oligochaetes exposed to spiked Lake Erie

sediments, hypothesis testing of sample means by ANOVA ($F_{10,34}$ =0.89; p =0.55) resulted in a failure to reject the null hypothesis of equal weights. In the two exposures of *H. azteca* to spiked sediments, separate ANOVAs for each sediment detected significant differences in mean wet weight/individual/beaker with respect to the main effect of time ($F_{5,36}$ =4.63; p =0.002 for Lake Huron; $F_{5,36}$ =4.76; p =0.002 for Lake Huron Erie). Tukey's HSD test determined that the mean wet weights of amphipods sampled from the Lake Huron exposures at 1 h were significantly (p <0.05) higher than the means for 3, 6, 24 and 48 h, whereas in the Lake Erie exposures mean wet weights from the at 12 h sampling time were significantly higher than the 3 and 48 h sample means.

The lipid contents of *L. variegatus* and *H. azteca* as a percent (± 1SD) of organism wet weight were determined at early (*e.g.,* 0 or 3 h) and the at the last time points in the experiments. The mean lipid contents from samples taken at zero and 96 h were not significantly different in the exposures of *L. variegatus* to spiked sediments from Lake Huron (ANOVA, $F_{1,3}$ =9.05; p =0.06) or Lake Erie (ANOVA, $F_{1,3}$ =5.15; p =0.11). The overall mean lipids for the oligochaetes were 1.05 ± 0.16% and 1.26 ± 0.03% for Lakes Huron and Erie experiments, respectively. These sample means were identified as significantly different between experiments by ANOVA ($F_{1,8}$ =9.16; p =0.02). For *H. azteca* exposed to the spiked sediments, mean lipid contents were not significantly different with respect to sample times for either Lakes Huron (ANOVA, $F_{1,4}$ =6.61; p =0.06) or Erie (ANOVA, $F_{1,4}$ =0.48; p =0.53) sediments, and there was no difference between mean lipids with respect to sediment types (ANOVA, $F_{1,10}$ =2.12;

p =0.18). The mean lipid contents determined for *H. azteca* samples taken at 3 and 48 h were 1.84 ± 0.43% in the Lake Huron exposure and 1.55 ± 0.23% in the Lake Erie experiment.

Bioaccumulation

The body burdens of FLU and TF in *L. variegatus* in experiments 1 and 2 typically increased rapidly over the first 24 h of exposure at each treatment concentration (Figures 2.5 and 2.6). Apparent steady state was reached by 48 h, except at the 200 mg/kg dose in Lake Huron sediments (experiment 1), where the tissue concentrations of both compounds peaked at 48 h (0.370 ± 0.020 µmol FLU/g wet wt; 0.154 ± 0.018 µmol TF/g wet wt) and then decreased by 96 h (0.261 ± 0.007 µmol FLU/g wet wt; 0.132 ± 0.009 µmol TF/g wet wt). The model-predicted plateaus for FLU and TF were between these concentrations and thus underestimated and overestimated the 48- and 96-h time point means, respectively. In the worms exposed to Lake Erie sediments at 200 mg/kg of the compounds (experiment 2), the observed C_{ss} values were 0.312 ± 0.037 µmol/g wet wt for FLU and 0.137 ± 0.018 µmol/g wet wt for TF. These values fell between the 48-h peak and the 96-h end-of-exposure tissue concentrations of the test compounds in the Lake Huron 200 mg/kg exposure group.

The observed tissue steady state concentrations (C_{ss}) in *L. variegatus*, calculated as the mean (± 1SD) of the 48 and 96 h samples, were similar for the 100 mg/kg treatment groups between experiments 1 and 2. These C_{ss} levels of FLU were 0.194 ± 0.027 µmol/g wet wt in the Lake Huron experiment and 0.161 ± 0.024 µmol/g wet wt in the Lake Erie exposure. TF was accumulated to C_{ss}

levels of 0.092 ± 0.015 and 0.067 ± 0.014 µmol/g wet wt by the 100 mg/kg exposure groups in Lakes Huron and Erie sediments, respectively. Model simulations for TF and FLU at 100 mg/kg were in good general agreement with observations.

Over the 48-h exposure period, the pattern of FLU accumulation by *H. azteca* showed a rapid increase over the first 12 h of the experiments and had reached an apparent steady state by 24 or 48 h for the Lakes Huron and Erie sediment exposures, respectively (Figures 2.7 and 2.8). Relative to the *L. variegatus* FLU accumulation kinetics described above, the data for *H. azteca* were more variable, especially at the 24 h time point in the Lake Erie exposure (experiment 4). The C_{ss} levels (calculated as the mean [± 1 SD] of the 24- and 48-h samples) for FLU in *H. azteca* from experiment 3 (Lake Huron) were 0.153 ± 0.033 µmol/g wet wt in the 100 mg/kg dose and 0.231 ± 0.051 µmol/g wet wt in the 200 mg/kg dose; and for Lake Erie were 0.242 ± 0.056 and 0.265 ± 0.087 µmol/g wet wt for the 100 and 200 mg/kg treatments, respectively. With the possible exception of the amphipods exposed in experiment 3 to spiked Lake Huron sediments at 100 mg/kg of the test compounds, TF accumulation did not exhibit saturation kinetics and appeared to be within the linear phase of uptake by the end of the exposure period for *H. azteca* in both exposure levels of spiked Lake Erie sediments. The amphipods exposed to spiked sediments from Lake Erie did not appear to accumulate FLU or TF in a dose-dependent manner and the tissue concentrations between dose groups were very similar. Tissue concentrations of TF were slightly higher in the *H. azteca* exposed at 100 mg/kg

than those exposed at 200 mg/kg of the compounds spiked onto Lake Erie sediments.

Estimates of toxicokinetic parameters (k_s and k_e) were obtained by nonlinear fits of the data to the two-compartment first-order kinetic model (Equations 2.2 and 2.3) and are listed in Table 2.8 for *L. variegatus* and Table 2.9 for *H. azteca*. The results of hypothesis testing of the equality of the rate estimates by Student's t-test procedures are shown in Tables 2.10 and 2.11 for *L. variegatus* and *H. azteca*, respectively. The collection of data for early time points during the rapid phase of uptake led to reliable estimates of the two fitted parameters, as reflected by the high values (range 0.746-0.973) of the adjusted coefficients of determination (R^2) and the small values (range 0.001-0.051) of the residual sum-of-squares (RSS) of the fits (Ratkowsky, 1983; Zar, 1999; Smyth, 2002). The R^2 values indicated that approximately 75-97% of the variability in the body burden data was accounted for by the least-squares nonlinear fit to the data and the RSS values (<1 and close to zero) indicated that the chosen model (Equation 2.2) was useful (Bailer, 1992).

In general, the conditional uptake clearance constants of FLU and TF from the sediments and pore water (k_s) exhibited similar trends for *L. variegatus* and *H. azteca*, with higher k_s estimates in the lower exposure concentration (100 mg/kg) than the 200 mg/kg dose. The exception was *H. azteca* exposed to FLU in Lake Huron sediments, in which k_s (± A.S.E.) was lower (0.064 ± 0.011 g dry sediment/g wet wt organism/h) at 100 mg/kg than in the 200 mg/kg dose (0.070 ± 0.013 g dry sediment/g wet wt organism/h), but this difference was not

significant ($p > 0.05$; Table 2.11). The ranges of k_s values for *L. variegatus* were 0.021 ± 0.003 to 0.031 ± 0.003 g dry sediment/g wet wt organism/h for FLU and 0.017 ± 0.002 to 0.024 ± 0.003 g dry sediment/g wet wt organism/h for TF, but no significant differences ($p >0.05$) were identified for k_s (Table 2.10). For *H. azteca*, the estimates of k_s ranged from 0.041 ± 0.009 to 0.070 ± 0.013 g dry sediment/g wet wt organism/h for FLU and 0.013 ± 0.001 to 0.047 ± 0.004 g dry sediment/g wet wt organism/h for TF. Numerous statistical contrasts between k_s estimates resulted in significant ($p <0.05$) differences (Table 2.11) and included: the 100 vs. 200 mg/kg treatments for TF in Lake Erie ($p <0.001$), FLU vs. TF in Lake Huron sediments at 100 mg/kg ($p <0.001$) and 200 mg/kg ($p <0.001$) and in Lake Erie sediments at 200 mg/kg ($p <0.01$), and Lake Huron vs. Lake Erie sediments for TF at 100 mg/kg ($p <0.001$).

The conditional rate constants for the elimination (k_e) of FLU and TF by *L. variegatus* in experiments 1 and 2 (Table 2.8) exhibited the same trends as k_s for this species. The values of k_e were higher in the lower dose (100 mg/kg) sediment exposures than those estimated at 200 mg/kg and there were no significant differences among any of the statistical contrasts ($p >0.05$; Table 2.10). The estimated elimination rates of both compounds by *L. variegatus* extended over a narrow range of values (0.047 ± 0.008 to 0.063 ± 0.007/h for FLU; 0.042 ± 0.007 to 0.059 ± 0.010/h for TF).

The estimated k_e values of FLU by *H. azteca* exposed to spiked sediments (Table 2.9) were more variable than (*i.e.,* higher A.S.E.s), and had opposite trends to those observed for *L. variegatus.* Although FLU elimination rates by

amphipods were higher in the 200 mg/kg treatments, these dose-related differences were not found to be significant ($p > 0.05$; Table 2.11). However, for both dose levels, the k_e values of FLU for amphipods exposed to spiked Lake Huron sediments were significantly ($p < 0.05$) higher than those estimated for the dosed sediments from Lake Erie, by a factor of approximately 2. Estimates of k_e for TF by *H. azteca* were not as variable as those for FLU and they were higher for the 100 mg/kg treatments compared to the 200 mg/kg dose. This dose-related difference was significant ($p < 0.05$) only in the exposure of amphipods to spiked Lake Erie sediments where elimination of TF was estimated to be less than 1% of the body burden per hour at 200 mg/kg. Like the differences in FLU k_e by amphipods between sediment types, TF elimination was significantly ($p < 0.001$) higher, by a factor of 3.8, at the 200 mg/kg treatment in the Lake Huron sediment exposure compared to the same dose spiked onto Lake Erie sediments. In contrasts between FLU and TF at each dose for each sediment, estimated elimination rates of FLU by *H. azteca* were significantly ($p < 0.01$) higher than TF in all comparisons by factors ranging from 3.5 (for 100 mg/kg in both Lakes Huron and Erie sediments) to 16 (for 200 mg/kg in Lake Erie sediments).

The median lethal residues (LR_{50} values) of FLU and TF were estimated for the *L. variegatus* exposed to Lake Erie sediments at 200 mg/kg by substituting the LT_{50} (55 h) into the fitted toxicokinetics relationship for this exposure group and solving for C_a (Equation 2.3; see Tables 2.4 and 2.8 for k_s, k_e and C_s values). The LR_{50} estimates with confidence intervals (CI) were

0.32 µmol/g wet wt (95% CI, 0.24-0.35 µmol/g wet wt) for FLU equivalents and 0.14 µmol/g wet wt (95% CI, 0.10-0.15 µmol/g wet wt) for TF equivalents. The LR_{50} for total test chemical equivalents (sum of FLU and TF molar equivalents) was 0.46 µmol/g wet wt (95% CI, 0.34-0.50 µmol/g wet wt).

Biotransformation of FLU and TF by *Lumbriculus variegatus*

After the exposures to FLU and TF in spiked Great Lakes sediments, pooled samples of *L. variegatus* from the final time point (96 h) of both Lake Huron treatments and the 100 mg/kg dose in Lake Erie sediments were extracted and analyzed for metabolites. For worms exposed to spiked Lake Huron sediments at 100 mg/kg, the percent of total [^{3}H]FLU body burden was 88.7% parent compound, 3.6% extractable metabolites and 7.7% unextractable. The total of the extractable metabolites and unextractable residue (11.3%) was assumed to represent the total metabolite burden (Kulkarni and Hodgson, 1980). The metabolism of FLU by *L. variegatus* in the Lake Huron exposure was less at 200 mg/kg, with 93.8% as parent compound, 0.1% as extractable metabolites and 6.1% residual. The percent of total [^{3}H]FLU body burden was 92.1, 2.7 and 5.2% parent compound, extractable metabolites and unextractable residues, respectively, for worms exposed to spiked Lake Erie sediments at 100 mg/kg.

Sediment-associated TF may have been metabolized by *L. variegatus*. Since the purity of TF in the sediments was estimated to range from only 80-90% parent compound, it was possible that the worms accumulated both parent TF and its breakdown products during the experiments. For worms exposed to the Lake Huron 100 and 200 mg/kg sediment treatments and the 100 mg/kg dose in

Lake Erie sediments, the respective [^{14}C]TF body burdens were 25.4, 25.3 and 34.5% parent compound, 38.9, 43.8 and 27.3% extractable products, and 37.5, 30.9 and 38.2% residual or unextractable. Biotransformation of FLU and TF in *H. azteca* was not determined due to an insufficient sample size for the analytical method.

Biota/sediment accumulation factors (BSAFs)

BSAFs for FLU were calculated from the end-of-exposure samples for *L. variegatus* and *H. azteca* (Figure 2.9). In *L. variegatus*, the BSAF (± 1 SD) for FLU ranged from 0.82 ± 0.12 to 1.75 ± 0.14 at 96 h and the 48-h BSAFs calculated for *H. azteca* ranged from 0.59 ± 0.08 to 1.06 ± 0.16. BSAFs were compared by one-way ANOVA and were significantly higher at 100 mg/kg for FLU in *L. variegatus* ($F_{1,3}$ =12.26; p =0.04) exposed to spiked Lake Huron sediments and in *H. azteca* ($F_{1,4}$ =11.67; p =0.03) exposed to contaminated Lake Erie sediments than at the higher dose. With respect to sediment type, there were numerous differences between BSAFs that resulted in rejection of the null hypothesis by ANOVA. *L. variegatus* FLU BSAFs were significantly higher in exposures to spiked Lake Huron sediments than those calculated from the Lake Erie sediment experiment. The results of ANOVA were as follows: FLU at 100 mg/kg, $F_{1,4}$ =75.43; p <0.001 and FLU at 200 mg/kg, $F_{1,2}$ = 21.48, p =0.04. The BSAF for FLU in *H. azteca* was not significantly different between sediment types. Due to the high levels of TF degradation products detected in the sediments and worm tissue samples, BSAFs were not calculated for this compound.

Indigenous tissues

Indigenous oligichaete worms (species not identified) collected at 96 h in the 100 mg/kg dose of experiment 1 (two individuals, 2.24 mg total wet wt) had tissue levels of 0.157 µmol FLU/g wet wt and 0.085 µmol TF/g wet wet. In the exposures of *H. azteca* to spiked Lake Huron sediments (experiment 3) the body burdens of FLU and TF were 0.174 and 0.098 µmol/g wet wt, respectively, in an indigenous oligochaete worm (3.72 mg wet wt) sampled from the 100 mg/kg treatment at 24 h. Three indigenous worm samples were taken during experiment 4 (*H. azteca*, Lake Erie sediments). A single indigenous worm (1.02 mg wet wt) was sampled from the 200 mg/kg dose at 3 h and its tissue concentrations were 0.214 µmol FLU/g wet wt and 0.038 µmol TF/g wet wt. In the 100 mg/kg treatment, concentrations of of FLU (0.469 µmol/g wet wt) and TF (0.386 µmol/g wet wt) in indigenous worms sampled at 12 h (0.99 mg wet wt) were higher than levels measured at 24 h (two individuals, 2.89 mg total wet wt,) (0.299 µmol FLU/g wet wt and 0.119 µmol TF/g wet wt).

DISCUSSION

Toxicity of sediment-associated FLU and TF

Sediment-associated fluoranthene (FLU) and trifluralin (TF) in binary mixtures were toxic to the infaunal oligochaete, *Lumbriculus variegatus,* at the highest nominal dose (200 mg/kg dry wt) but not to the epibenthic amphipod, *Hyalella azteca* in short term exposures (=96 h). The measured mean dry wt concentrations of the compounds in the 200 mg/kg treatments ranged from

0.659-0.746 µmol FLU-equivalents/g (133-151 mg FLU/kg) and 0.352-0.386 µmol TF-equivalents/g (118-129 mg TF/kg) in sediments with total organic carbon (TOC) contents ranging from 2.00-3.66% (Tables 3 and 4). Concentrations of FLU in this range were reported to cause mortality in the estuarine copepod, *Coullana* sp. (LC_{50} = 132 mg/kg dry sediments; Lotufo, 1998a). In studies with the marine amphipod, *Rhepoxynius abronius*, percent mortality ranged from 23.3-91.7% in exposures to sediment concentrations of FLU (12.09-26.4 mg/kg dry wt) that were below measured levels in the present study (DeWitt et al., 1992). The 10-d LC_{50} for exposure of *H. azteca* to sediment-associated FLU ranged from 2.3-7.4 mg/kg dry wt in three different sediments (Suedel et al., 1993) and these concentrations were between 20-60 fold below the levels of FLU that were associated with mortality to *L. variegatus* at 96 h. However, the sediments used in the DeWitt et al. (1992) and Suedel et al. (1993) studies were of low TOC (=0.50%) and high sand content (42.7-98.8%) and thus FLU would be expected to be more bioavailable (Power and Chapman, 1992) than it was in the present study of FLU and TF mixtures.

More recently, single-compound studies with *H. azteca* and *L. variegatus* have demonstrated that toxic effects do not occur for these species at the levels of FLU spiked onto sediments in the present 48- and 96-h long exposures. Kane Driscoll and Landrum (1997) determined a 16-d LC_{50} of 719 mg/kg dry wt for *H. azteca* exposed to FLU-spiked sediments originating from Lake Michigan (1.14% TOC) that were more similar to the sediments used in the present study. Further, a high mean percent survival (=90%) was observed at 10 d in sediment

concentrations up to 210 mg/kg dry wt. In a study of the sediment reworking rate by *L. variegatus* exposed to FLU-spiked sediments of approximately 2% TOC, there were no significant effects on the survival and growth of worms up to 355 mg/kg dry wt (Landrum et al., 2002). Furthermore, the concentrations of FLU in Lake Huron and Erie sediments were almost 4-fold below the consensus-based threshold effects concentration sediment quality guideline of 423 mg/kg dry wt (MacDonald et al., 2000). Therefore, the levels of FLU incorporated into the Lakes Huron and Erie sediments should not have been toxic to *L. variegatus* based on the available literature for single-compound exposures.

Toxicity data for sediment-associated TF were not available, so the interstitial (*i.e.,* pore) water concentrations of TF (log K_{ow} =5.3; Mackay et al., 1997) were estimated for the sediments in the present study using equilibrium partitioning theory (Di Toro et al., 1991). Then, these estimates were compared to toxic aqueous concentrations of TF obtained from the literature. Estimated pore water concentrations of TF in the 100 and 200 mg/kg treatments of Lakes Huron and Erie sediments ranged from 10-39 µg/L. This concentration range is 1-2 orders of magnitude below the acute toxicity (*e.g.,* 24-, 48-, 96-h LC_{50} or EC_{50}) values reported for many aquatic insect and crustacean species including amphipods, crayfish, daphnids, shrimp, sowbugs and stoneflies (Spacie, 1975; Parrish et al., 1978; Johnson and Finley, 1980; Mayer and Ellersieck, 1986; Nowell et al., 1999). Spacie (1975) observed chronic effects in *Daphnia magna* exposed to 7.2 µg/L and emergence of the midge, *Chironomus riparius*, was observed to decline only at TF concentrations >9000 µg/L in the water overlying

previously clean sediments (Hamer and Heimbach, 1996). Therefore, the TF levels that were spiked onto the test sediments in the present study should not have been toxic to *L. variegatus* based on equilibrium partitioning and single-compound exposures.

Since the above discussion suggests that for each compound, FLU and TF, there should be little or no toxicity, then a possible explanation for the observed mortality in *L. variegatus* may be the simultaneous administration (*i.e.,* mixture) of both chemicals. The nature of this potential interaction (*e.g.,* additivity, synergism) is unknown but may be related to the modes of action of the compounds (Pape-Lindstrom and Lydy, 1997). Because FLU is a PAH and TF is a dinitroaniline herbicide, their modes of action may have been different in the exposed animals. PAHs, in the absence of their photoinduced toxicity (Oris and Giesy, 1986; Ankley et al., 1995; Hatch and Burton, 1999), are thought to affect aquatic organisms through narcosis (anesthesia) (van Wezel and Opporhuizen, 1995). Narcosis is defined as a nonspecific reversible disruption of the functioning of the lipid membrane resulting in loss of selective permeability that can lead to death (Kane Driscoll et al., 1998; Fisher et al., 1999). TF acts as an inhibitor of cell and nuclear division in plants (Montgomery, 1997), as a mitotic blocking agent and as a disruptor of cell membrane structure and integrity in the fertilized eggs of the sea urchin *Lytechinus variegatus* (Medina, 1986; Medina et al., 1994) and via narcosis in the algae *Scenedesmus vacuolatus* (Schmitt et al., 2000). Therefore, on the hypothesis that both FLU and TF exerted their toxic effects to *L. variegatus* by narcosis, an evaluation of

the total molar equivalents of the compounds accumulated by the oligochates (*i.e.,* the additive internalized dose) may explain the observed mortality.

The critical body residue (CBR) approach relates toxic effects to measured body burdens. For lethality, the LR_{50} is the tissue concentration at which 50% mortality of an exposed population occurs (McCarty et al., 1992a). The acute toxicity and mortality to aquatic vertebrates and invertebrates exposed to neutral, lipophilic (nonpolar) chemicals that act by narcosis is commonly observed at body burdens (LR_{50} values) of 2-8 µmol/g wet wt (McCarty and MacKay, 1993; van Wezel and Opperhuizen, 1995). This reproducible, additive model of critical or lethal body burdens has been applied to numerous nonpolar organic compounds, including mixtures of chlorinated aromatics and alkanes in fish exposed for 96 h (McCarty et al., 1992b), mixtures of 4-nonylphenol with PAHs or PCBs in 10-d exposures of the marine amphipod *Ampelisca abdita* (Fay et al., 2000), chlorinated benzenes accumulated by sand crabs, *Portunus pelagicus,* for 7 d (Mortimer and Connell, 1994) and *D. magna* exposed for 24 or 48 h (Pawlisz and Peters, 1995), separate chronic (35-d) exposures of *L. variegatus* to various PCBs congeners (Fisher et al., 1999), and exposures of benthic copepods (for 10 d) and amphipods (for 8-32 d) to PAHs (Landrum et al., 1994; Kane Driscoll and Landrum, 1997; Kane Driscoll et al., 1998; Lotufo, 1998a). The observed LR_{50}s for narcosis in fish exposed to polar organic chemicals (0.7-1.9 µmol/g wet wt) for various durations are slightly below the general range for nonpolar chemicals (McCarty and MacKay, 1993).

Other compounds that elicit toxicity by more specific modes of action for which LR_{50}s have been measured include a number of pesticides. LR_{50}s of DDT, which causes nervous system effects via blockage of voltage-gated sodium channels, were recently reported for mortality in the amphipods *H. azteca* (4- and 10-d LR_{50} range, 0.006-0.008 µmol/g wet wt) and *Diporeia sp*. (10- and 28-d LR_{50} range, 0.041-0.090 µmol/g wet wt) (Lotufo et al., 2000). These LR_{50}s for DDT are 1-3 orders of magnitude below body burdens that cause polar and nonpolar narcosis. McCarty and MacKay (1993) reviewed body residue-effects relationships of pesticides in fish and found that acute LR_{50}s ranged widely from 0.000048-2.7 µmol/g wet wt. The chemicals and specific modes of action included: respiratory uncouplers such as 2,4-dintrophenol; acetylcholine esterase inhibitors such as parathion, chlorpyrifos and carbaryl; central nervous system convulsants such as fenvalerate, endrin and permethrin; and respiratory blockers including rotenone (McCarty and MacKay, 1993).

The LR_{50} for the summed FLU and TF equivalents measured in *L. variegatus* (0.46 µmol/g wet wt) and the total molar body residues in *H. azteca* at the end-of-exposure (˜0.37-0.53 µmol/g wet wt, both FLU and TF) were less than the lower bound (2 µmol/g wet wet) CBR for lethality by narcosis. Therefore, when the tissue residues are expressed on a wet weight concentration basis, the hypothesis that the mixture of FLU and TF acted by narcosis to cause mortality in *L. variegatus* was not supported by the data. These wet weight body burdens were near the range for polar narcosis and within the range of the many specific mechanisms reviewed above. However,

the lower-bound CBR for narcosis (2 µmol/g wet wt) was derived for fish with approximately 5% lipid contents and thus when the body residue is normalized to lipid, the value is 40 µmol/g lipid (van Wezel and Opporhuizen,1995). The lipid-normalized LR_{50} for *L. variegatus* (36 µmol/g lipid) is similar to the reported body burdens for lethal narcosis.

The discussion above underscores the current issue regarding the the uncertainties surrounding predictions of toxicity based on exposure or tissue concentrations. When the body residue approach was applied, the lipid-normalized tissue concentrations in *L. variegatus* suggested that their mortality in the spiked Lake Erie sediments may have been due to narcosis. However, evaluation of the FLU and TF concentrations measured in the sediments and predicted for pore water suggested that TF and FLU, when considered individually, should not have been acutely toxic, and thus the compounds may have exerted their joint toxicity through different modes of action (*i.e.,* not only by narcosis). Therefore, further study is needed to identify the specific mode of TF toxicity to invertebrates and whether its simultaneous administration with FLU led to an enhancement over their individual effects, or synergism, as has been recently observed in invertebrates, fish and avians exposed to pesticide or PAH mixtures (Johnston et al., 1994; Levine and Oris, 1999; Belden and Lydy, 2000; Verrhiest et al., 2001).

Bioaccumulation

The accumulation patterns of FLU and TF in exposures to spiked Lake Erie sediments were different for *H. azteca* compared to other exposures of both

the amphipods and *L. variegatus*. *H. azteca* exposed to the spiked sediments from Lake Erie showed poor separation of the body burdens of TF and FLU between the 100 and 200 mg/kg nominal doses. Furthermore, *H. azteca* in the 100 mg/kg dose group of the Lake Erie exposure (Figure 2.8) accumulated FLU (0.270 ± 0.047 µmol/g wet wt) and TF (0.243 ± 0.028 µmol/g wet wt) equivalents by 48 h that were higher, by factors of 1.6 and 3.5, respectively, than the end-of-exposure tissue levels of these compounds measured for the amphipods exposed at the same dose in Lake Huron sediments. These tissue levels also exceeded the C_{ss} of FLU and TF equivalents in *L. variegatus* exposed to 100 mg/kg of the test chemicals in both Lakes Huron and Erie sediments by factors of approximately 1.5 for FLU and up to 3.6 for TF. Finally, compared to the apparent C_{ss} of TF in *L. variegatus* exposed at 200 mg/kg to Lake Huron sediments, the TF body burden in *H. azteca* exposed to Lake Erie sediments at 100 mg/kg were higher by a factor of 1.6.

Even with the accumulation differences observed between species in the present investigation, the higher body burdens achieved by *H. azteca*, did not lead to toxicity. Although toxicity was observed in *L. variegatus* at lower body residues, the exposure duration for the worms was twice that of the amphipods and thus may have been an important factor in the mortality. Recent investigations have shown that the tissue concentrations associated with a response metric can be inversely related to exposure duration (Chaisuksant et al., 1997). The exposure times were selected in order to obtain kinetic data that

would result in good fits to the accumulation model (Equation 2.3) and not to allow for direct comparison of toxicity between species.

Although good agreement between the data and the accumulation model predictions was obtained, it is important to note that the measured concentrations of FLU and TF represented equivalents of the compounds (*i.e.,* both parent and non-parent material). Therefore, degradation of FLU and TF in the sediments and biotransformation of the compounds by the organisms adds uncertainty to the estimated kinetic constants. Since the degradation of TF in the sediments was significant (10-20% degraded) and that of FLU was minimal (=1%), the potential impacts of chemical degradation on the accumulation kinetics most likely affected the estimates of k_s for TF. Assuming that the degradation products were more polar than parent TF, the values of k_s for the TF-equivalents may underestimate uptake for only the parent compound. The potential biotransformation of TF by *L. variegatus* (only 25-37% parent compound in the tissues) and known ability of *H. azteca* to metabolize both FLU and TF (Kane Driscoll et al., 1997b; see Chapter 3) also adds uncertainty to the estimates of the elimination rates. Previous investigators have shown that in aquatic invertebrates, polar and aqueous metabolites of hydrophobic organic contaminants are eliminated more slowly than the parent compound (Landrum and Crosby, 1981; Lydy et al., 2000). Therefore, the estimates of k_e for parent FLU and TF may be faster than the rates reported here based on their total equivalents. These issues of degradation and biotransformation are discussed further as they relate to BSAFs (see below).

The observations of behavior during experiment 4 indicated that the *H. azteca* avoided the sediments in the exposure to spiked Lake Erie sediments at 200 mg/kg. Although avoidance behavior was not specifically measured using a preference test in which animals are presented clean and dosed sediments within the same exposure arena and then their distribution is analyzed following exposure (Gossiaux et al., 1993; Lotufo, 1997), it was evident that the amphipods in the higher dose of spiked Lake Erie sediments spent most of their time in the overlying water as their recovery from the test beakers did not require sieving the sediments. This perceptible avoidance likely reduced their exposure to the sediments and resulted in the accumulation of both FLU and TF to similar concentrations as those exposed to the lower concentration.

Avoidance of contaminated sediments by amphipod species has been observed in exposures to various contaminants including petroleum hydrocarbons and PAHs (Lenihan et al., 1995; Hatch and Burton, 1999; Kravitz et al., 1999). This avoidance behavior, however does not explain why the *H. azteca* of the present study were still able to accumulate similar, or in some cases more, of the test compounds than either the amphipods or *L. variegatus* in experiments 1-3. Perhaps the lower organic carbon contents of the Lake Erie sediments (used in experiments 2 and 4), relative to the Lake Huron sediments, favored more partitioning of the compounds into the interstitial water which diffused to the overlying waters. This hypothesis was partially supported by estimates of the pore water concentrations of FLU (14-32 µg/L for Lake Huron; 26-57 µg/L for Lake Erie) and TF (10-22 µg/L for Huron; 17-39 µg/L for Lake

Erie) in the sediments using equations given by Di Toro et al. (1991). Such a scenario may have exposed the *H. azteca* without their constant, direct interaction with the sediments. These higher expected pore water concentrations of FLU and TF in the Lake Erie sediments may also help explain the significant mortality observed at the 24, 48 and 96 h time points in experiment 2 with *L. variegatus*. Unfortunately, even though the FLU and TF concentrations in the Lake Erie sediments were considered to be reasonably constant during the experiment, the interstitial and overlying waters were not directly measured leaving these issues unresolved.

Peak concentrations before the end of the 96-h exposures were observed for *L. variegatus* exposed to FLU and TF in Lake Huron sediments at 200 mg/kg (Figure 2.5). This peak may have been due to the changes in worm behavior between the 48 and 96 h samples as a high degree of mortality (>66%) was observed at the last sampling point. However, this observation may also be explained by the combined effects of low rates of feeding (influx) with continual elimination (efflux), and depletion of the bioavailable pool of the chemicals from pore water as explained in the paragraphs below.

A number of recent studies have demonstrated the importance of ingested sediment as a primary route of contaminant uptake by benthic deposit-feeding species. For example, Weston et al. (2000) reported that after 24 h, up to 38% of the body burden of benzo[a]pyrene (BaP) in the polychaete, *Abarenicola pacifica,* was derived from ingested material while after 72 h this proportion increased to 77%. The bioaccumulation of FLU from ingested sediment by the polychaete

Capitella sp. was predicted to be the dominant uptake pathway based on a model incorporating dietary assimilation efficiency and feeding selectivity data (Forbes et al., 1998). In a study that utilized feeding vs. non-feeding individuals, approximately 39 and 61% of the bioaccumulation of pyrene (PYR) by *L. variegatus* was due to uptake from pore water and ingested material, respectively (Leppänen and Kukkonen, 1998b). Although feeding rate was not measured in the present study, behavioral observations (*e.g.,* presence of fecal pellets in beakers and gut contents in worms) made at each sample time during the experiment 1 (Lake Huron sediments) indicated that worms in the 200 mg/kg dose group were feeding less than the control and 100 mg/kg exposure groups. The significantly reduced wet weights of worms in the 200 mg/kg treatment compared to the control and lower dose groups support this observed lack of feeding. Therefore, it is assumed that for these worms that appeared to be non-feeding, or at least feeding at reduced rates, the uptake of FLU and TF was via passive diffusion. Specifically, this would include uptake from the pore water and by integument contact with contaminated sediment particles from which FLU and TF desorbed (Landrum and Robbins, 1990).

Previous bioaccumulation studies conducted with *L. variegatus* and other species have shown peaks in uptake of contaminants followed by a decline to a steady state value or continuous reduction in body burdens. Landrum (1989) first introduced the concept of a limited pore water source of freely dissolved, bioavailable contaminant that is controlled by desorption rates from sediment particles and by uptake by the exposed organisms. He observed that uptake of

the PAH phenanthrene (PHE; log Kow =4.57) by the amphipod *Pontoporei hoyi* (now known as *Diporeia* spp.) peaked at approximately 8 d during a 30-d exposure and that serial 2-wk exposures of groups of amphipods to the same sediment resulted in reduction in bioaccumulation and hence bioavailablitly (Landrum, 1989). However, these trends were not observed for higher log K_{ow} PAHs including PYR, benzo[a]anthracene and BaP (Landrum, 1989). Later investigations of the uptake of sediment-associated PYR by *L. variegatus* (Kukkonen and Landrum, 1994) and FLU by *H. azteca* (Harkey et al., 1997) and *L. variegatus* (Landrum et al., 2002) also demonstrated peaks at early time points, which suggested that low-molecular weight PAHs (*e.g.,* <230 mol. wt., FLU, PHE, PYR) can be rapidly depleted from the bioavailable pore water fraction at a rate that exceeds desorption in exposure concentrations ranging from trace levels to hundreds of ppm. In such a scenario, within the biologically active region of the sediments (*i.e.,* the area inhabited by organisms), desorption of contaminants from particles is not rapid enough to maintain the initial pore water concentrations. This same trend was observed in studies of PAH bioaccumulation by *L. variegatus* exposed to contaminated sediments that were obtained from the historically contaminated Little Scioto River, Marion, OH, USA (Van Hoof et al., 2001). These authors noted that low-molecular weight PAHs such as FLU, PHE and PYR peaked by day four along a 14-d time course. Van Hoof et al. (2001) suggested that the accumulation patterns of these specific PAHs was a result of their uptake from pore water dominating the early period of the assay and that the freely dissolved, rapidly desorbed amounts of these

compounds in the pore water were depleted during the exposure. Meador et al. (1995) also reported that bioaccumulation of FLU and PYR by a nonselective deposit-feeding marine polychaete (*Armanda brevis*) and the amphipod *R. abronius* occurred through uptake from pore water and that ingestion was an insignificant route of uptake for these species.

Given the assumption that the *L. variegatus* exposed to spiked Lake Huron sediments at 200 mg/kg in the present study were feeding at a negligible rate, the peaks in body burdens of both FLU and TF may have occurred by the mechanism of depletion of the labile, bioavailable fraction in the pore water as described above. In addition, the oligochaetes exposed to the 100 mg/kg sediments were feeding throughout the study and presumably would have accumulated FLU and TF by uptake from both pore water and ingested sediment, yet they still accumulated less of the test compounds than the *L. variegatus* exposed at 200 mg/kg. This observation suggests the importance of the pore water route of uptake for FLU and TF in these exposures and that it was likely sufficient to result in the dose-dependent accumulation of the compounds by *L. variegatus*. The impact of the peaks in the body burdens on the estimates of the conditional uptake clearance rate constants (k_s) of FLU and TF for the worms exposed at 200 mg/kg in experiment 1 should be minimal since the four sampling points up to 24 h of the exposure provided a good estimate of the initial rate of uptake, where elimination is negligible.

Apparent steady state FLU BSAF values calculated for *L. variegatus* in exposures to spiked Lake Huron sediments reached levels that were either near

or greater than the theoretical maximum BSAF value (1.7) predicted for all neutral organic compounds. This value was based on the equilibrium partitioning of contaminants between organic carbon and lipid and empirical relationships between organic carbon-water partitioning (K_{oc}) and lipid-normalized bioaccumulation factors (BAFs) (USEPA, 1989; Boese and Lee, 1992; Lee, 1992). BSAFs may be below this value if metabolism of the compound occurs, if steady state body burdens of the test organisms are not reached during the exposure, or if only a fraction of the contaminant associated with sediment orgainic carbon is available for uptake (Lee, 1992).

Since tissue and sediment concentrations in the present study were expressed as equivalents of the test compounds (*i.e.,* they may represent both parent compound and metabolite/degradation products) there is a degree of uncertainty regarding the BSAF calculations. Because relatively large amounts of non-parent TF equivalents were measured in the *L. variegatus* (65-75%) and estimated in the sediments (up to 20%), BSAFs were not calculated for TF. However, since the amounts of parent FLU measured in *L. variegatus* (88-92%%) and in the sediments (88% to >95%) were high, the uncertainty in the BSAFs for the worms was most likely minimal. Less certainty is associated with the BSAFs calculated for *H. azteca* since biotransformation was not measured in the amphipods. *H. azteca* is known to be capable of metabolizing FLU (Kane Driscoll et al., 1997b) and a companion experiment to the present study also indicates their ability to metabolize both FLU and TF (see Chapter 3). Therefore,

the BSAFs of FLU equivalents reported here for *H. azteca* may be overestimated if biotransformation occurred over the 48-h exposures.

BSAFs for FLU have been reported for a number of benthic species, including *L. variegatus* and *H. azteca*. The marine polychaete, *Nereis virens*, was exposed to FLU-contaminated sediments in the presence or absence of amphipods and the steady state BSAFs from these experiments ranged from 0.97-1.6 (Ciarelli et al., 2000). In a study of the bioaccumulation of contaminants associated with Mississippi River sediments, Brunson et al. (1998) measured mean (± 1 SD) BSAF values for FLU of 1.6 ± 0.34 (range 0.9-3.9) in field-collected oligochaetes and 1.8 ± 0.27 (range 0.6-4.9) in *L. variegatus* that were exposed to the sediments for 28 d. The BSAF values reported in these previous studies were similar to the those calculated for FLU in the *L. variegatus* exposed for 4 d to spiked Great Lakes sediments (0.82-1.8) in the present investigation. The lower values of the BSAF ranges for FLU reported by Brunson et al. (1998) were similar to the observed values for the worms exposed to spiked Lake Erie sediment.

Mean BSAF values of FLU obtained for *H. azteca* ranged from 0.59-0.80 in the present study except for the 100 mg/kg exposure to spiked Lake Erie sediments in which the mean was >1. In studies in which the ranges of FLU exposure concentrations contained the levels used in the present study, the mean BSAFs for *H. azteca* were 0.13-0.67 and for *Diporeia* sp. were 0.11-1.33 after 16 d or 30 d (Kane Driscoll and Landrum, 1997; Kane Driscoll et al., 1997a). The maximum BSAF for the esturarine amphipod, *Leptocherius plumulosus*, was

0.38 following a 26-d exposure to FLU-contaminated sediments (Kane Driscoll et al., 1998), which is below the range measured in the present study. However, for the marine amphipod, *Corophium volutator*, BSAFs for FLU were approximately 1.5 in field-collected sediments, and 2.25 in the same sediments that were spiked with a mixture of PAHs (Kraaij et al., 2001). Bioaccumulation data from field monitoring studies of freshwater isopods, of which *Asellus aquaticus* was the dominant species, resulted in BSAFs for FLU ranging from 0.10 to 0.70 (van Hattum et al., 1998) and benthic copepods exposed to sediment-associated FLU in the laboratory had BSAFs in the range of 0.22 to 0.80 (Lotufo, 1998b). Therefore, the BSAF values for the *H. azteca* in the present study are at the high end, or above the range of the BSAFs measured for *H. azteca* and isopods in other studies but are within the range reported for other species of amphipods and copepods.

Only one report of the bioaccumulation of TF by invertebrates appears in the literature and this study was for terrestrial species. In this mesocosm study, the dry weight concentrations of TF in spiked soil and in exposed isopods, *Porcellio scaber*, and earthworms, *Eisenia fetida* were reported (Staak et al., 1998). Based on the data of Staak et al. (1998) lipid-normalized tissue concentrations of TF were calculated using data on the lipid contents of *P. scaber* (7.15-24% by dry wt) and *E. fetida* (7.2% by dry wt) obtained from other literature sources (Lavy et al., 1997; Fitzgerald et al., 1996). These TF concentrations ranged from 0.04 to 0.28 µmol/g lipid in *P. scaber* and from 0.82 to 2.1 µmol/g lipid in *E. fetida*. Lipid-normalized C_{ss} values of TF in *L. variegatus*

(4.2-37 µmol/g lipid) and 48-h body burdens of TF in *H. azteca* (2.4-23 µmol/g lipid) were generally 1-2 orders of magnitude higher than the levels reported for the terrestrial invertebrates.

The calculated BSAFs for FLU were at the theoretical maximum value of 1.7 for *L. variegatus* exposed at 100 mg/kg of the test compounds in Lake Huron sediments, whereas for the oligochaetes exposed at 200 mg/kg the BSAF values were significantly lower. BSAFs for FLU bioaccumulation by *L. variegatus* exposed to Lake Erie sediments were significantly less than those calculated for the Lake Huron exposure; however, a higher degree of toxicity and less overall activity was observed in the former exposure with this species. Regarding the instances where BSAFs were significantly higher in the lower dose (*e.g., L. variegatus* exposed to Lake Huron sediments; *H. azteca* exposed to Lake Erie sediments), this observation was not unexpected. Higher BSAFs in less contaminated sediments were observed for *L. variegatus* exposed *in situ* for 7 d to a PCB concentration gradient in the Housatonic River, MA, USA (Burton et al., 2001). Similar trends were reported by Bremle and Ewald (1995) who studied indigenous midge larvae and oligochaetes collected from PCB-contaminated lakes. Moreover, it has been frequently observed in field studies and in laboratory-spiked sediment tests, that an inverse relationship exists between BSAF and sediment contaminant concentration (Rubenstein *et al*., 1987; McElroy and Means, 1988; Ferraro *et al.,* 1990a,b). Thus, “cleaner” sediments often result in higher BSAFs than more contaminated sediments. This may be due to sublethal physiological responses or changes in organism

behavior (*e.g.,* decreased feeding rate, decreased sediment reworking, contaminant avoidance) with increasing levels of sediment contamination (Keilty *et al*., 1988a,b). Both decreased feeding and avoidance behavior were qualitatively observed in the present study.

The BSAFs for *H. azteca* in all exposures except the Lake Erie 100 mg/kg treatments resulted in values that would be predicted from the published values for FLU (*i.e.,* <1.0, see review above; Lee, 1992). It was surprising that values of approximately 1 for FLU were obtained in this specific case, but these values are not extreme, as mean BSAFs for chlorinated organic compounds (e.g, PCBs, chlordane, hexachlorobenzene) are frequently >2 and can be as high as 10 for aquatic bivalves, polychaetes and crustaceans (Lee, 1992). However, since BSAFs for FLU and other PAHs in various aquatic species are usually below 1.0, the BSAFs calculated for *H. azteca* should be viewed with caution. Possible reasons for the higher-than-expected value are outlined by (Lee, 1992) and include: 1) the active uptake of carbon and associated contaminants in the gut, 2) additivity of multiple contaminant uptake routes, 3) compartmentalization of pollutants within an organism such that organs or tissue compartments are not at thermodynamic equilibrium, and 4) a lack of metabolism and/or elimination of the compound in species known to biotransform the chemical, or a slow release of metabolites by a species once they are formed (Landrum and Crosby, 1981; Lydy et al., 2000). It is unknown what the exact mechanism was that led to the high BSAF values in the Lake Erie low-dose-exposed amphipod group, but a plausible explanation based on the above discussion of the dominance of pore

water over ingestion as the primary uptake route, is that these *H. azteca* ingested highly contaminated particles that added to the body burden obtained by uptake from dissolved contaminants in the pore water.

Kinetics and biotransformation

The original hypothesis was that the doses chosen would not be toxic to *L. variegatus* or *H. azteca* and that kinetic rates should be similar between compounds based on their similar log Kow values. As discussed above, mortality in the exposures of *L. variegatus* was observed, the worms appeared to be less active in the higher-dose sediments and *H. azteca* avoided highly contaminated sediments, therefore the first part of this hypothesis was refuted. Even with this mortaility in *L. variegatus*, there were no significant differences among the rate constants, and therefore the second part of the hypothesis is generally supported by the oligochaete data. However, the mortality and avoidance may have led to the trend toward a decline in conditional uptake rate (k_S) at the higher treatment concentration that was observed for both FLU and TF in both test species, although this was only significant for *H. azteca* uptake of TF in the lake Erie exposure (experiment 4). The cause of this trend may simply have been the toxicity of the compounds to the *L. variegatus* (Van Hoof et al., 2001), or the ability of amphipods such as *H. azteca* to sense PAH contamination and thus avoid it (Landrum et al., 1991). Landrum et al. (1991) observed an increase in k_s for *Diporeia* sp. with increasing concentrations of sediment-associated PAHs, but, the highest dose used (0.327 µmol/g dry wt) was about equal to the low dose of the present study. The trend of decreasing uptake with

increasing contaminant concentration was observed for *L. variegatus* exposed to sediment-associated FLU (Landrum et al., 2002) and PYR (Kukkonen and Landrum, 1994) at concentration ranges containing the levels used in the present study. These findings indicated that the organisms needed to clear less sediment per unit time to achieve their steady state tissue burdens. *L. variegatus* were also observed to be feeding less in the high dose treatments of both Lakes Huron and Erie sediments compared to the low dose and control groups. Such behavior would also lead to decreased uptake rate coefficients (Keilty et al., 1988a,b). The inverse relationship between k_s and contaminant concentration also supports the hypothesis that the dominant route of uptake of FLU and TF was from desorbed amounts of the chemicals in the pore water (see discussion above).

The elimination rate constant obtained by nonlinear curve fitting of the accumulation data did not exhibit a similar general trend for both species. For *L. variegatus*, k_e values for FLU and TF were not significantly higher for the 100 mg/kg treatment than the for higher dose in all cases. In *H. azteca*, FLU was eliminated at a higher rate in the low-dose sediments, but TF was eliminated faster in the higher dose, although this was significant only for the Lake Erie exposure. Estimates of k_e from the nonlinear fits of accumulation data are prone to error, and direct measurement of elimination following exposures is preferable (Van Hoof et al., 2001). However, examination of the kinetic BSAF (for FLU only) as described by Kraaij et al. (2001), can give an indication of the adequacy of the kinetic constant estimates if the calculated BSAFs are in good agreement with

the values determined from the ratios of organism lipid and organic carbon normalized tissue and sediment concentrations as described by Equation 2.1. The kinetic BSAF was calculated as follows (Kraaij et al., 2001):

$$BSAF = \frac{k_s}{k_e} \bullet \frac{f_{OC}}{f_{lipid}} \quad (2.7).$$

In general, the kinetic BSAF was in good agreement with the mean BSAFs calculated from measured tissue and sediment concentrations of FLU equivalents. The kinetic BSAFs were between 94 and 112% of the values calculated from measured tissue and sediment concentrations for FLU accumulation by *L. variegatus*. For *H. azteca*, the kinetic BSAFs for FLU were between 93 and 110% of the measured concentration-derived BSAFs.

Kinetic BSAFs were not calculated for TF because of the uncertainties surrounding the kinetic rate estimates obtained from fitting the accumulation data. The source of this uncertainty was the high degree of degradation of the compound in the sediments (only 80-90% estimated to be parent TF during the exposures). Therefore, it was possible that a large proportion of the accumulated TF equivalents were degradation products, and thus the estimates of k_s may not have been very representative of parent TF. Likewise, since it is unknown if the (sediment) degradation products that presumably accumulated by *L. variegatus* and *H. azteca* were eliminated more rapidly or more slowly than parent TF, the values of k_e must also be viewed with caution. Unfortunately, there are no studies on the toxicokinetics of TF available in the literature for comparison to these results. Future investigations of the toxicokinetics of TF in benthic invertebrates should include measurements of the concentration-time profiles of

both parent TF and its degradation products in the sediments and metabolites in exposed organisms to improve upon these estimates.

Bioavailability of FLU to amphipods appeared to be greater than TF as indicated by statistical comparisons between chemicals except for the Lake Erie 100 mg/kg dose. This may be due to more FLU being available via pore water as its hydrophobicity (log K_{ow}, 5.2; MacKay et al., 1992) is slightly less than that of TF (log K_{ow}, 5.3; MacKay et al., 1997), or may be related to degradation of the TF in the sediments. FLU in the sediments had degraded little (=8%) from its initial purity at 6-7 months after the experiments, whereas TF spiked onto the sediments had degraded relatively rapidly. TF was estimated to have degraded by 10-20% of its initial purity at spiking by the time of the bioaccumulation tests, and by 54% and 40% of its initial purity in the Lakes Huron and Erie sediments, respectively, 6-7 months following the experiments. Extensive degradation (up to 80% within one year; not attributed to volatilization) of TF has been observed in soils and sediments (Camper et al., 1980; Walker et al., 1988; Dzantor and Felsot, 1991; Diaz et al., 1995), which is in concordance with the observed amount of degradation in Great Lakes sediments over 6-7 months.

Assuming that degradation at the time of the experiments was high for TF and that *H. azteca* obtained FLU and TF from pore water or overlying water, as suggested above for the Lake Erie exposure, then this could explain the apparent difference in bioavailability. Another concern regarding the high proportion of TF degradation products in the sediments is the uncertainty of their potential role in the toxicity observed for *L. variegatus*. It should be noted that since the extent to

which uptake and accumulation of TF is represented by degradation products is unknown, the kinetic modeling on a total equivalents basis for both FLU and TF may not have provided the most accurate estimates of uptake clearance and elimination rates for the parent compounds. This is of concern because *L. variegatus* was shown to metabolize both FLU and TF in the present sediment exposures, and *H. azteca* is known to extensively metabolize FLU (Kane Driscoll et al. 1997b; see Chapter 3) and can metabolize TF (see Chapter 3). Therefore, it was possible that polar or aqueous TF metabolites were taken up by the test organisms and were eliminated, or they were taken up and then represented some unknown fraction of the body burden at each sample point. Since the assumption that metabolism of the test compounds should be negligible during the experiment was violated by both degradation in the sediments (TF only) and biotransformation by the organisms (both FLU and TF), time course evaluations of metabolite formation and elimination of these compounds in binary mixture by the oligochaetes and amphipods need to be attempted to resolve both the relative bioavailability and parent compound issues.

The values of k_s and k_e for FLU were compared to estimates reported in the literature and were generally within the ranges obtained in investigations of spiked sediments. Landrum et al. (2002) observed mean k_s values of 0.022-0.067 g dry sediment/g wet organism/h and mean elimination rates of 0.013-0.074/h. These values contain the conditional kinetic rates determined for the accumulation data in the present study. Uptake clearance rates for PAHs, including FLU, in *L. variegatus* exposed to a PAH mixture that was resident to

contaminated field-collected sediments (Van Hoof et al., 2001) were an order of magnitude below the values obtained in the present study, and the elimination rate (0.026/h) was about half of the values reported here. However, Van Hoof et al. (2001) obtained k_s values for spiked PYR and BaP in the same field-collected sediments, indicating that the aging of the contaminants and/or their contact time with sediment particles may be important to toxicokinetics and bioavailability.

This research is the first to report kinetic rate estimates for the uptake and elimination of FLU by *H. azteca* and it represents the first report of TF toxicokinetics in aquatic invertebrates. FLU uptake from sediments by other amphipod species has been reported and ranges from 0.038 g dry sediment/g wet organism/h for the marine species, *Corophium volutator*, to 0.099 g/g/h for estuarine *L. plumulosus* (Kraaij et al., 2001; Kane Driscoll et al., 1998). These same authors reported elimination (k_e) of FLU at rates of 0.042/h and 0.01/h, for *C. volutator* and *L. plumulosus*, respectively. Uptake of FLU from sediments by the freshwater amphipod, *Diporeia* sp. exposed at 4 ºC ranged from 0.006 to 0.057 g/g/h and elimination in this species averaged 0.003/h (Kane Driscoll and Landrum, 1997; Kane Driscoll et al., 1997a,b). Therefore, the uptake clearance of *H. azteca* in the present study is similar to rates reported for other amphipods, but the elimination appears to be fastest in *H. azteca*. This is may partly be due to the ability of *H. azteca* to extensively biotransform FLU (half life 4-6 h; Kane Driscoll et al., 1997b).

The elimination of TF by *H. azteca* exposed at 200 mg/kg to spiked Lake Erie sediments was an order of magnitude below any of the other k_e values for

either *H. azteca* or *L. variegatus*. The elimination of FLU was also significantly reduced for *H. azteca* in this treatment compared to elimination in Lake Huron sediments. There is no good explanation for this. Their avoidance of the sediments in the high concentration treatment in Lake Erie sediments could have potentially reduced the possibility of the sediments acting as a reverse sink of contaminants by competing for lipid-associated contaminants in the body. The role of sediment in enhancing elimination of PAHs from *H. azteca* and *L. variegatus* has been demonstrated in the presence of, or by active ingestion of clean sediment particles (Landum and Scavia, 1983; Kukkonen and Landrum, 1994; Lotufo and Landrum, 2002). Another possible explanation assumes that the sediments in this treatment elicited a specific sublethal effect on amphipod metabolism such that active elimination of TF via biotransformation to excretable forms or active transport from the organism was hindered. This seems possible based on the mortality observed in *L. variegatus* to this same sediment.

Conclusions

L. variegatus and *H. azteca* accumulated FLU and TF and reached an apparent steady state for FLU. Steady state for TF was only reached in exposures of *L. variegatus*. Based on the accumulation data and observations on organism behavior during the bioassays, it appeared that the dominant role of uptake was from dissolved FLU and TF in the pore water. Organisms in the low dose treatments were seen interacting with and feeding upon the sediments, so ingestion also contributed to the body burdens. Toxicity of the mixtures of FLU and TF was observed in exposures of *L. variegatus* to spiked Great Lakes

sediments (LT_{50}, 55 h; LR_{50}, 0.46 µmol/g wet wt combined total FLU and TF equivalents), but the exact nature of the toxicity (*i.e.,* mode of action) could not be described.

The uptake rate constant (k_s) generally declined with increasing dose, but this was significant only for TF accumulation by *H. azteca* exposed to Lake Erie sediments. In general, the conditional uptake and elimination rate coefficients of FLU obtained for *L. variegatus* in the present study are in good agreement with the values reported in other published kinetic studies. This study is the first to report k_s and k_e values for TF in either *L. variegatus* or *H. azteca* and is the first record of the uptake and elimination of sediment-associated FLU by *H. azteca*. Literature values of k_s and k_e for FLU by other species of amphipods are in most cases similar to those reported here for *H. azteca*. Since there was degradation of TF (9.7-20%) in the sediments used in this study, the kinetic values should be viewed with caution as the estimates likely do not represent the kinetics of only parent TF.

Apparent steady state BSAF values of FLU calculated for *L. variegatus* and *H. azteca* in this study ranged from around 0.6 to values that were either near or greater than the theoretical maximum BSAF value (1.7) predicted for all neutral organic compounds. Kinetics-derived BSAFs generally reflected those obtained by the ratio of the tissue concentrations to the organic carbon-normalized sediment concentrations.

Table 2.1. Toxicokinetic studies conducted with two benthic invertebrates exposed to sediments spiked with fluoranthene and trifluralin.

Experiment	Organism	Sediment	Conc. of FLU and TF (mg/kg dry sed)	Test duration (h)	No. of sampling points	Initial no. animals per beaker	No. of replicates per conc. x time point
1	*L. variegatus*	Lake Huron	0, 100, 200	96	6	10	3
2	*L. variegatus*	Lake Erie	0, 100, 200	96	6	10	3
3	*H. azteca*	Lake Huron	0, 100, 200	48	6	20	3
4	*H. azteca*	Lake Erie	0, 100, 200	48	6	20	3

Abbreviations:
FLU = fluoranthene
TF = trifluralin
Conc. = concentrations
No. = number

Table 2.2. Experiment set up, initiation and sampling (termination) schedule.

		Set-up		Initiation		Termination	
Experiment	Organism/ sediment	Date	Amount of wet sediments added to beakers (g)	Date	Time points (h)	Date	Time points (h)
1	*L. variegatus*/ Lake Huron	25-Jun-01	50	27-Jun-01	4, 8, 13, 24, 48, 96	27-Jun-01	4, 8
						28-Jun-01	13, 24
						29-Jun-01	48
						01-Jul-01	96
2	*L. variegatus*/ Lake Erie	03-Jul-01	50	05-Jul-01	4, 8, 13, 24, 48, 96	05-Jul-01	4, 8, 13
						06-Jul-01	24
						07-Jul-01	48
						09-Jul-01	96

Table 2.2. (Continued).

Experiment	Organism/ sediment	Set-up		Initiation		Termination	
		Date	Amount of wet sediments added to beakers (g)	Date	Time points (h)	Date	Time points (h)
3	*H. azteca*/ Lake Huron	20-Jul-01	30	24-Jul-01	1, 6, 24, 48	24-Jul-01	1, 6
				25-Jul-01	3, 12	25-Jul-01	3, 24
						26-Jul-01	12, 48
4	*H. azteca*/ Lake Erie	03-Aug-01	30	07-Aug-01	1, 6, 24, 48	07-Aug-01	1, 6
				08-Aug-01	3, 12	08-Aug-01	3, 24
						09-Aug-01	12, 48

Table 2.3. Physical and chemical characteristics of water and sediments used in sediment-borne contaminant exposures of *Lumbriculus variegatus* and *Hyalella azteca*. Data are presented as means ± 1 SD. TOC = total organic carbon; TN = total nitrogen; and C:N ratio = carbon to nitrogen ratio.

	Experiment 1[a]		Experiment 2		Experiment 3		Experiment 4	
Characteristics	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n
Water								
Temperature (°C)	22.17 ± 0.41	6	22.00 ± 0.00	5	22.00 ± 0.00	3	22.00 ± 0.00	3
Dissolved oxygen (mg/L)	6.43 ± 0.88	6	6.84 ± 0.65	5	5.33 ± 1.18	3	7.20 ± 0.38	4
pH	7.97 ± 0.26	2	8.26 ± 0.01	2	7.92 ± 0.14	2	8.30 ± 0.18	2
Hardness (mg/L)	183 ± 2.9	2	180 ± 5.8	2	198 ± 3.3	2	169 ± 8.7	2
Alkalinity (mg/L)	184 ± 0.0	2	148 ± 5.7	2	158 ± 2.8	2	120 ± 39.6	2
Conductivity (µS/cm)	400 ± 28.3	2	315 ± 7.1	2	355 ± 7.1	2	310 ± 28.3	2
Total Ammonia (mg/L)	0.62 ± 0.16	2	0.01 ± 0.01	2	0.37 ± 0.08	2	0.02 ± 0.00	2
Sediments								
TOC (% of dry weight)	3.66 ± 0.20	3	2.00 ± 0.03	3	3.64 ± 0.08	3	2.08 ± 0.20	3
TN (% of dry weight)	0.56 ± 0.07	3	0.35 ± 0.04	3	0.62 ± 0.06	3	0.33 ± 0.07	3
C:N ratio	6.61 ± 1.08	3	5.67 ± 0.48	3	5.87 ± 0.67	3	6.31 ± 0.69	3
Wet:dry weight ratio	5.10 ± 0.19	42	4.00 ± 0.13	42	5.29 ± 0.19	41	4.23 ± 0.11	42

[a]Experiment 1: *Lumbriculus variegatus* exposed to spiked Lake Huron sediments, 96 h
Experiment 2: *Lumbriculus variegatus* exposed to spiked Lake Erie sediments, 96 h
Experiment 3: *Hyalella azteca* exposed to spiked Lake Huron sediments, 48 h
Experiment 4: *Hyalella azteca* exposed to spiked Lake Erie sediments, 48 h

Table 2.4. Summary of the concentrations of fluoranthene and trifluralin in sediment samples taken at all time points in the exposures of *Lumbriculus variegatus* to spiked Lake Huron and Lake Erie sediments. Treatment doses of the test compounds were 100 and 200 mg/kg dry sediment. Concentrations are expressed as µmol/g dry sediment and the data are presented as means ± 1 SD. Hypothesis testing by analysis of variance (ANOVA) with respect to the main effects of dose and time and their interaction was performed at α =0.05. ANOVA results that indicated significant differences (p <0.05) are shown. Results of post-hoc multiple comparison procedures are described in the text.

	Fluoranthene (µmol/g dry sediment)				Trifluralin (µmol/g dry sediment)			
	100 mg/kg		200 mg/kg		100 mg/kg		200 mg/kg	
Time point (h)	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n
Experiment 1: *Lumbriculus variegatus* exposed to spiked Lake Huron sediments, 96 h								
-48	0.415 ± 0.015	3	0.725 ± 0.029	3	0.227 ± 0.004	3	0.405 ± 0.018	3
4	0.398 ± 0.005	3	0.691 ± 0.026	3	0.210 ± 0.005	3	0.368 ± 0.014	3
8	0.410 ± 0.017	3	0.713 ± 0.043	3	0.211 ± 0.009	3	0.383 ± 0.018	3
13	0.410 ± 0.019	3	0.860 ± 0.193	3	0.206 ± 0.008	3	0.441 ± 0.096	3
24	0.406 ± 0.005	3	0.729 ± 0.060	3	0.207 ± 0.006	3	0.369 ± 0.020	3
48	0.393 ± 0.014	3	0.788 ± 0.039	3	0.198 ± 0.008	3	0.393 ± 0.028	3
96	0.407 ± 0.022	3	0.713 ± 0.047	3	0.210 ± 0.009	3	0.344 ± 0.005	3
Overall Mean	0.406 ± 0.015	21	0.746 ± 0.089	21	0.210 ± 0.010	21	0.386 ± 0.045	21
	ANOVA: $F_{1,28}$ = 342.4, p < 0.001 (Dose)				$F_{1,28}$ = 388.8, p < 0.001 (Dose)			
Experiment 2: *Lumbriculus variegatus* exposed to spiked Lake Erie sediments, 96 h								
-48	0.338 ± 0.004	3	0.666 ± 0.061	3	0.182 ± 0.001	3	0.385 ± 0.024	3

4	0.333 ± 0.005	3	0.639 ±	0.048	3	0.174 ± 0.006	3	0.347 ± 0.010	3
8	0.339 ± 0.009	3	0.707 ±	0.167	3	0.176 ± 0.003	3	0.379 ± 0.084	3
13	0.337 ± 0.010	3	0.677 ±	0.019	3	0.180 ± 0.005	3	0.367 ± 0.029	3
24	0.341 ± 0.014	3	0.686 ±	0.069	3	0.171 ± 0.002	3	0.351 ± 0.021	3
48	0.332 ± 0.005	3	0.597 ±	0.037	3	0.168 ± 0.005	3	0.319 ± 0.023	3
96	0.339 ± 0.001	3	0.643 ±	0.042	3	0.158 ± 0.003	3	0.315 ± 0.020	3
Overall Mean	0.337 ± 0.007	21	0.659 ±	0.073	21	0.173 ± 0.008	21	0.352 ± 0.041	21
	ANOVA: $F_{1,28} = 358$, $p < 0.001$ (Dose)					$F_{1,28} = 469$, $p < 0.001$ (Dose) $F_{6,28} = 2.55$, $p = 0.043$ (Time)			

Table 2.5. Summary of the concentrations of fluoranthene and trifluralin in sediment samples taken at all time points in the exposures of *Hyalella azteca* to spiked Lake Huron and Lake Erie sediments. Treatment doses of each compound were 100 and 200 mg/kg dry sediment. Concentrations are shown as µmol/g dry weight and data are presented as means ± 1 SD. Hypothesis testing by analysis of variance (ANOVA) with respect to the main effects of dose and time and their interaction was performed at α =0.05. ANOVA results that indicated significant differences (p <0.05) are shown. Results of post-hoc multiple comparison procedures are described in the text.

	Fluoranthene (µmol/g dry sediment)				Trifluralin (µmol/g dry sediment)			
	100 mg/kg		200 mg/kg		100 mg/kg		200 mg/kg	
Time point (h)	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n
Experiment 3: *Hyalella azteca* exposed to spiked Lake Huron sediments, 48 h								
-96	0.440 ± 0.029	3	0.755 ± 0.051	3	0.236 ± 0.007	3	0.408 ± 0.016	3
1	0.390 ± 0.019	3	0.837 ± 0.106	3	0.199 ± 0.007	3	0.417 ± 0.052	3
3	0.400 ± 0.027	3	0.704 ± 0.027	3	0.212 ± 0.021	3	0.367 ± 0.014	3
6	0.390 ± 0.016	3	0.707 ± 0.037	3	0.194 ± 0.002	3	0.363 ± 0.033	3
12	0.371 ± 0.010	3	0.683 ± 0.004	3	0.197 ± 0.003	3	0.350 ± 0.001	3
24	0.383 ± 0.012	3	0.702 ± 0.040	3	0.194 ± 0.005	3	0.354 ± 0.012	3
48	0.356 ± 0.042	3	0.750 ± 0.038	3	0.183 ± 0.017	3	0.373 ± 0.020	3
Overall Mean	0.390 ± 0.032	21	0.734 ± 0.066	21	0.202 ± 0.019	21	0.376 ± 0.033	21
	ANOVA: $F_{6,28}$ = 2.69, p = 0.034 (Dose*Time)				$F_{1,28}$ = 783.4, p < 0.001 (Dose) $F_{6,28}$ = 5.24, p < 0.001 (Time)			
Experiment 4: *Hyalella azteca* exposed to spiked Lake Erie sediments, 48 h								
-96	0.361 ± 0.025	3	0.610 ± 0.040	3	0.204 ± 0.017	3	0.355 ± 0.010	3

1	0.366 ± 0.018	3	0.681 ± 0.113	3	0.174 ± 0.005	3	0.346 ± 0.054	3
3	0.369 ± 0.013	3	0.635 ± 0.053	3	0.168 ± 0.003	3	0.342 ± 0.024	3
6	0.338 ± 0.012	3	0.883 ± 0.176	3	0.166 ± 0.004	3	0.428 ± 0.067	3
12	0.352 ± 0.007	3	0.634 ± 0.022	3	0.179 ± 0.002	3	0.324 ± 0.015	3
24	0.355 ± 0.003	3	0.573 ± 0.024	3	0.172 ± 0.002	3	0.306 ± 0.021	3
48	0.355 ± 0.012	3	0.710 ± 0.076	3	0.169 ± 0.004	3	0.366 ± 0.028	3
Overall Mean	0.357 ± 0.016	21	0.675 ± 0.122	21	0.176 ± 0.014	21	0.352 ± 0.048	21
	ANOVA: $F_{6,28} = 4.45$, $p = 0.003$ (Dose*Time)				$F_{6,28} = 3.97$, $p = 0.005$ (Dose*Time)			

Table 2.6. Summary of the wet weight measurements of *Lumbriculus variegatus* in exposures to spiked Lake Huron and Lake Erie sediments. Treatment doses of fluoranthene and trifluralin were 100 and 200 mg/kg dry sediment. Wet weights are expressed as mg/individual/beaker and the data are presented as means ± 1 SD[a]. Hypothesis testing by analysis of variance (ANOVA) with respect to the main effects of dose and time and their interaction was performed at α =0.05. ANOVA results that indicated significant differences (p <0.05) are shown. Results of post-hoc multiple comparison procedures are described in the text.

	Control		100 mg/kg		200 mg/kg	
Time point (h)	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n
Experiment 1: *Lumbriculus variegatus* exposed to spiked Lake Huron sediments, 96 h						
4	4.38 ± 0.33	3	4.73 ± 0.27	3	3.95 ± 0.07	3
8	4.59 ± 0.47	3	4.20 ± 0.27	3	3.94 ± 0.43	3
13	4.25 ± 0.42	3	4.11 ± 0.30	3	3.99 ± 0.08	3
24	4.46 ± 0.43	3	4.20 ± 0.27	3	3.78 ± 0.31	3
48	4.59 ± 0.44	3	3.94 ± 0.39	3	3.02 ± 0.72	3
96	5.01 ± 0.45	3	4.41 ± 0.30	3	3.91 ± 0.78	2
Overall Mean	4.55 ± 0.43	18	4.26 ± 0.36	18	3.76 ± 0.52	17
ANOVA: $F_{2,35} = 17.12$, $p < 0.001$ (Dose)						
Experiment 2: *Lumbriculus variegatus* exposed to spiked Lake Erie sediments, 96 h						
4	4.11 ± 0.28	3	4.11 ± 0.56	3	3.94 ± 0.19	3
8	3.96 ± 0.26	3	4.00 ± 0.47	3	4.24 ± 0.15	3
13	4.24 ± 0.08	3	4.08 ± 0.27	3	4.22 ± 0.52	3

24	4.19 ± 0.30	3	4.02 ± 0.13	3	3.41 ± 0.66	3
48	4.61 ± 0.40	3	4.05 ± 0.73	3	4.63 ± 0.99	2
96	4.45 ± 0.85	3	4.12 ± 0.44	3	3.73 ± 0.06	2
Overall Mean	4.26 ± 0.42	18	4.06 ± 0.40	18	4.01 ± 0.57	16

[a]Units are mg/individual/beaker.

Table 2.7. Summary of the wet weight measurements of *Hyalella azteca* in exposures to spiked Lake Huron and Lake Erie sediments. Treatment doses of fluoranthene and trifluralin were 100 and 200 mg/kg dry sediment. Wet weights are expressed as mg/individual/beaker and the data are presented as means ± 1 SD[a]. Hypothesis testing by analysis of variance (ANOVA) with respect to the main effects of dose and time and their interaction was performed at α =0.05. ANOVA results that indicated significant differences (p <0.05) are shown. Results of post-hoc multiple comparison procedures are described in the text.

	Control		100 mg/kg		200 mg/kg	
Time point (h)	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n
Experiment 3: *Hyalella azteca* exposed to spiked Lake Huron sediments, 48 h						
1	0.239 ± 0.118	3	0.295 ± 0.025	3	0.266 ± 0.074	3
3	0.203 ± 0.051	3	0.156 ± 0.025	3	0.185 ± 0.027	3
6	0.181 ± 0.028	3	0.212 ± 0.029	3	0.179 ± 0.022	3
12	0.216 ± 0.022	3	0.195 ± 0.025	3	0.219 ± 0.075	3
24	0.156 ± 0.016	3	0.175 ± 0.032	3	0.211 ± 0.022	3
48	0.189 ± 0.040	3	0.181 ± 0.011	3	0.200 ± 0.030	3
Overall Mean	0.197 ± 0.055	18	0.202 ± 0.051	18	0.210 ± 0.050	18
ANOVA: $F_{5,36} = 4.63$, $p = 0.002$ (Time)						
Experiment 4: *Hyalella azteca* exposed to spiked Lake Erie sediments, 48 h						
1	0.275 ± 0.074	3	0.251 ± 0.079	3	0.260 ± 0.032	3
3	0.216 ± 0.032	3	0.216 ± 0.053	3	0.249 ± 0.027	3
6	0.320 ± 0.071	3	0.243 ± 0.050	3	0.236 ± 0.018	3

12	0.300 ± 0.046	3	0.300 ± 0.037	3	0.322 ± 0.010	3
24	0.255 ± 0.033	3	0.266 ± 0.041	3	0.258 ± 0.049	3
48	0.199 ± 0.016	3	0.179 ± 0.037	3	0.242 ± 0.079	3
Overall Mean	0.261 ± 0.061	18	0.242 ± 0.058	18	0.261 ± 0.046	18

ANOVA: $F_{5,36} = 4.76$, $p = 0.002$ (Time)

[a]Units are mg/individual/beaker.

Table 2.8. Summary of uptake clearance (k_s) and elimination (k_e) constants[a] (± asymptotic standard errors; A.S.E.) for fluoranthene and trifluralin by *Lumbriculus variegatus*. Also shown are the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Treatment	k_s	± A.S.E.	k_e	± A.S.E.	RSS	R^2
Fluoranthene	Lake Huron, 100 mg/kg	0.027	± 0.003	0.055	± 0.007	0.006	0.891
	Lake Huron, 200 mg/kg	0.021	± 0.003	0.049	± 0.010	0.032	0.825
	Lake Erie, 100 mg/kg	0.031	± 0.003	0.063	± 0.007	0.003	0.907
	Lake Erie, 200 mg/kg	0.025	± 0.003	0.047	± 0.008	0.026	0.876
Trifluralin	Lake Huron, 100 mg/kg	0.022	± 0.003	0.050	± 0.008	0.002	0.872
	Lake Huron, 200 mg/kg	0.017	± 0.002	0.042	± 0.007	0.004	0.880
	Lake Erie, 100 mg/kg	0.024	± 0.003	0.059	± 0.010	0.001	0.814
	Lake Erie, 200 mg/kg	0.019	± 0.003	0.045	± 0.008	0.005	0.863

[a]Values were obtained by fitting Equation 2.3 to the organism wet-weight-normalized data. Units for k_s are in g dry sediment/g wet organism/h. Units for k_e are 1/h.

Table 2.9. Summary of uptake clearance (k_s) and elimination (k_e) constants[a] (± asymptotic standard errors; A.S.E.) for fluoranthene and trifluralin by *Hyalella azteca*. Also shown are the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Treatment	k_s	± A.S.E.	k_e	± A.S.E.	RSS	R^2
Fluoranthene	Lake Huron, 100 mg/kg	0.064	± 0.011	0.161	± 0.034	0.010	0.768
	Lake Huron, 200 mg/kg	0.070	± 0.013	0.212	± 0.046	0.028	0.746
	Lake Erie, 100 mg/kg	0.061	± 0.009	0.082	± 0.016	0.018	0.874
	Lake Erie, 200 mg/kg	0.041	± 0.009	0.095	± 0.026	0.051	0.764
Trifluralin	Lake Huron, 100 mg/kg	0.018	± 0.003	0.046	± 0.012	0.001	0.864
	Lake Huron, 200 mg/kg	0.013	± 0.001	0.038	± 0.008	0.002	0.927
	Lake Erie, 100 mg/kg	0.047	± 0.004	0.023	± 0.005	0.003	0.973
	Lake Erie, 200 mg/kg	0.015	± 0.001	0.006	± 0.005	0.003	0.969

[a]Values were obtained by fitting Equation 2.3 to the organism wet-weight-normalized data. Units for k_s are in g dry sediment/g wet organism/h. Units for k_e are 1/h.

Table 2.10. Summary of statistical comparisons of the uptake (k_s) and elimination (k_e) rate constants[a] for fluoranthene and trifluralin by *Lumbriculus variegatus*. Student's t-test was used to compare estimated values of the rate constants (± asymptotic standard errors) determined by toxicokinetic modeling. Separate hypothesis tests of kinetic rate equality with respect to dose, chemical and sediment type were performed at α =0.05.

Categories		Statistical comparison	Kinetic constant	*df*[b]	t_{crit}[c]	t_{obs}[d]	Significance[e]
Between dose for each compound in each sediment							
Lake Huron	FLU[f]	100 vs. 200 mg/kg	k_s	31	2.04	1.23	NS
			k_e	31	2.04	0.49	NS
	TF[g]	100 vs. 200 mg/kg	k_s	31	2.04	1.81	NS
			k_e	31	2.04	0.72	NS
Lake Erie	FLU	100 vs. 200 mg/kg	k_s	31	2.04	1.47	NS
			k_e	31	2.04	1.46	NS
	TF	100 vs. 200 mg/kg	k_s	31	2.04	1.04	NS
			k_e	31	2.04	1.06	NS
Between compounds at each dose in each sediment							
Lake Huron	100 mg/kg	FLU vs. TF	k_s	32	2.04	1.10	NS
			k_e	32	2.04	0.47	NS
	200 mg/kg	FLU vs. TF	k_s	30	2.04	1.29	NS
			k_e	30	2.04	0.52	NS

Lake Erie	100 mg/kg	FLU vs. TF	k_s	32	2.04	1.81	NS
			k_e	32	2.04	0.37	NS
	200 mg/kg	FLU vs. TF	k_s	30	2.04	1.30	NS
			k_e	30	2.04	0.14	NS
Between sediments for each compound at each dose							
FLU	100 mg/kg	Lake Huron vs. Erie	k_s	32	2.04	1.08	NS
			k_e	32	2.04	0.77	NS
FLU	200 mg/kg	Lake Huron vs. Erie	k_s	30	2.04	0.73	NS
			k_e	30	2.04	-0.15	NS
TF	100 mg/kg	Lake Huron vs. Erie	k_s	32	2.04	0.27	NS
			k_e	32	2.04	0.71	NS
TF	200 mg/kg	Lake Huron vs. Erie	k_s	30	2.04	0.89	NS
			k_e	30	2.04	0.25	NS

[a]Kinetic constants, k_s (conditional uptake clearance rate from sediments; g dry sediment/g wet wt organism/h) and ke (conditional elimination rate constant; 1/h). Estimates of k_s and k_e for *Lumbriculus variegatus* are given in Table 2.8.

[b]*df* = degrees of freedom.

[c]t_{crit} = critical value of t at α =0.05

[d]t_{obs} = observed value of t.

[e]Designations of significance are as follows: NS = no significant difference; * = significant (*p* <0.05); ** = strongly significant (*p* <0.01); *** = highly significant (p <0.001).

[f]FLU = fluoranthene.
[g]TF = trifluralin.

Table 2.11. Summary of statistical comparisons of the uptake (k_s) and elimination (k_e) rate constants[a] for fluoranthene and trifluralin by *Hyalella azteca*. Student's t-test was used to compare estimated values of the rate constants (± asymptotic standard errors) determined by toxicokinetic modeling. Separate hypothesis tests of kinetic rate equality with respect to dose, chemical and sediment type were performed at α =0.05.

Categories		Statistical comparison	Kinetic constant[a]	*df*[b]	t_{crit}[c]	t_{obs}[d]	Significance[e]
Between dose for each compound in each sediment							
Lake Huron	FLU[f]	100 vs. 200 mg/kg	k_s	32	2.04	-0.38	NS
			k_e	32	2.04	-0.88	NS
	TF[g]	100 vs. 200 mg/kg	k_s	32	2.04	1.39	NS
			k_e	32	2.04	0.53	NS
Lake Erie	FLU	100 vs. 200 mg/kg	k_s	32	2.04	1.61	NS
			k_e	32	2.04	-0.43	NS
	TF	100 vs. 200 mg/kg	k_s	32	2.04	7.91	***
			k_e	32	2.04	2.52	*
Between compounds at each dose in each sediment							
Lake Huron	100 mg/kg	FLU vs. TF	k_s	32	2.04	4.02	***
			k_e	32	2.04	3.15	**
	200 mg/kg	FLU vs. TF	k_s	32	2.04	4.46	***
			k_e	32	2.04	3.73	***

Lake Erie	100 mg/kg	FLU vs. TF	k_s	32	2.04	1.45	NS
			k_e	32	2.04	3.53	**
	200 mg/kg	FLU vs. TF	k_s	32	2.04	3.07	**
			k_e	32	2.04	3.36	**
Between sediments for each compound at each dose							
FLU	100 mg/kg	Lake Huron vs. Erie	k_s	32	2.04	-0.18	NS
			k_e	32	2.04	-2.09	*
FLU	200 mg/kg	Lake Huron vs. Erie	k_s	32	2.04	-1.86	NS
			k_e	32	2.04	-2.21	*
TF	100 mg/kg	Lake Huron vs. Erie	k_s	32	2.04	6.28	***
			k_e	32	2.04	-1.70	NS
TF	200 mg/kg	Lake Huron vs. Erie	k_s	32	2.04	0.75	NS
			k_e	32	2.04	-3.44	***

[a]Kinetic constants, k_s (conditional uptake clearance rate from sediments; g dry sediment/g wet wt organism/h) and k_e (conditional elimination rate constant; 1/h). Estimates of k_s and k_e for *Hyalella azteca* are given in Table 2.9.

[b]*df* = degrees of freedom.

[c]t_{crit} = critical value of t at α =0.05

[d]t_{obs} = observed value of t.

[e]Designations of significance are as follows: NS = no significant difference; * = significant (p <0.05); ** = strongly significant (p <0.01); *** = highly significant (p <0.001).

[f]FLU = fluoranthene.
[g]TF = trifluralin.

Figure 2.1. Mean percent survival of *Lumbriculus variegafus* exposed to Lake Huron sediments spiked with 100 and 200 mg/kg fluoranthene and trifluralin (experiment 1). Error bars represent standard deviation. Letters that are different denote significant differences between mean percent survival.

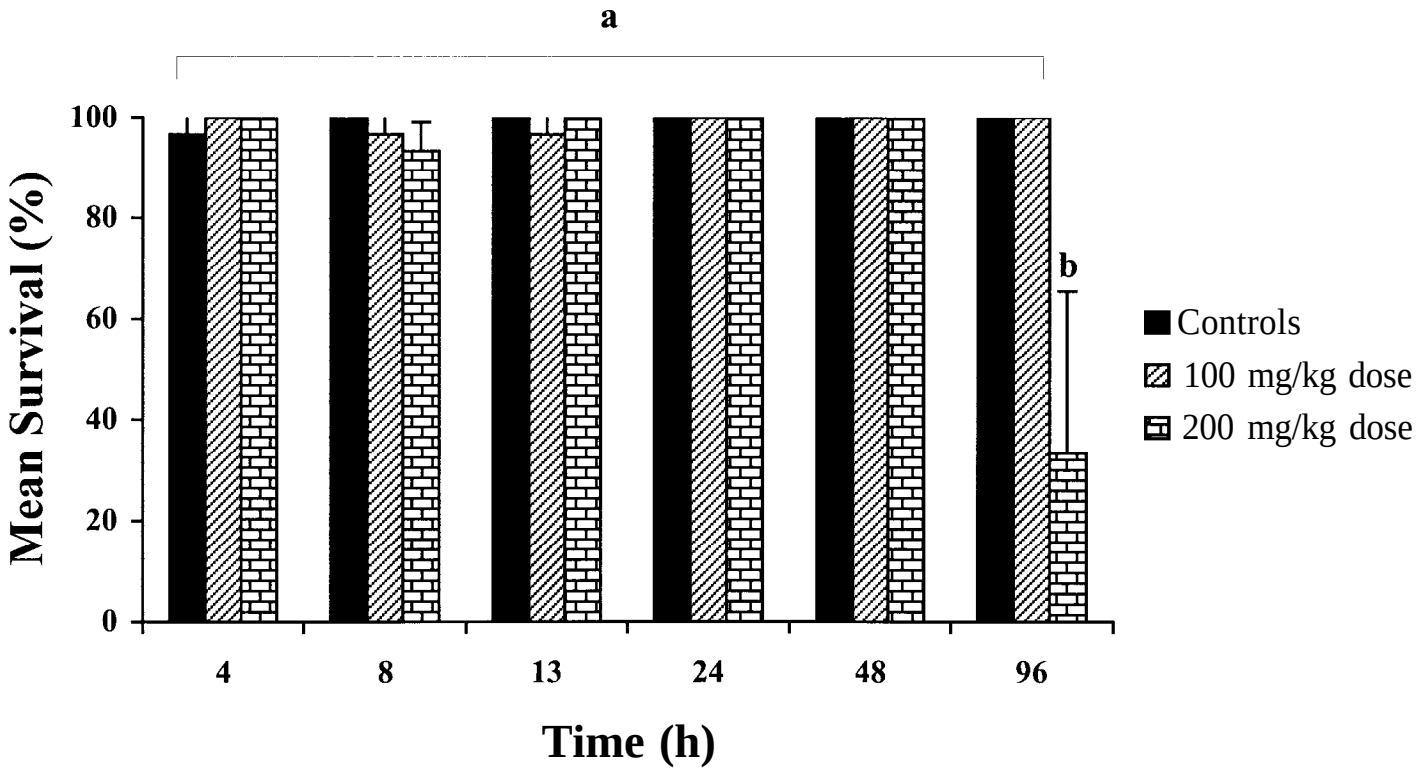


Figure 2.2. Mean percent survival of *Lumbriculus variegatus* exposed to Lake Erie sediments spiked with 100 and 200 mg/kg fluoranthene and trifluralin (experiment 2). Error bars represent standard deviation. Letters that are different denote significant differences between mean percent survival.

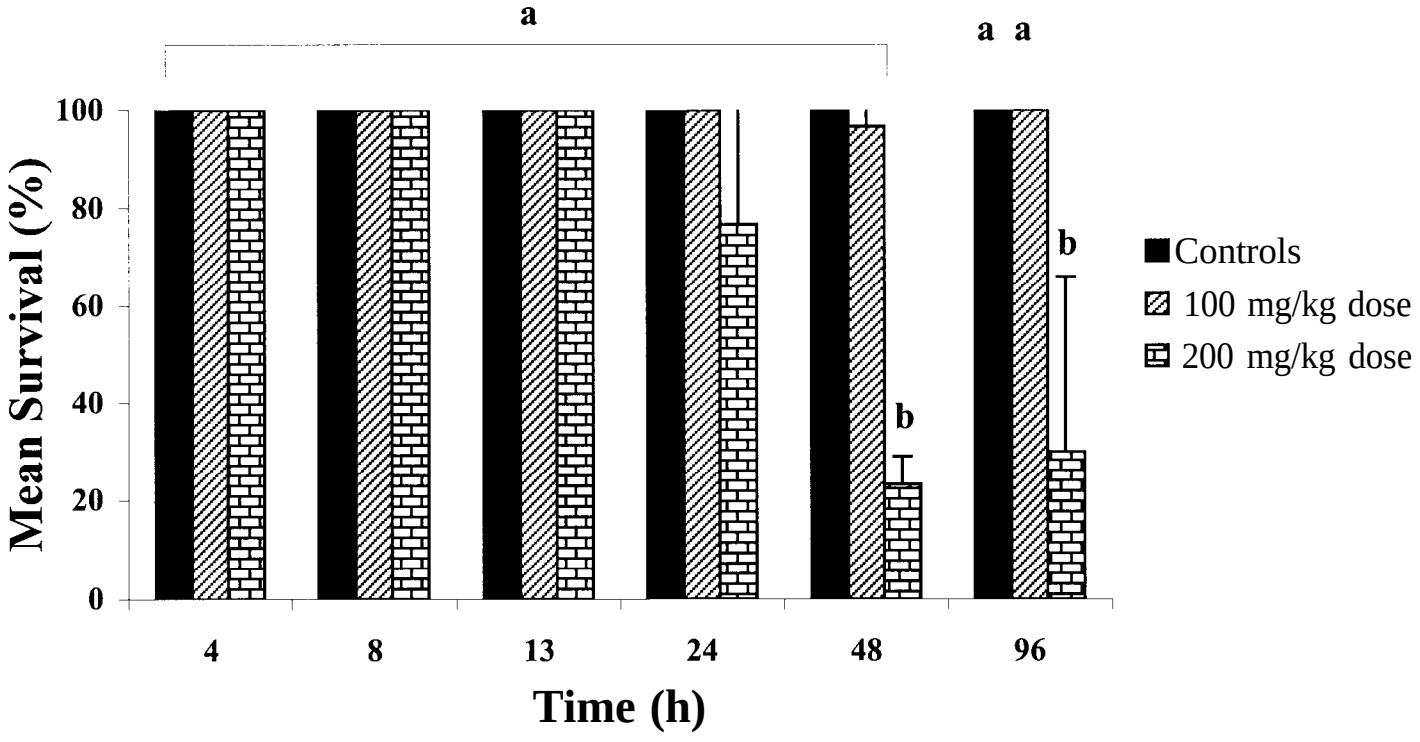


Figure 2.3. Mean percent survival of *Hyalella azteca* exposed to Lake Huron sediments spiked with 100 and 200 mg/kg fluoranthene and trifluralin (experiment 3). Error bars represent standard deviation.

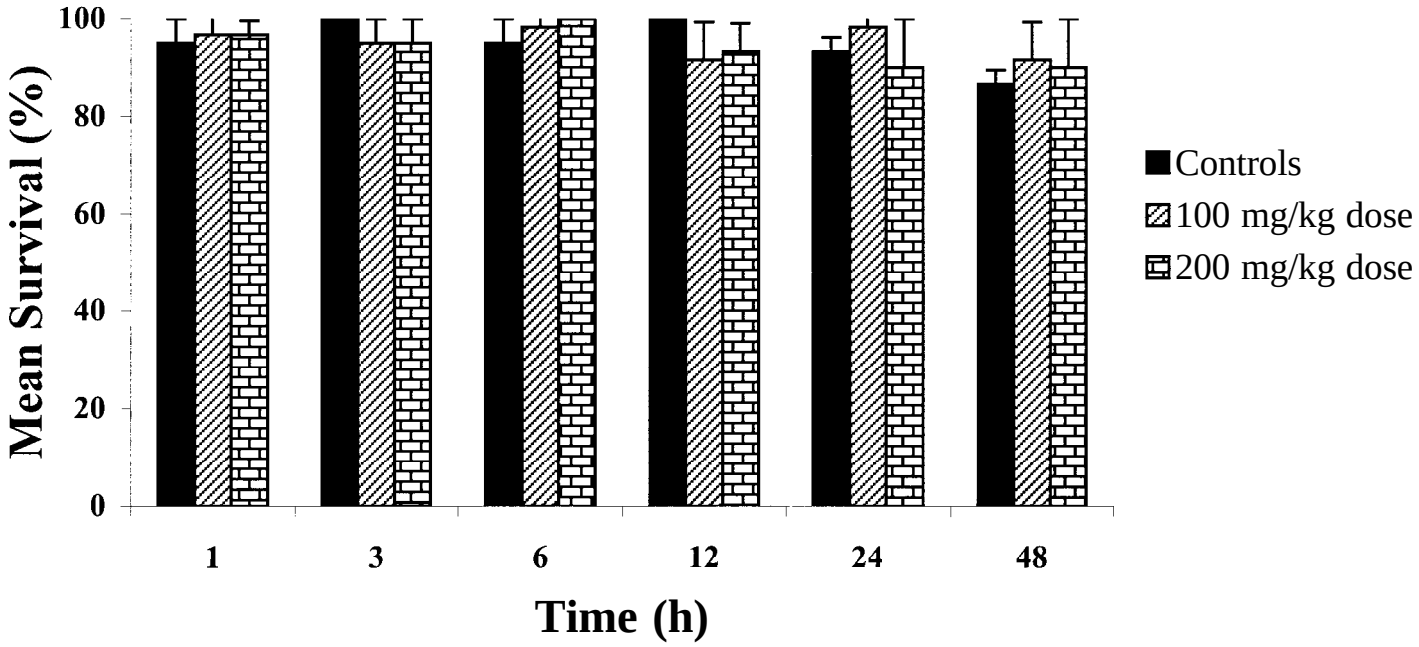


Figure 2.4. Mean percent survival of Hyalella azteca exposed to Lake Erie sediments spiked with 100 and 200 mg/kg fluoranthene and trifluralin (experiment 4). Error bars represent standard deviation.

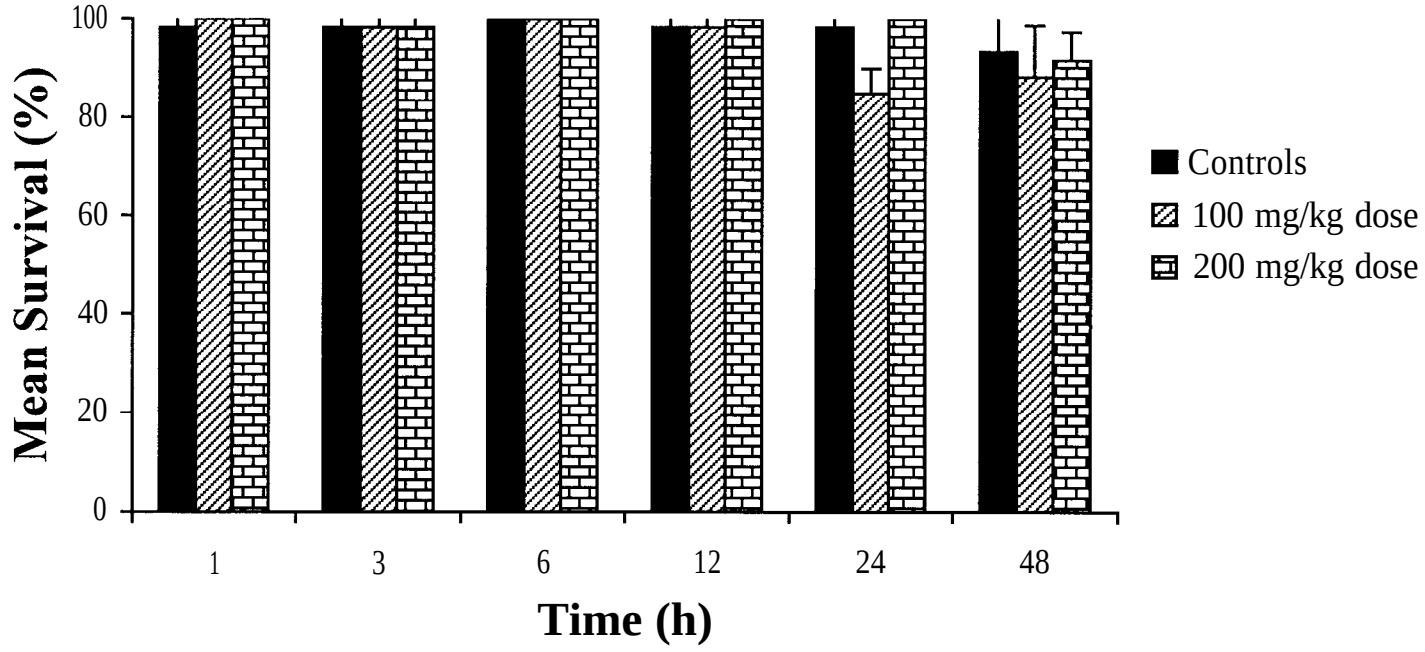


Figure 2.5. Body burdens of (**A**) fluoranthene and (**B**) trifluralin in *Lumbriculus variegatus* over a 96 h exposure to nominal sediment concentrations of 100 and 200 mg/kg dry weight of the test compounds spiked onto Lake Huron sediments (experiment 1). Error bars represent standard deviations of three samples. Lines represent best-fit model results.

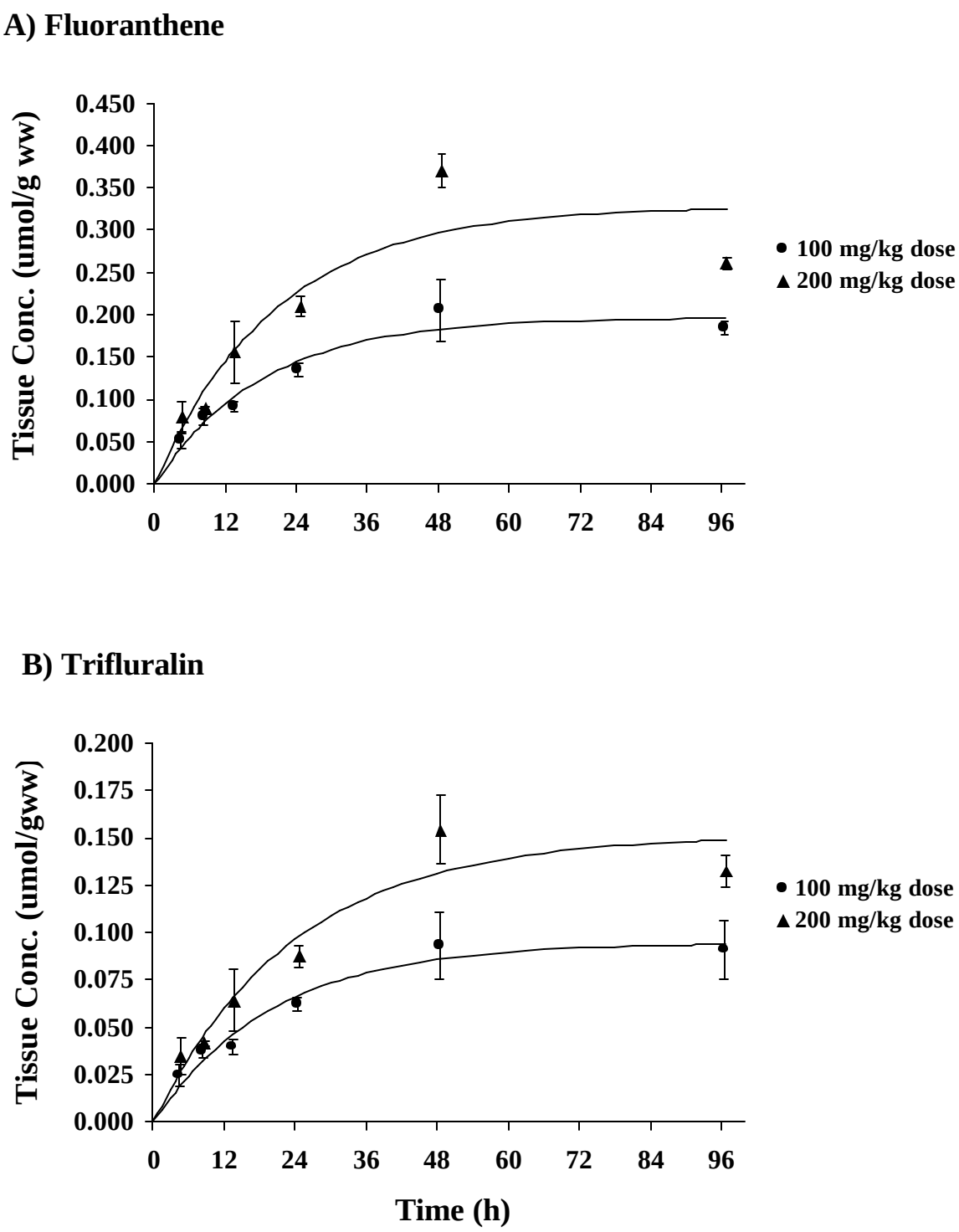


Figure 2.6. Body burdens of (**A**) fluoranthene and (**B**) trifluralin in *Lumbriculus variegatus* over a 96 h exposure to nominal sediment concentrations of 100 and 200 mg/kg dry weight of the test compounds spiked onto Lake Erie sediments (experiment 2). Error bars represent standard deviations of three samples. Lines represent best-fit model results.

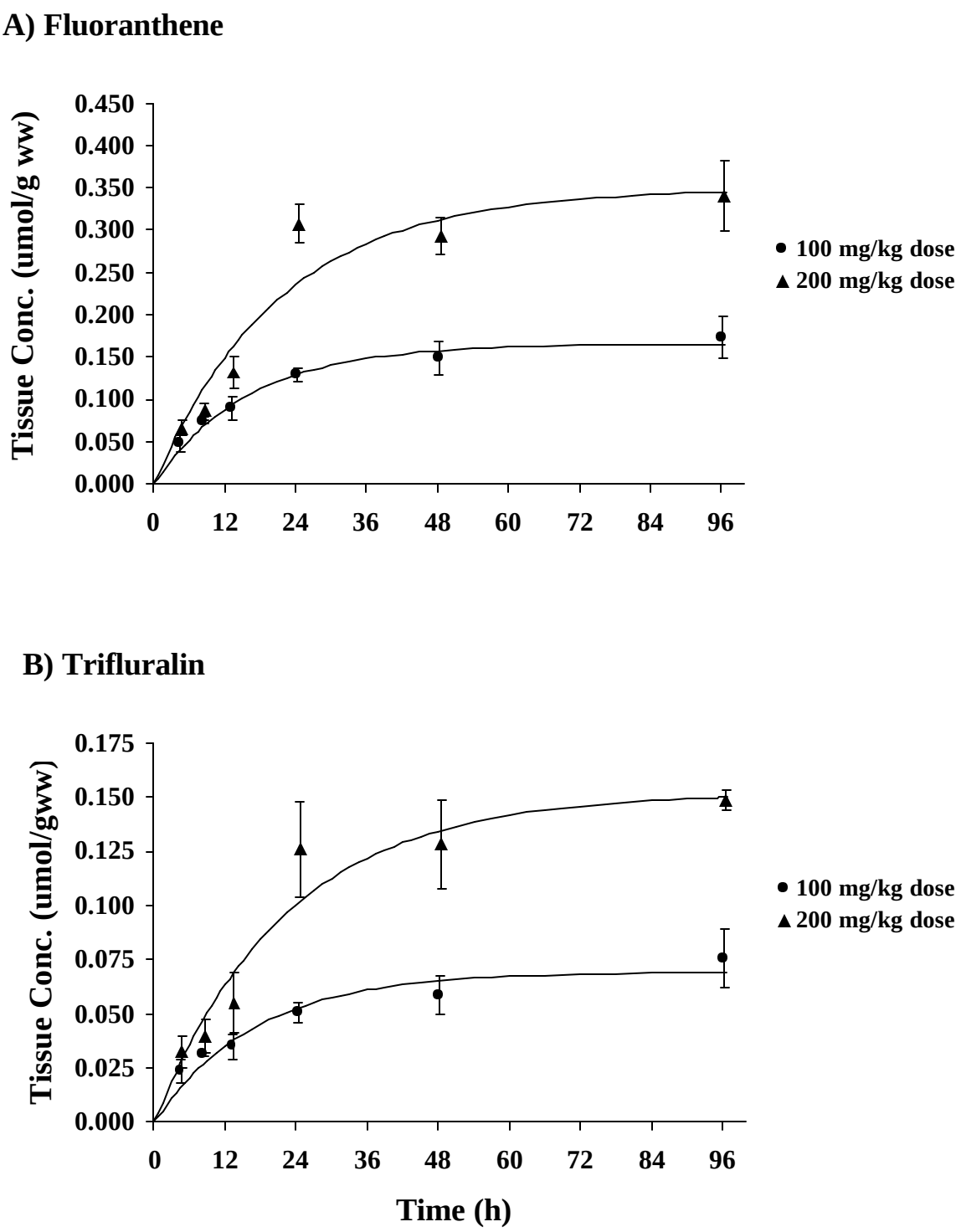


Figure 2.7. Body burdens of (**A**) fluoranthene and (**B**) trifluralin in *Hyalella azteca* over a 48 h exposure to nominal sediment concentrations of 100 and 200 mg/kg dry weight of the test compounds spiked onto Lake Huron sediments (experiment 3). Error bars represent standard deviations of three samples. Lines represent best-fit model results.

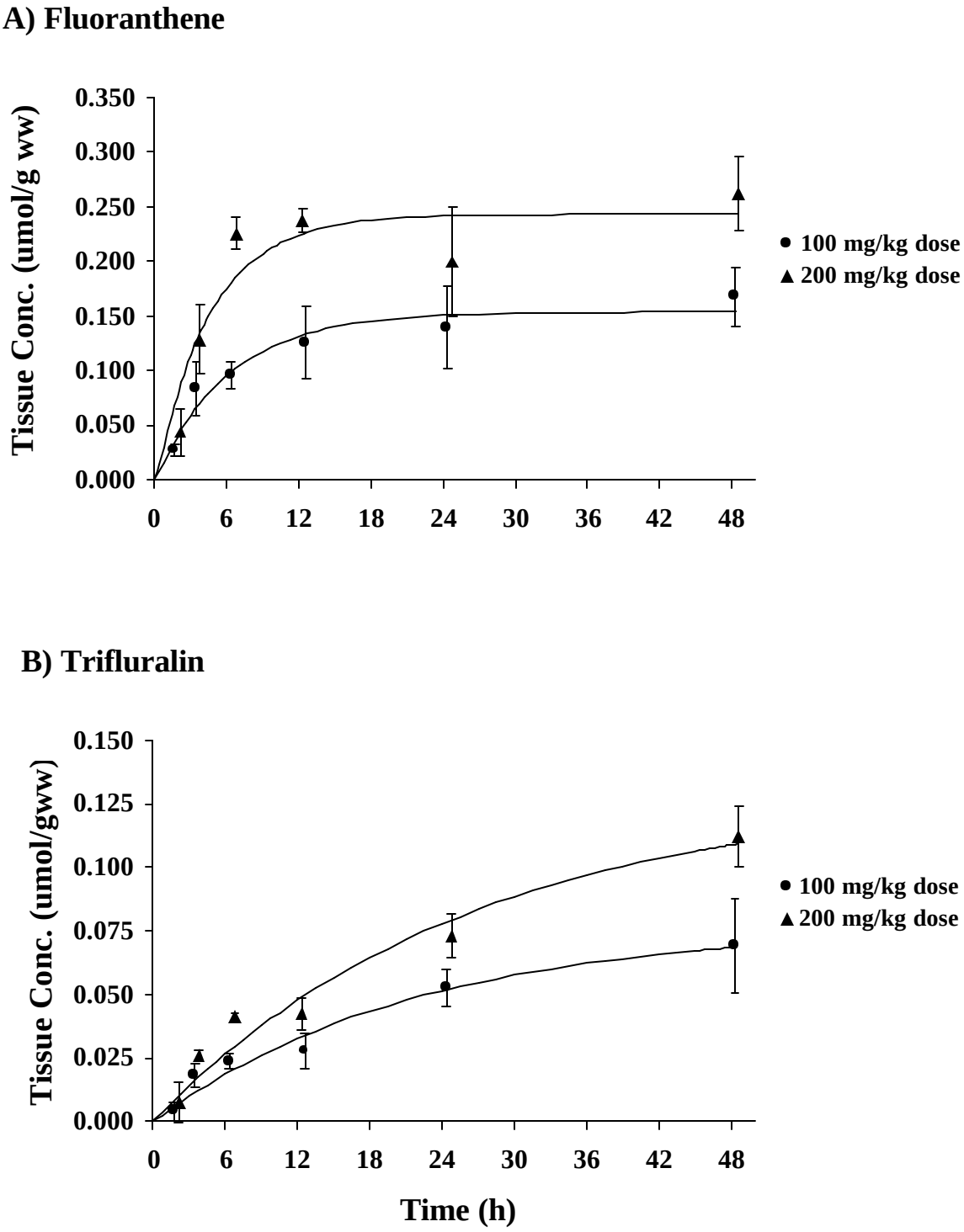
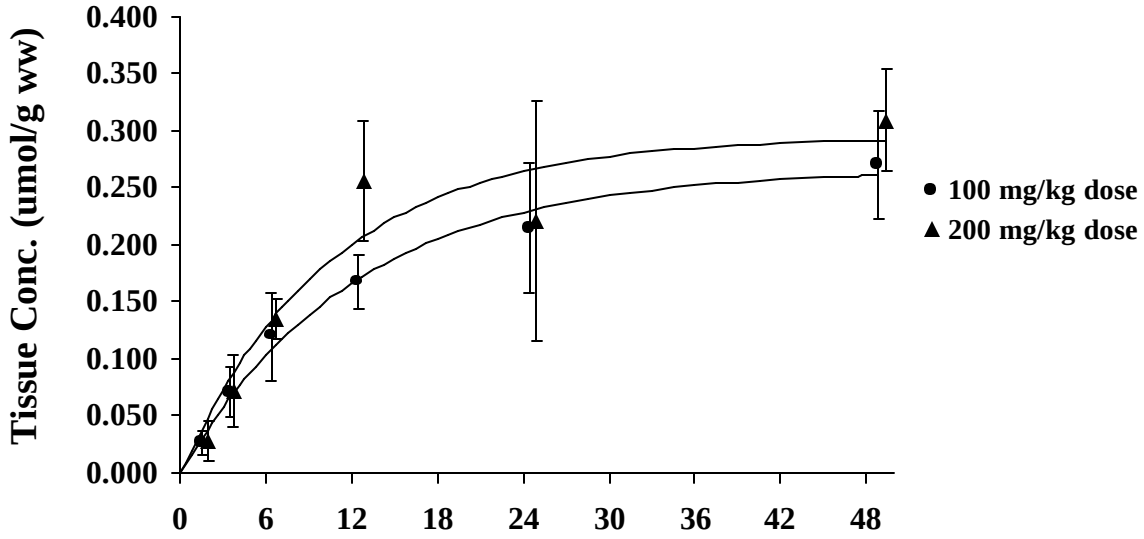


Figure 2.8. Body burdens of (**A**) fluoranthene and (**B**) trifluralin in *Hyalella azteca* over a 48 h exposure to nominal sediment concentrations of 100 and 200 mg/kg dry weight of the test compounds spiked onto Lake Erie sediments (experiment 4). Error bars represent standard deviations of three samples. Lines represent best-fit model results.

A) Fluoranthene



B) Trifluralin

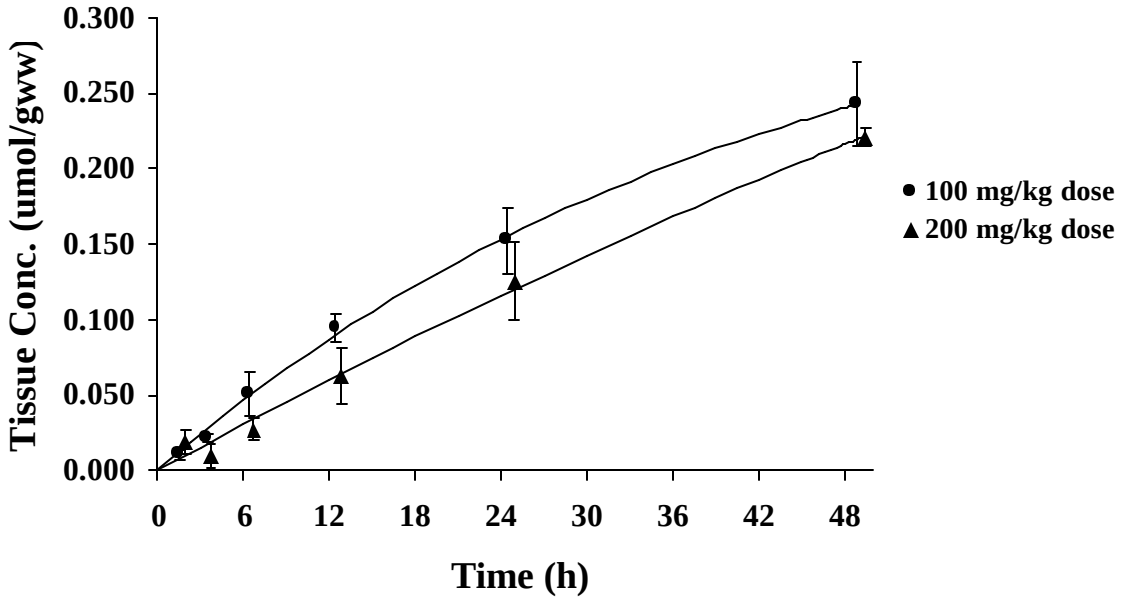
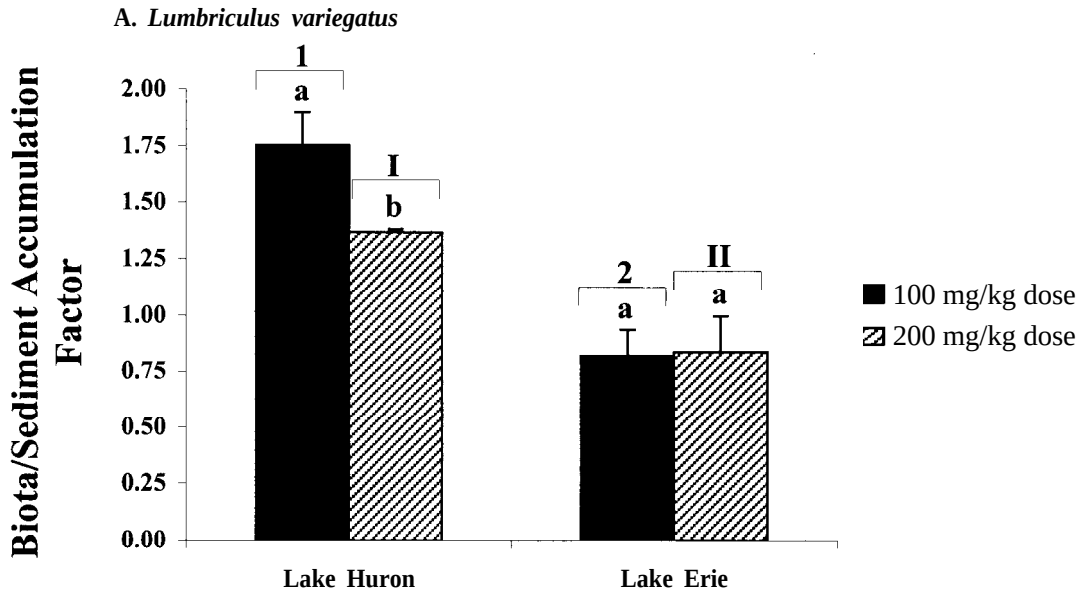
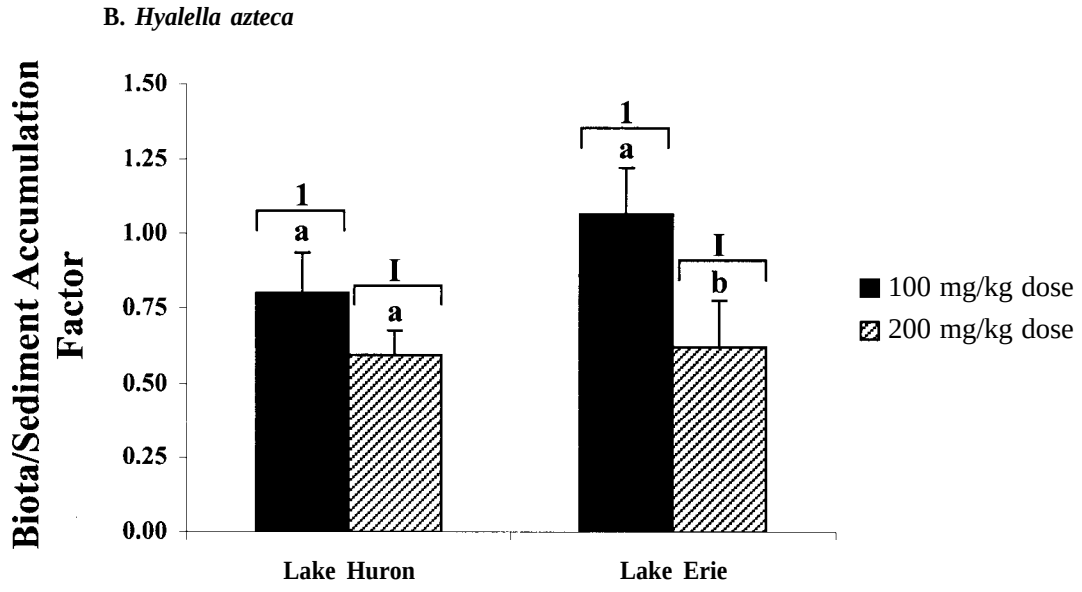


Figure 2.9. Mean (± 1SD) biota/sediment accumulation factors (BSAFs) of fluoranthene calculated for **(A)** *Lumbriculus variegatus* exposed to spiked sediments from Lakes Huron and Erie for 96 h and **(B)** *Hyalella azteca* exposed for 48 h. Letters that are different denote significant differences between the BSAFs for the 100 and 200 mg/kg doses within a sediment type. Arabic numerals that are different denote significant differences between BSAFs in the 100 mg/kg treatments of the Lakes Huron and Erie exposures. Roman numerals that are different denote significant differences between BSAFs in the 200 mg/kg treatments. The factors of dose and sediment type were considered in the one-way ANOVA models used to test for significant differences between BSAFs at a =0.05.





CHAPTER 3

Bioconcentration and Toxicokinetics of Waterborne Fluoranthene and Trifluralin in *Lumbriculus variegatus* and *Hyalella azteca*

INTRODUCTION

Contamination of aquatic environments by inputs of hydrophobic organic chemicals including polycyclic aromatic hydrocarbons (PAHs) and pesticides from industrial, agricultural and residential activities is a well-documented contributing factor to the problem of environmental degradation in modern society (Hudiburgh, 1995; Nitschke and Schussler, 1998; Burton, 1999; USGS, 1999). While these lipophilic substances often accumulate in the bottom sediments of oceans, lakes, rivers and streams (Karickhoff et al., 1979), small quantities of these chemicals will remain in solution for uptake by aquatic organisms from both surface and pore waters (Di Toro et al., 1991). The toxicokinetics of aqueous concentrations of the PAH fluoranthene (FLU) and the dinitroaniline herbicide trifluralin (TF) in benthic invertebrates such as the infaunal oligochaete, *Lumbriculus variegatus* and the epibenthic amphipod, *Hyalella azteca*, has received little attention with only one published study for FLU (Kane Driscoll et al., 1997b). Since PAHs and pesticides in aquatic environments are often present in complex mixtures (Burton, 1995; Nowell et al., 1999; USGS, 1999), the study of mixtures of chemicals from different classes is necessary.

The goals of this study were to measure the bioconcentration and toxicokinetics of the PAH FLU and the dinitroaniline herbicide TF in *L. variegatus* and *H. azteca* that were exposed to equivalent-mass mixtures of these compounds at sublethal aqueous concentrations. The hypothesis was that: 1) the compounds would not elicit toxicity and hence would provide good estimates of the conditional rates of uptake and elimination, 2) that these rate constants would not be significantly different between dose for each test species, and 3) that both *L. variegatus* and *H. azteca* would be capable of biotransformation of FLU and TF. The hypothesis was tested through the measurement of the uptake rates in short term exposures of *L. variegatus* (24 h) and *H. azteca* (12 h) to water-borne FLU and TF followed by measurement of their elimination of the test compounds in uncontaminated systems containing water and sediments. Sediments were used in the elimination phase because it was assumed that this design would most closely simulate the elimination of contaminants in the natural environments of *L. variegatus* and *H. azteca*. It was assumed that regardless of the specific route of uptake (*i.e.,* uptake from ingested sediments, pore or surface water), elimination by these species would occur in sediment-water systems.

MATERIALS AND METHODS

Experimental design

Uptake and elimination experiments were conducted with *H. azteca* and *L. variegatus* in order to determine the bioconcentration and toxicokinetics of

aqueous FLU and TF. In addition, biotransformation of these compounds following water-only exposures was evaluated for each of the test species. Nominal water-borne exposures concentrations of FLU (0, 0.025, 0.099 and 0.25 µmol/L) and TF (0, 0.015, 0.060 and 0.15 µmol/L) corresponded to 0, 5, 20 and 50 µg/L of each compound in equal-mass binary mixtures and were below the limits of solubility of FLU (260 µg/L at 25 ºC; Karickhoff, 1981; Verschueren, 1983) and TF (4 mg/L at 25 ºC, Mackay et al., 1997) in water. These concentrations of FLU were chosen as they were expected to allow for near 100% survival of the organisms, based on published LC_{50} values for *H. azteca* (Suedel et al., 1993; Kane Driscoll et al., 1997b) and recent bioconcentration studies with *L. variegatus* (Sheedy et al., 1998). The concentrations of TF chosen for this study were below the 48-h LC_{50} values (range 193-1800 µg/L) reported for a number of aquatic invertebrates including daphnids (*e.g., Daphnia pulex, D. magna, Simocepahalus serrulatus*) and amphipods (*e.g., Gammarus fasciatus*) (Parrish et al., 1978; Mayer and Ellersieck, 1986; Nowell et al., 1999). Therefore, TF was not expected to be toxic to *L. variegatus* and *H. azteca.* These aqueous concentrations were also below or within the ranges of pore water concentrations of FLU (21.2–75.8 µg/L) and TF (16.9–60.5 µg/L) that were estimated using equilibrium partitioning theory (Di Toro et al., 1991) for the nominal doses (100 and 200 mg/kg dry wt, each chemical) spiked onto Lakes Huron and Erie sediments in an earlier study (see Chapter 2).

Two experiments were conducted and are described in Table 3.1. *L. variegatus* were exposed to water-borne FLU and TF for 24 h and their uptake

kinetics were determined by sampling organisms at 1, 2, 5, 10, 18, and 24 h. The elimination kinetics of *L. variegatus* following the 24-h exposure were determined by transferring the organisms to beakers containing uncontaminated (unspiked, Lake Huron) sediments and water and then sampling them at 27, 33, 43, 51, 72, and 96 h. Samples of *L. variegatus* for metabolite analysis were collected at the end of exposure (24 h) and during the elimination phase, at 48 h. *H. azteca* were exposed for 12 h and sample times for uptake kinetics were 1, 2, 4, 6, 9, and 12 h. The elimination kinetics of FLU and TF by *H. azteca* following transfer of the amphipods to uncontaminated sediments and water at 12 h were measured at 13, 15, 18, 24.5, and 38 h. Biotransformation of FLU and TF by *H. azteca* was measured following an exposure of 19.5 h. Test set up, initiation (*i.e.,* additions of animals), sampling and end dates are shown in Table 3.2. For both species, three beakers for each concentration were sampled destructively at each time point.

Chemicals

Radiolabeled [G-^{3}H]fluoranthene (FLU) and [Ring-UL-^{14}C]trifluralin (TF) and unlabelled FLU and TF were obtained from suppliers as described in Chapter 2, Chemicals. The purity of the radiolabeled TF was determined to be >98% by the manufacturer (September, 2001) and the radiolabeled FLU was determined to be >96% pure by TLC prior to the preparation of the test solutions. Both radiolabeled compounds were used without further purification. The volumetric activities of the radiolabeled chemical stocks in acetone (HPLC-grade; Aldrich Chemical Co.) were checked on November 27, 2001 by liquid scintillation

counting (LSC) of duplicate, 2-µL samples. The mean activities and concentrations were 132.1 µCi/mL and 0.038 mg/mL for [^{3}H]FLU and 12.56 µCi/mL and 0.251 mg/mL for [^{14}C]TF. Acetone (HPLC-grade) was used to prepare spiking solutions. All reagents used for extractions and analyses, and the scintillation cocktail and solubilizer used for radionuclide analysis were as described in Chapter 2, Chemicals.

Preparation of test solutions

Test solutions were prepared in laboratory culture water. The culture water that was prepared as described in Chapter 1, General Methods was used in these experiments. Test solutions of nominal concentrations of 5, 20 and 50 mg FLU and TF per liter were prepared by adding appropriate amounts of [^{3}H]FLU and [^{14}C]TF and the unlabeled compounds from stock solutions (1 mg/mL each in acetone) to 4 L of culture water that had been vigorously aerated for 24 h prior to spiking. Target activity levels of the radioisotopes in the water were 2,000 disintegrations per minute (DPM) per mL of water for tritium and 500 DPM/mL of carbon-14. Separate 10- or 100-µL Gastight® syringes and 200-µL pipets (Eppendorf Series 2000, Hamburg, Germany) were used to dispense the radiolabeled and unlabeled chemicals to the water and the 4-L solutions were prepared in large (4.1-L) borosilicate glass bottles. Control (0 mg/L) test solutions were prepared with similar amounts of acetone (120 µL/mL). Then, stir-bars were added to the bottles, the solutions were sealed with glass covers and they were gently mixed on magnetic stir-plates. Triplicate 2-mL samples of each test solution were placed into 12 mL of

scintillation cocktail then analyzed by LSC and the mean values were used to calculate the specific activities of the spiking solutions (µCi of radiolabeled compound/µmol of total nominal compound) and their concentrations (µmol of total nominal compound/mL). Coefficients of variation (CV; %) for the triplicate samples of each solution were low (=9.3%). All solutions were prepared at 22 ºC under constant yellow light (λ > 500 nm) to avoid potential photodegradation of FLU and TF.

Test Organisms

Lumbriculus variegatus. The oligochaete worms used in the exposures to waterborne FLU and TF were taken from the WSU laboratory culture. *L. variegatus* were selected based on their size (1-2 cm length, 4.29 ± 0.56 mg wet wt/individual; n=9 measurements of 10 individuals each), the presence of a fully developed head and tail, and gut contents indicating active feeding. Prior to their introduction into test beakers, the animals were allowed to purge their guts for 5 hours. The intent of this procedure was to reduce uncertainties surrounding the water concentration measurements that could arise if the organisms produced fecal pellets during the exposure (*i.e.,* material capable of binding the chemicals thus reducing the dissolved amounts) (Mount et al., 1999). For ease of rapid addition of the *L. variegatus* to the test beakers, ten individuals each were gently added to 50-mL centrifuge tubes that contained 2 mL of culture water that was free of debris. The small volume of culture water in the centrifuge tubes insured that dilution of the test solutions in the beakers would be minimal upon organism addition.

Hyalella azteca. The WSU laboratory cultures used in the water-only exposure of *H. azteca* to FLU and TF were initiated on July 9 and 18, 2001 and August 2, 2001. Amphipods were harvested on December 16, 2001 for use in the experiment. Animals that were retained on an ASTM-approved U.S. standard #40 sieve (425 µm) were used. They were placed in 1-L beakers with 900 mL of culture water that was gently aerated and maintained overnight as previously described. Organisms from these batches were approximately 1-3 weeks old. Amphipods were randomly selected for testing and their mean individual wet weight was 0.12 ± 0.03 mg (n=3 measurements of 20 individuals each). For ease of rapid addition of the amphipods to the test beakers, 20 (uptake exposure beakers) or 100 (biotransformation beakers) individuals each were gently added to 50-mL centrifuge tubes that contained 5 mL of culture water. The small volume of culture water in the centrifuge tubes insured that dilution of the test solutions in the beakers would be minimal upon organism addition.

Exposures and sampling

The design of the *L. variegatus* and *H. azteca* experiments was such that there were three groups of randomly selected organisms with each group corresponding to a component of the experiment (Table 3.1). The first group of organisms was exposed to spiked water and their uptake kinetics were determined. The second group was exposed to spiked water and then transferred to beakers containing uncontaminated sediments and water to determine the elimination kinetics. The third group was used to measure

biotransformation of FLU and TF following water-only exposures of 24 h (*L. variegatus*) and 19.5 h (*H. azteca*). The three components of the *L. variegatus* experiment were all completed within a contiguous 96-h period (November 29-December 3, 2001). Since sampling the *H. azteca* was logistically more difficult and required substantially more time than sampling the worms, the three components of the amphipod experiment were not conducted simultaneously. Therefore, the uptake kinetics were measured over 12 h on December 17, 2001 and the elimination kinetics and biotransformation components were conducted over a 38-h period that included a 12 or 19.5 h uptake exposure, respectively, from December 20-22, 2001. These latter exposures were begun within 36 h of the last sample time for determination of the uptake kinetics of FLU and TF by *H. azteca* and the same culture of amphipods was used. The experimental dates including test set-up, initiation and sample time points are given in Table 3.2. All experiments were run on a 16:8-h light:dark photoperiod at room temperature (22 ± 1 ºC) under yellow light (λ > 500 nm) to avoid photodegradation of the FLU or TF.

Uptake kinetics. For the uptake exposures, dosed water (185 mL) was added to each 237-mL exposure jar (Ball® type, Alltrista Corporation, Muncie, IN, USA) and loosely covered to reduce chemical loss by volatilization. Immediately prior to the addition of animals (Time =0 h), the jars were randomly placed in a shallow (5 cm depth) water bath to maintain a constant temperature (21 °C). At this time, a water sample (300 mL) was taken from the culture water that was used in the preparation of the test solutions for determination water quality

characteristics including temperature (°C), pH, dissolved oxygen (DO; mg/L), hardness (mg/L as $CaCO_3$), alkalinity (mg/L as $CaCO_3$), conductivity (µS/cm) and ammonia (mg/L). Ten *L. variegatus* or 20 *H. azteca* were added to each test jar. Distribution of animals to the test jars for each experiment took <10 min. Each jar in the *H. azteca* experiment contained a 1-cm square of sterile cotton gauze for substrate that was presoaked for 12-24 h in culture water. The exposures were static with no aeration, water was not renewed during the *L. variegatus* (24 h) or *H. azteca* (12 h) uptake periods and the organisms were not fed. Control (0 µg/L exposed) organisms were sampled only at the end of the uptake phase for the determination of background levels of the radionuclides by LSC and percent survival which verified that the organisms used in the kinetic tests were healthy. In addition, samples of *L. variegatus* and *H. azteca* from WSU cultures were analyzed for background levels of the radionuclides.

At each time point, triplicate jars from each concentration were selected at random and were sampled. Duplicate 2-mL water samples were taken from each jar in the 5, 20 and 50 µg/L treatments for measurement of [^{3}H]FLU and [^{14}C]TF by LSC. Water from the controls (0 µg/L of the test compounds) was sampled for LSC only at test initiation and was sampled at the end of the uptake phase for measurement of the full suite of water quality characteristics. Mean percent survival and standard deviation was calculated based on the number of live organisms recovered divided by the initial number added to each jar. The concentrations of [^{3}H]FLU and [^{14}C]TF were measured by LSC in all surviving *L. variegatus* and *H. azteca* from each jar.

Elimination kinetics. *L. variegatus* and *H. azteca* were exposed to FLU and TF as described above in *Uptake kinetics* and were then transferred to 300-mL tall-form beakers containing uncontaminated Lake Huron sediments (7 g, *L. variegatus*; 2 g, *H. azteca*) and culture water (250 mL) for measurement of the elimination time course. The sediments and water were added to the elimination beakers where they were vigorously stirred and then gently aerated for 24-48 h prior to the transfer of the organisms (Table 3.2). This allowed the sediments to settle as thin (3-5 mm), even layer covering the bottom of the beaker. In the *H. azteca* test, 1.0 mL of YCT was added to each beaker after the sediments had settled to provide food for the amphipods over the 26-h elimination period. *L. variegatus* were expected to feed on the organic carbon in the sediments over their 72-h elimination period. Prior to the transfer of the exposed organisms, the aeration was removed from the elimination beakers and a pooled water sample (300 mL) was taken from 10 randomly sampled beakers for water quality characterization.

Following the exposure period (24 h for *L. variegatus*, 12 h for *H. azteca*), the animals were carefully transferred to the elimination beakers containing uncontaminated sediments and water. The organisms were transferred by gently pouring the contents of an exposure jar through an ASTM-approved U.S. standard #80 sieve (180 µm) and then the worms or amphipods that were retained by the sieve were thoroughly cleaned with culture water prior to being rinsed into an elimination beaker. The transfer process took approximately 1 h for all beakers. To prevent large differences in elimination times between

replicates at a given sample time, triplicate exposure jars of each test concentration (5, 20, 50 µg/L) were randomly arranged into groups (6 groups for *L. variegatus*, 5 groups for *H. azteca*) such that each group took no more than 10 min for the transfers and thus each replicate beaker within a time point group would have nearly equal elimination times.

At each time point, triplicate jars from each concentration were sampled. Water samples of 3-mL total volume were composited by combining 1 mL x 3 beakers per treatment concentration (5, 20 and 50 µg/L) for measurement of [^{3}H]FLU and [^{14}C]TF by LSC. Water from the control elimination beakers (0 µg/L of the test compounds) was sampled for LSC only at the end of the elimination phase. In addition, a 300-mL composite sample was taken at the end of the elimination period for measurement of the full suite of water quality characteristics. Mean percent survival and standard deviation was calculated as described above in *Uptake kinetics*. The concentrations of [^{3}H]FLU and [^{14}C]TF were measured by LSC in all surviving *L. variegatus* and *H. azteca* from each jar.

Biotransformation. Triplicate exposure jars were sampled for the measurement of FLU and TF metabolites in the *L. variegatus* and *H. azteca*. Samples were taken at the end of the uptake kinetics exposures for *L. variegatus* (24 h) and after 19.5 h of exposure for *H. azteca*. The organisms were blotted dry on paper towels, and then wet weighed and frozen at –20 ºC, as described in Chapter 2, until extraction and analysis of metabolites.

Lipids. Samples of the *L. variegatus* (n =3 samples of 10 individuals each) and the *H. azteca* (n =3 samples of 20 individuals each) removed from the WSU

laboratory cultures for use in these experiments were stored frozen at –20 ºC in chloroform-rinsed (3 x 0.5 mL) 1-mL borosilicate tubes prior to extraction and determination of lipid contents by following the methods described in Chapter 2, Analytical Methods. It was assumed that the lipid contents of the organisms used in the exposures would not change significantly during the experiments.

Analytical methods

Water samples that were collected for the measurement of ^{3}H and ^{14}C were placed into 20-mL borosilicate glass scintillation vials with 12 mL of scintillation cocktail. The contents were vortexed for 10 sec and the samples were stored for >48 h in the dark at room temperature. After subsidence of chemiluminescence (=48 h), radioactivity was quantified by LSC. The measurement of FLU and TF equivalents in tissue samples by LSC, the extraction and analysis of metabolites from *L. variegatus* and *H.* azteca by TLC followed by LSC, and general LSC analysis procedures were all carried out by following the methods described in Chapter 2, Analytical Methods. Modifications to those detailed methods included: 1) that the *L. variegatus* sampled during the elimination phase in this study were not allowed to purge their guts prior to their preparation for LSC and 2) that the *L. variegatus* in this study were placed into 7-mL scintillation vials and 500 µL of solubilizer was added to each vial.

Tissue and aqueous concentrations

Concentration values are reported as mean (± 1 SD) in units of µmol/g wet wt for *L. variegatus* and *H. azteca,* and µmol/mL for water. The

concentrations represent measured equivalents of [^{3}H]FLU and [^{14}C]TF in the samples either as parent compound for total or as specific metabolites where determined.

Modeling

The experimental design was such that separate components of the experiments provided specific measurements for each the accumulation and elimination kinetics of FLU and TF by *L. variegatus* and *H. azteca* in water-only exposures. Therefore, it was assumed that the conditional elimination rate constant measured in the presence of sediments would provide an estimate more representative of field conditions.

Uptake kinetics. The accumulation data for FLU and TF were fit to a two-compartment first-order kinetic model that accounted for the slight decline in the aqueous concentration of the contaminants with time as a result of chemical from the exposure system. The losses from the water were assumed to be due to: 1) accumulation of the compounds into organisms, 2) adsorption to the glass walls of the exposure jars and 3) volatilization. The decline of FLU and TF in water was described by a single-exponential model (Newman, 1995):

$$C_{w}^{t} = C_{w}^{0} e^{-\lambda t}, \quad (3.1)$$

where C_{w}^{t} is the concentration of the contaminant in the water (µmol/L) at time t, C_{w}^{0} is the initial water concentration (µmol/L), λ is the rate constant for the loss of the chemical from the water (1/h) and t is time (h). This type of correction also provided an estimate for C_{w}^{0}, which was assumed to be more reflective of the exposure conditions than the concentration determined from the samples taken

immediately following the preparation of each test solution (see *Preparation of test solutions* above). This assumption was made because those samples were collected prior to the distribution of the test solutions to the exposure jars and thus would not have accounted for any losses due to the instantaneous sorption of FLU and TF to the glassware or their volatilization during the pouring of the solutions into the jars or during the time before organisms were added to the exposures. Therefore, the model estimated values of C_w^0 and λ were used as input values for the modeling of the accumulation data.

Then, the accumulation data were fit by nonlinear least squares regression to the following two-compartment model (Landrum, 1989):

$$\frac{dC_a}{dt} = k_u C_w^0 e^{-lt} - k_e C_a, \quad (3.2)$$

with the integrated form

$$C_a = \frac{k_u C_w^0}{k_e - l}(e^{-lt} - e^{-k_e t}), \quad (3.3)$$

where C_a is the concentration in the organism (µmol/g wet wt), k_u is the conditional uptake clearance coefficient (mL/g wet wt organism/h), k_e is the conditional elimination rate constant (1/h), and t is time (h). To apply this model, it was assumed that: 1) there was no growth of the organisms, 2) the material in the water was parent compound; and 3) all uptake was from water. The total molar equivalents of FLU and TF in both water and organisms were modeled to obtain the values of k_u and k_e.

The water concentration and accumulation data were modeled by least squares nonlinear regression using *SYSTAT for Windows, Version 9* (SYSTAT,

Evanston, IL, USA). The use of the Gauss-Newton algorithm and exact sample times for the replicates with their corresponding measured C_a values were as previously described in Chapter 2, Modeling. The initial water concentration (C_w^0, µmol/mL) and the rate constant for the loss of the chemical from the water (λ, 1/h) were determined by Equation 3.1 and these values were used to model the accumulation data (Equation 3.3). This fit yielded least-squares estimates for k_u that is reported as the estimated value (± asymptotic standard error, A.S.E.).

Elimination kinetics. The FLU and TF were eliminated by *L. variegatus* and *H. azteca* to static systems containing culture water and uncontaminated sediments. Because the water in the elimination beakers was not exchanged, concentrations of FLU and TF in the water (C_w) generally increased over the course of elimination period and thus violated assumptions of negligible or zero concentration levels of the contaminants in the elimination water. Therefore, it was assumed that the most accurate prediction of the tissue concentration-time profile would be obtained by using a model that allowed for the re-uptake of the eliminated compounds.

Initial estimates of the experimentally measured elimination rate constant ($k_{e(m)}$, 1/h) were obtained by fitting the elimination data to the following simple, single-exponential equation (Newman, 1995):

$$C_a^t = C_a^0 e^{-k_{e(m)} t}, \tag{3.4}$$

where C_a^t is the concentration of the contaminant in the organism (µmol/g wet wt) at time t, C_a^0 is the initial concentration in the organism (*i.e*., at the beginning of the elimination phase) (µmol/g wet wt) and was estimated by the fit to the

elimination data, $k_{e(m)}$ is the experimentally measured conditional elimination rate constant (1/h) and *t* is time (h). The data were fit to this integrated equation (Equation 3.4) by least squares nonlinear regression using *SYSTAT for Windows, Version 9* (SYSTAT, Evanston, IL, USA). This simple model resulted in an overprediction of elimination at the later timepoints and confirmed the concern mentioned above that the FLU and TF in the water should be considered in the modeling of elimination. It was assumed that FLU and TF present in the water was due to the elimination of parent compound from the organisms and that it was bioavailable (*i.e.,* dissolved) for re-uptake by the organisms. Therefore, the estimates of C_a^0 and $k_{e(m)}$ from Equation 3.4 were used as initial values for a more complicated elimination model as described below.

A two-compartment model was used to describe the elimination and re-uptake of FLU and TF by *L. variegatus* and *H. azteca* in sediment-water elimination beakers. The first-order model was identical to those used to describe accumulation and bioconcentration (Spacie and Hamelink, 1979):

$$\frac{dC_a}{dt} = k_u C_w - k_{e(m)} C_a, \qquad (3.5)$$

where where C_a is the concentration in the organism (µmol/g wet wt), k_u is the conditional uptake clearance coefficient (mL/g wet wt organism/h), C_w is the concentration in the water (µmol/mL), $k_{e(m)}$ is the experimentally measured conditional elimination rate constant (1/h), and *t* is time (h). Since the concentrations of FLU and TF in the water were measured at each time point and they generally increased asymptotically over the elimination period, the change in C_w was described by the following logarithmic equation:

$$C_w = a \bullet \ln(t) + b \quad (3.6)$$

where *a* and *b* are constants and *t* is time (h). This equation was solved in Excel® for Macintosh, Version 2000 (Microsoft, Redmond, WA, USA) and the values of *a* and *b* were then used to calculate values of C_w for each time-step (0.01 h) of the numeric integration of Equation 3.5.

The numeric integration of Equation 3.5 and the time-step calculations of C_w by Equation 3.6 were performed to provide a least-squares fit to the elimination data using *Scientist®, Version 2.01* (MicroMath Scientific Software, Salt Lake City, UT, USA). The fit was obtained by first simulating the elimination data using the Bulirsch-Stoer algorithm with starting values for $k_{e(m)}$ and C_a (*i.e.,* C_a^0) that were obtained by fitting Equation 3.4 as described above. The starting value of k_u for these first iterations was set to 200 mL/g/h. Then, the values of k_u and $k_{e(m)}$ that were estimated by the Bulirsch-Stoer method were used as starting values for the numeric integration of Equation 3.5 by the fourth order Runga-Kutta approach. This final estimate of $k_{e(m)}$ is reported as the estimated value (± asymptotic standard error, A.S.E.).

Bioconcentration Factor (BCF)

The bioconcentration factor (BCF) is a unitless value describing the steady state concentration of a chemical in an organism relative to the chemical concentration in the aquatic environment (*i.e.,* aqueous concentration) (Rand et al., 1995). The BCF is often defined as a point estimate from the conditional uptake and elimination rate constants and was calculated as follows:

$$\mathrm{BCF} = \frac{k_u}{k_e}, \tag{3.7}$$

The values of k_u and k_e were taken from the model fit to the accumulation data (Equation 3.3). Bailer et al. (2000) reported a series of calculations for approximating the confidence interval of the BCF beginning with the log of the estimated BCF:

$$\log(\mathrm{BCF}) = \log(k_u) - \log(k_e) \tag{3.8}.$$

The standard error (SE) for the log BCF is approximated by:

$$\mathrm{SE}(\log \mathrm{BCF}) \approx \sqrt{\frac{s_u^2}{k_u^2} - \frac{2s_{ue}}{k_u k_e} + \frac{s_e^2}{k_e^2}}, \tag{3.9}$$

where s_u and and s_e represent the asymptotic standard error (A.S.E.) values of the k_u and k_e estimates and thus the square of these errors are the variances. The value of s_{ue} represents the covariance between the estimates of the rate constants. The term "log" in the notation of Bailer et al. (2000) represents the natural logarithm (*i.e., ln*). The log BCF and its SE yield a confidence interval for BCF:

$$\exp\{\log(\mathrm{BCF}) \pm z_{a/2}\mathrm{SE}(\log \mathrm{BCF})\} \tag{3.10}$$

where $z_{\alpha/2}$ at α =0.05 is 1.96 and thus provides a 95% confidence interval (CI) for the BCF.

Statistical analysis

Significant differences between estimated conditional rate constants (k_u, k_e) from the nonlinear fitting of the tissue-time course data by Equations 3.3 and 3.5 were tested with the Student's t-test as described in Chapter 2, Statistical

analysis. Hypothesis tests of k_u, k_e and $k_{e(m)}$, with respect to species, were performed to test for significant differences in the rates of each kinetic parameter across the three concentration treatments (*i.e.,* 5, 20 and 50 µg/L). These multiple, two-tailed Student's t-tests (3) were conducted using a Bonferroni-adjusted critical value (t_{crit}) at an experiment-wise error (α) of 0.05 (Newman, 1995). The per comparison error rate was $\alpha/3$. Hypothesis testing of the equality of the rate constants between species at a given dose (*i.e.,* standard pairwise comparisons) were performed at a significance level (α) of 0.05.

The procedures recommended by Bailer et al. (2000) were followed for the statistical comparisons of the BCFs of FLU and TF in *L. variegatus* and *H. azteca*. An approximate Z statistic was used to test for significant differences between the BCFs (Bailer et al., 2000):

$$Z_{obs} = \frac{\log(BCF_1) - \log(BCF_2)}{\sqrt{[SE(\log BCF_1)]^2 + [SE(\log BCF_2)]^2}}. \qquad (3.11)$$

The null hypothesis (H_0: $\log(BCF_1) - \log(BCF_2) = 0$) was rejected if $|Z_{obs}| = Z_{crit}$, where Z_{crit} is the critical value of a standard normal distribution with an upper tail probability of $\alpha/2$ (Bailer, 1988). As described above for the t-tests performed for the kinetic rate constants, when statistical contrasts with respect to species were conducted to test for significant differences in the BCFs across the three concentration treatments, a Bonferroni-adjusted critical value ($Z_{crit} = 2.395$, per comparison error of $\alpha/3$, two-tailed test) was used. For planned pairwise comparisons of the BCFs between species for a given dose, the value of Z_{crit} was 1.96 Bailer, 1988; Bailer et al., 2000).

Significant differences between the means of the fractions of FLU and TF determined to be parent compound, extractable metabolites or unextractable residues in *L. variegatus* were tested with two-factor analysis of variance (ANOVA; Zar, 1999) followed by pairwise comparisons among treatments (Tukey's honest significant difference [HSD] test). The two factors included in the ANOVA model were time and dose (5, 20, 50 µg/L). Differences due to the interaction of or the main effects of time and dose were considered significant if $p = \alpha = 0.05$. The biotransformation data for *H. azteca* were analyzed by one-way ANOVA (Zar, 1999) for the effect of dose and differences were considered significant if $p = \alpha = 0.05$. Prior to testing with ANOVA, data normality was verified using Kolmogorov-Smirnov and Sharpiro-Wilk tests of normality, and homogeneity of variances were tested with Levene's and Bartlett's tests. Statistical analysis by ANOVA was performed using *Statistica for Windows, Version 5* (STATSOFT, Tulsa, OK, USA).

RESULTS

Test conditions

The physical-chemical characteristics of the water in the uptake exposures and elimination rate determinations are summarized in Table 3.3. Temperatures during the experiments were stable (21-22 ºC) and dissolved oxygen concentrations remained high (mean range 6.64-7.45 mg O_2/L). Total ammonia levels (mean =0.70 mg/L) were below concentrations that would be expected to cause toxicity to either *L. variegatus* or *H. azteca* (Whiteman et al., 1996).

Overall, the water within the test beakers was of high quality throughout the experiments.

Survival, wet weight, lipid contents and observations

The percentage survival of *L. variegatus* and *H. azteca* in the water-only exposures to FLU and TF was recorded at each time point and the data are summarized below. *L. variegatus* survival was 100% in all concentrations during both uptake and elimination phases of the experiment except in the 5 µg/L treated organisms during elimination where the mean (± 1SD) percentage survival was 99.4 ± 2.4%. The mean (± 1SD) wet weights of individual worms within the uptake phase exposure jars were similar between concentrations and were 3.98 ± 0.39 (n =6 beakers), 3.56 ± 0.51 (n=18), 3.35 ± 0.81 (n =18) and 3.64 ± 0.80 mg/worm (n =18) in the zero (control), 5, 20 and 50 µg/L treatments, respectively. In the post-exposure elimination component of the *L. variegatus* experiment, individual wet weights (mg/worm) were 3.99 ± 0.39 (control, n =3), 4.03 ± 0.94 (5 µg/L, n =18), 3.73 ± 0.77 (20 µg/L, n =17) and 4.10 ± 0.67 (50 µg/L, n =18).

H. azteca survival was high (mean >96%) in all concentrations during the 12-h uptake exposures to waterborne FLU and TF but was reduced (mean range 58-68%) in the post-exposure elimination phase. Mean percentage survival (± 1SD) during uptake was 96.7 ± 5.77 (n =3), 99.2 ± 2.57 (n =18), 98.3 ± 2.97 (n =18) and 97.5 ± 5.49% (n =18) in the zero (control), 5, 20 and 50 µg/L treatments, respectively. After transfer to the elimination beakers, the survival (%) was 60.0 ± 5.00 (control, n =3), 64.0 ± 18.1 (5 µg/L, n =15), 68.8 ± 13.5

(20 µg/L, n =15) and 58.0 ± 19.3 (50 µg/L, n =15). Since control survival was below 80%, the mortality data in the treatments was normalized to control values using Abbot's formula (Abbot, 1925). These control-adjusted mortality values were -6.11, -7.98 and 15.6% in the 5, 20 and 50 µg/L treatments, respectively. The mean (± 1SD) wet weights of individual amphipods within the uptake phase exposure jars were not observed to differ between concentrations and were 0.118 ± 0.027 (n =3 beakers), 0.131 ± 0.008 (n=18), 0.129 ± 0.012 (n =18) and 0.136 ± 0.017 mg/amphipod (n =18) in the zero (control), 5, 20 and 50 µg/L treatments, respectively. Amphipod wet weights (mg/amphipod) during the elimination phase were 0.118 ± 0.027 (control, n =3), 0.147 ± 0.028 (5 µg/L, n =18), 0.133 ± 0.032 (20 µg/L, n =17) and 0.147 ± 0.027 (50 µg/L, n =18).

The lipid contents of *L. variegatus* and *H. azteca* as a percent (± 1SD) of organism wet and dry weight were determined for samples taken from the cultures used in these experiments. The mean lipids for the oligochaetes were 1.69 ± 0.005% on a wet weight basis and 12.0 ± 0.03% of dry weight. *H. azteca* lipid contents were 3.15 ± 0.59% of wet weight and 5.36 ± 1.00% of dry weight.

The organisms appeared to be healthy throughout the experiments. *L. variegatus* generally spread themselves across the bottom surface of the substrate-free uptake exposure jars. However, in the highest dose (50 µg/L) the worms were amassed into balls until the 12 h sampling point at which time they were observed to be spread along the bottom for the remainder of the test. Upon their transfer to the elimination beakers, *L. variegatus* quickly burrowed into the thin layer (7 g) of sediments and began feeding. For *H. azteca*, at each exposure

sample time point, the amphipods were observed to be tightly clung to the gauze substrate but they swam quickly when prodded with a disposable transfer pipet prior to their removal from the exposure jars. With the exception of the *H. azteca* that presumably died upon transfer to the elimination beakers, the amphipods swam freely during the elimination phase and were seen grazing on the sediments to which food (YCT) had been added previously.

Water concentrations of the test compounds

L. variegatus experiment. The aqueous concentration-time profiles of FLU and TF in the uptake exposures of *L. variegatus* are shown in Figure 3.1. The concentrations declined in the uptake beakers as the worms were accumulating FLU and TF over the 24-h exposure period. The mean concentrations (± 1 SD) of FLU that were measured at the first (1 h) time point were 0.021 ± 0.0002, 0.085 ± 0.001 and 0.20 ± 0.003 µmol/L in the 5, 20 and 50 µg/L (0.025, 0.099 and 0.25 µmol/L) treatments, respectively. The concentrations decreased to final (24 h) levels of 0.014 ± 0.001 µmol/L (at 5 µg/L), 0.056 ± 0.002 µmol/L (at 20 µg/L) and 0.15 ± 0.005 µmol/L (at 50 µg/L). The loss rates (λ; 1/h) and initial aqueous concentrations (C_w^0) of FLU and TF in these exposures were determined from the measured concentration-time profiles by Equation 3.1 and these estimates and best-fit lines are respectively shown in Table 3.4 and Figure 3.1. The λ values were similar for the treatments and ranged from 0.011 to 0.018/h for FLU and from 0.021 to 0.024 for TF. The low sum-of-squares values (RSS; =0.0006) and high adjusted coefficients of determination (R^2; =0.82) indicated a good fit of the equation to the data. These values of λ and C_w^0 were

used as input parameters for the fitting of the body burdens of FLU and TF in the 24-h exposed *L. variegatus* (Equation 3.3) to estimate k_u.

The appearance of the test chemicals in overlying water during the elimination time course is shown in Figure 3.2 for FLU equivalents and in Figure 3.3 for TF equivalents. Radioactivity associated with FLU was detected in the first elimination samples (27 h from T=0 h of uptake, 3 h of elimination) at levels of 0.017, 0.31 and 0.42 nmol/L for *L. variegatus* from the 5, 20 and 50 µg/L uptake exposures (Figure 3.2). The highest mean (± 1 SD) concentrations of FLU in the elimination beakers were 0.32 ± 0.003 nmol/L at 72 h of elimination (96 h from T=0 of exposure) in worms exposed to 5 µg/L, 1.1 ± 0.086 nmol/L (48 h elimination; 20 µg/L exposure group) and 3.1 ± 0.15 nmol/L (72 h elimination; 50 µg/L group). TF was not detected in the aqueous samples of any of the elimination beakers until the 9 h post-exposure elimination time point (Figure 3.3). The concentrations of TF in these samples were 0.045 ± 0.021, 0.060 ± 0.025 and 0.25 ± 0.17 nmol/L for the 5, 20 and 50 µg/L treatments, respectively. TF in the elimination waters rose to maximum concentrations (nmol/L) of 0.15 ± 0.025 (27 h elimination; 5 µg/L treatment group), 0.68 ± 0.13 (48 h elimination; 20 µg/L group) and 1.3 ± 0.15 (27 h elimination; 50 µg/L group). Equation 3.6 was applied to these data to obtain coefficients (*a* and *b*) that were later used in the numeric integration modeling of the elimination of FLU and TF by *L. variegatus*. The values of the coefficients are shown in Table 3.5 and the r^2 values ranged from 0.32-0.87 for FLU and 0.50-0.78 for TF. These values of *a* and *b* were used as input parameters for the numeric integration

modeling (Equations 3.5 and 3.6) of the elimination phase body burden time course data in order to estimate $k_{e(m)}$.

H. azteca experiments. The measured concentrations of FLU and TF in the samples taken during the water-only uptake exposures of *H. azteca* to the test chemicals are shown in Figure 3.4. Similar to the observations with *L. variegatus*, the mean water concentrations (± 1SD) declined with time as the amphipods accumulated FLU and TF but the losses were less than for the worms. The declines in the 5 µg/L treatment group over the period from 1-12 h were very slight for FLU (from 0.022 ± 0.0003 to 0.022 ± 0.001 µmol/L) and TF (0.012 ± 0.0003 to 0.010 ± 0.001 µmol/L). The concentrations of FLU in water samples at 1h were 0.094 ± 0.001 µmol/L at the 20 µg/L and 0.24 ± 0.007 µmol/L at 50 µg/L and these levels declined to 0.086 ± 0.003 and 0.22 ± 0.004 µmol/L, respectively, by 12 h. TF concentrations were 0.044 ± 0.003 at 1 h in the 20 µg/L treatment and were 0.13 ± 0.004 µg/L at the same time in the 50 µg/L dose and by 12 h their corresponding concentrations had declined to 0.034 ± 0.003 and 0.11 ± 0.004 µmol/L. The estimated λ values for these losses were <1% of FLU per hour and between 1.4 and 2.2% of TF per hour (Table 3.6). The values of λ and C_w^0 (Table 3.6) were used as input parameters for the model (Equation 3.3) estimation of k_u for FLU and TF accumulation by *H. azteca*.

The concentrations of the test chemical equivalents measured in the water samples collected during the elimination phase following 12-h water-only exposures of *H. azteca* are shown in Figure 3.5 for FLU and Figure 3.6 for TF. The mean (± 1 SD) concentrations in the samples taken at 1h of elimination were

0.30 ± 0.016, 0.25 ± 0.018 and 0.84 ± 0.37 µmol/L for FLU, and for TF were 0.10 ± 0.011, 0.10 ± 0.003 and 0.59 ± 0.37 nmol/L in the 5, 20 and 50 µg/L treatment groups, respectively. Maximum levels of FLU in the water during elimination were 0.63 ± 0.074 nmol/L at 12 h of elimination in the lowest treatment group, 2.1 ± 0.004 nmol/L at 6 h of elimination in the 20 µg/L exposure group and 5.3 ± 0.33 nmol/L for the 50 µg/L group at 6 h into the elimination phase. Peak TF levels in the water were measured at 12 h of elimination in the 5 µg/L (0.15 ± 0.005 nmol/L) and 20 µg/L (0.46 ± 0.094 nmol/L) exposure groups, and at 6 h of elimination in the 50 µg/L exposure group (1.3 ± 0.19 nmol/L). Estimates of the coefficients *a* and *b* were obtained (Equation 3.6) and are shown in Table 3.7. These values were used as input parameters for the numeric integration modeling (Equations 3.5 and 3.6) of the *H. azteca* elimination time course that provided estimates of $k_{e(m)}$.

Toxicokinetics

Uptake and elimination by L. variegatus. In the *L. variegatus* experiment, uptake data were collected during a 24-h water-only exposure to 5, 10 and 50 µg/L of FLU and TF. The tissue concentration-time profiles of FLU and TF equivalents in *L. variegatus* and model predictions by Equation 3.3 are shown in Figure 3.7. The body burdens of FLU and TF increased rapidly over the first 10 h of exposure in the 5 and 20 µg/L doses, but appeared to level off between 3 and 6 h in the 50 µg/L exposure group before again rising. The observed apparent steady state tissue concentrations (C_{ss}) were reached for both FLU and TF by 18 h and are expressed as the mean (± 1 SD) of the 18 and 24 h samples.

These C_{ss} levels of FLU in *L. variegatus* were 0.032 ± 0.002 µmol/g wet wt at 5 µg/L, 0.127 ± 0.017 µmol/g wet wt at 20 µg/L and 0.216 ± 0.036 at 50 µg/L. TF was accumulated to C_{ss} levels of 0.009 ± 0.001, 0.037 ± 0.003 and 0.074 ± 0.010 µmol/g wet wt in the worms exposed to 5, 20 and 50 µg/L of the test chemicals. The model predicted body burdens of FLU and TF at treatment levels of 5 and 20 µg/L were in very close agreement with the data, whereas the fits were not as good for the highest dose. This discrepancy at 50 µg/L was likely due to the plateau in the tissue concentrations of both FLU and TF between the early (3 and 6 h) time points which was concurrent with observations of clumping behavior by the worms in this treatment.

The estimates of the uptake (k_u) and elimination (k_e) rate constants from the fitting of the uptake data by Equation 3.3 are shown in Table 3.8. The estimates of k_u across experimental concentrations were not significantly different for either FLU ($|t_{obs}|<2.74$, df =32, p >0.05; Table 3.9) or TF ($|t_{obs}|<2.74$, df =32, p >0.05). Likewise, k_e estimates for FLU or TF were not different across the three treatment concentrations (all $|t_{obs}|<2.74$, df =32 p >0.05). The values of k_u ranged from 138-165 mL/g wet organism/h for FLU and 112-129 mL/g wet organism/h for TF. The ranges of k_e were from 0.046-0.115/h and 0.094-0.142/h for FLU and TF respectively. The low residual sum-of-squares (RSS; =0.002) and high r^2 (=0.942) values for the modeling of the 5 and 20 µg/L treatments indicated a good fits to the FLU and TF body burden data. The fits to the 50 µg/L data were not as good (r^2 =0.675 for FLU; r^2 = 0.494 for TF).

Experimentally measured elimination by L. variegatus. Elimination was measured in *L. variegatus* that had been allowed to accumulate FLU and TF for 24 h during water-only exposures prior to their transfer to beakers containing undosed water and a small amount (7 g) of uncontaminated Lake Huron sediments. The elimination profiles of FLU and TF equivalents by *L. variegatus* and model predictions by Equation 3.5 are shown in Figures 3.8 and 3.9 respectively. The body burdens of FLU and TF decreased rapidly over the first 27 h of elimination (*i.e.,* 24-51 h from T=0 h of exposure) in a typical negative exponential fashion. After 3 h of elimination, the mean (± 1SD) tissue concentrations of FLU in the worms were 0.020 ± 0.0003 µmol/ g wet wt (5 µg/L exposure group), 0.083 ± 0.008 µmol/g wet wt (20 µg/L group) and 0.140 ± 0.012 µmol/g wet wt (50 µg/L group). For TF the respective body burdens at 3 h of elimination were 0.005 ± 0.0002, 0.023 ± 0.001 and 0.046 ± 0.003 µmol/g wet wt. Therefore, the amounts the test chemicals remaining in the tissues of *L. variegatus* at 3 h of elimination were approximately 64% (FLU) and 60% (TF) of the apparent C_{ss} values measured during uptake exposures. By 27 h of elimination, body burdens in the worms exposed to 5, 20 and 50 µg/L FLU and TF fell to 0.002 ± 0.0004, 0.007 ± 0.001 and 0.015 ± 0.003 µmol/g wet wt, respectively, for FLU and 0.001 ± 0.0001, 0.003 ± 0.001 and 0.006 ± 0.001 µmol/g wet wt for TF. These concentrations represented only 6% of the FLU and 9% of the TF apparent C_{ss} values in worms from the uptake test.

Prior to the modeling the elimination data for estimations of $k_{e(m)}$ by the numeric integration of Equation 3.5, starting values for the parameters C_a^0 and

$k_{e(m)}$ were derived from the elimination phase tissue concentration-time profile by least squares nonlinear regression of Equation 3.4. The results of these fittings for *L. variegatus* are shown in Table 3.10. The estimates of C_a^0 from this fitting (Equation 3.4) of the elimination data were in very close agreement with the measured apparent C_{ss} levels that were calculated as the mean body burdens of each FLU and TF at 18 and 24 h of uptake (see above). These starting values of C_a^0 and $k_{e(m)}$ (Table 3.10) were used in the primary iterations of Equation 3.5 by the Bulirsch-Stoer method which resulted in new estimates of both k_u and $k_{e(m)}$ (Table 3.11). Finally, the values of k_u and $k_{e(m)}$ from Table 3.11 were input as the starting values for these parameters in the final iterations of the model (Equation 3.5) using a fourth-order Runga-Kutta integration method. The elimination kinetics resulting from this final simulation are shown in Table 3.12. The model predictions of the elimination data were in very close agreement with the data and the goodness-of-fit indicators (*e.g.,* RSS =0.0005, r^2 =0.981) suggest that the model provided an accurate estimate of $k_{e(m)}$ (Table 3.12). The estimated $k_{e(m)}$ for FLU in *L. variegatus* exposed to 20 µg/L (0.128/h) was significantly higher than the value for worms exposed at 50 µg/L (0.112/h) ($|t_{obs}|$ =2.99, *df* =31, p <0.05; Table 3.9). The values of $k_{e(m)}$ for TF in the worms (range 0.099-0.106/h) were not significantly different ($|t_{obs}|$<2.74, *df* =31, p >0.05) across the treatment concentrations (Table 3.9).

The measured elimination rates can be used to easily calculate half-lives (*i.e.,* $t_{1/2} = \ln(2)/k_{e(m)}$) of FLU and TF under field conditions (*i.e.,* in the presence of sediments) and the times required for the compounds to reach 95% steady state

(*i.e.,* $Tss_{95} = 2.99/k_{e(m)}$; Meador et al., 1995). The half-lives corresponding to the elimination rates of the test chemicals by *L. variegatus* ranged from 5.40-6.19 h for FLU and 6.53-7.01 h for TF. The values of Tss_{95} ranged from 23.3-26.7 h for FLU and 28.2-30.2 h for TF.

Uptake and elimination by H. azteca. Uptake data for *H. azteca* exposed to aqueous FLU and TF at 5, 20 and 50 µg/L were measured over a 12-h exposure period. The tissue concentration-time profiles and best model fits for the *H. azteca* exposures to FLU and TF are shown in Figure 3.10. The body burdens of FLU and TF equivalents increased over the entire 12-h time course and did not reach an apparent steady state. The end-of-exposure mean (± 1SD) concentrations of FLU in *H. azteca* exposed to 5, 20 and 50 µg/L of the test compounds, were 0.026 ± 0.004, 0.133 ± 0.013 and 0.391 ± 0.053 µmol/g wet wt, respectively. For TF, the corresponding concentrations were 0.007 ± 0.001, 0.030 ± 0.003 and 0.099 ± 0.014 µmol/g wet wt. In general, the lines representing the model fits to the data were in good agreement with the accumulation data.

The estimated values of the uptake (k_u) and elimination (k_e) rate constants from the fitting of the uptake data by Equation 3.3 are shown in Table 3.13. The estimated of k_u values for the test chemicals in water-only exposed *H. azteca* ranged from 168-199 mL/g wet organism/h for FLU and 75.4-94.0 mL/g wet organism/h for TF. The estimates of k_e ranged from 0.084-0.118/h and 0.053-0.069/h for FLU and TF, respectively. The values values of RSS (=0.004) and r^2 (=0.784) indicated that the fits of the FLU and TF data were in close agreement

with the uptake data. The exception was FLU in the 50 µg/L exposure, for which the RSS (0.038; marginally high) and r^2 (0.758) indicated only an adequate fit to the data. However, even with this less-than-optimal fit for FLU at the highest treatment concentration, neither the k_u or k_e values for *H. azteca* were significantly different for FLU ($|t_{obs}|<2.74$, $df=32$, $p>0.05$) or for TF ($|t_{obs}|<2.74$, $df=32$, $p>0.05$) across the doses (Table 3.9).

Experimentally measured elimination by H. azteca. Elimination of FLU and TF by 12-h exposed *H. azteca* was measured after the organisms were transferred to beakers containing culture water and 3 g of uncontaminated Lake Huron sediments. The elimination profiles of equivalents of the test compounds and model predicted lines (Equation 3.5) are given in Figures 3.10 (FLU) and 3.11 (TF). The data for *H. azteca* elimination was more variable than the elimination profile generated for *L. variegatus* (Tables 3.7 and 3.8*)*. This variability was most likely due to the analytical error introduced by the comparatively lower sample biomass of the amphipods that were losing chemicals from their bodies through time. However, the body burdens exhibited a rapid, decrease over the first 12.5 h of elimination (*i.e.,* 12-24.5 h from the initiation of exposure) that appeared to reach an asymptote thereafter. By the 12.5 h of elimination, the body burdens of *H. azteca* were reduced by approximately 79% for FLU and 30% for TF from the end-of-exposure tissue concentrations summarized above. These body burdens of FLU at 12.5 h of elimination were 0.006 ± 0.001, 0.027 ± 0.011 and 0.083 ± 0.017 µmol/g wet wt for *H. azteca* exposed to 5, 20 and 50 µg/L of FLU and TF, respectively. The

corresponding TF concentrations in the amphipods were 0.005 ± 0.003, 0.024 ± 0.005 and 0.094 ± 0.006 µmol/g wet wt.

The elimination kinetics of FLU and TF in *H. azteca* were first modeled with Equation 3.4 in order to obtain initial parameter values for C_a^0 and $k_{e(m)}$ that were then used for the numeric integration of Equation 3.5. Table 3.14 contains these starting parameter values and they generally described the data (RSS, 0.022-0.0003; r^2, 0.563-0.916). This initial simulation of the body burden time courses using a single-exponential model (Equation 3.4; Table 3.14) overpredicted the last two timepoints of elimination in all cases (predicted line not shown) which was likely responsible for the range in quality of the fits to the data. Even so, the estimated values of C_a^0 were in good agreement with the body burdens of FLU and TF that were measured in in *H. azteca* at 12 h or uptake (see above). Then, these starting values for C_a^0 and $k_{e(m)}$ (Table 3.14) were used in the primary iterations of Equation 3.5 which produced new estimates of both k_u and $k_{e(m)}$ (Table 3.15). Lastly, the the values of k_u and $k_{e(m)}$ from Table 3.15 were used as starting values for final iterations of the modeling of elimination with re-uptake of the contaminants (Equation 3.5) and the results are shown in Table 3.16. The model predictions of the elimination kinetics adequately described the data, although the estimates of $k_{e(m)}$ for FLU (0.124-0.220/h; r^2 range 0.819-0.955) were better than the estimated elimination rates for TF (0.043-0.109/h; r^2 range 0.645-0.778). Inspection of the best-fit lines to the TF data (Figure 3.11) shows that for all three test concentrations, elimination was overpredicted for the first measured time point at 1 h post-exposure and this is especially so for the 5

and 50 µg/L exposed amphipods (Figure 3.11A and C, respectively). The $t_{1/2}$ values corresponding to the $k_{e(m)}$ estimates for the elimination of the test chemicals by *H. azteca* were 3.16-5.60 h for FLU and 6.35-16.3 for TF. The values of TSS_{95} ranged from 13.6-24.1 h for FLU and 27.4-70.2 h for TF. There were no significant differences between the elimination rates ($k_{e(m)}$) of FLU or TF across the treatments (Table 3.9).

Species differences. The kinetic rates of uptake (k_u; obtained from the accumulation kinetics) and elimination ($k_{e(m)}$; obtained from the elimination data) were compared between species using a t-test (Table 3.9). FLU was accumulated at similar rates by both *H. azteca* and *L. variegatus* (p >0.05); however, elimination was significantly faster for *H. azteca* following exposure to 5 µg/L (t_{obs} =-2.30, df =29, p <0.05) and 20 µg/L (t_{obs} =-4.07, df =28, p <0.001) of the test chemicals by factors of 1.8 and 1.4, respectively, but was not different at 50 µg/L (p >0.05). Overall, the estimated values of k_u and $k_{e(m)}$ of TF were highest for *L. variegatus*. Uptake of TF by the oligochaetes was significantly faster than the rate in amphipods for the 5 µg/L (t_{obs} =5.25, df =32, p <0.001) and 20 µg/L (t_{obs} =2.56, df =32, p <0.05) treatments by factors of approximately 1.5. Elimination of TF by *L. variegatus* was more than twice the rate measured for *H. azteca* at 20 µg/L (t_{obs} =3.33, df =28, p <0.01) and 50 µg/L (t_{obs} =2.93, df =29, p <0.01).

Bioconcentration factors (BCFs)

BCFs for FLU and TF in *L. variegatus* (Table 3.17) and *H. azteca* (Table 3.18) were estimated from the accumulation kinetics-based k_u and the k_e

(Equations 3.7-3.10). The kinetics-based BCFs for FLU in *L. variegatus* ranged from 1375 (95% CI, 1143–1654) to 2995 (95% CI, 2297-3904) and were significantly different for comparisons to the high treatment concentrations (*i.e.,* 5 vs. 50 µg/L, 20 vs. 50 µg/L) in multiple comparisons using a z-statistic ($|z_{obs}| > 2.40$, $p < 0.05$; Table 3.19). The kinetics-derived BCFs for TF in the oligochaetes ranged from 850 (95% CI of 735-982) to 1194 (95% CI of 1085–1314). As for FLU, the BCFs for TF in *L. variegatus* were significantly different for comparisons to the 50 µg/L concentration ($|z_{obs}| > 2.40$, $p < 0.05$; Table 3.19). These kinetics-based BCFs were in very close agreement with the empirical BCFs calculated from the steady state tissue concentrations of FLU and TF relative to the aqueous concentrations of the chemicals (Rand et al., 1995; Table 3.17).

BCFs for FLU and TF in *H. azteca* are shown in Table 3.18, and for FLU they increased with dose from 765 (95% CI, 462–1264) to 1606 (95% CI, 1059–2435). TF BCFs in *H. azteca* also increased in a dose-dependent manner from 691 (95% CI. 271–1762) to 2207 (95% CI, 818–5953). There were no significant differences for the BCFs of FLU or TF between the three exposure concentrations (all $|z_{obs}| < 2.40$, $p > 0.05$; Table 3.19).

The equality of the BCFs (estimated by Equation 3.7) between species with respect to dose was tested in pairwise statistical contrasts (Table 3.19). The bioconcentration of FLU by *L. variegatus* was higher than *H. azteca* by a factor of 1.7 at 5 µg/L ($z_{obs} = 2.22$, $p < 0.05$). However, the bioconcentration of TF by the

amphipods was greater than the worms by a factor of 1.6 at 50 µg/L (z_{obs} =-2.14, p <0.05).

Biotransformation of FLU and TF

The biotransformation of FLU and TF by *L. variegatus* was measured by extraction followed by TLC and LSC for samples taken at the end-of-exposure (24 h) and at 48 h of post-exposure elimination, whereas the metabolism of the compounds by *H. azteca* was assessed only at the end of a 19.5-h exposure. The percentages of the total [^{3}H]FLU and [^{14}C]TF body burdens that were determined to be the parent compounds, extractable metabolites and unextractable residues are shown in Table 3.20 for *L. variegatus* and Table 3.21 for *H. azteca*. The total of the extractable metabolites and unextractable residue were assumed to represent the total metabolite burden (Kulkarni and Hodgson, 1980). An overall mass balance was achieved in all measurements. Fluoranthene was not metabolized by *L. variegatus* after 24 h exposures to the test chemicals as indicated by the equivalence between the starting purity of the radiolabeled compound (96%) and the body burden as parent compound (96-97%; Table 3.20). The remainder of the FLU body burden in the worms was 1.1-1.2% as extractable metabolites and 1.8-2.2% residual; however, these fractions may actually represent breakdown products of radiolabeled FLU that were taken up during the exposures. The biotransformation of TF by the worms at the end-of-exposure was minimal, with 93-94, 4.1-4.2 and 2.3-2.6% as parent compound, extractable metabolites and unextractable residues, respectively. However, by 48-h of elimination, the time at which only approximately 6 and 9%

of the total accumulated FLU and TF equivalents, respectively, remained in the tissues, metabolites in *L. variegatus* comprised a larger proportion of the body burdens. The total [^{3}H]FLU body burden at this sample time was 31-50% parent compound, 11-15% extractable metabolites and 39-55% unextractable residues. The [^{14}C]TF in the worms at 48 h of elimination was 52-68, 12-18 and 20-30% parent compound, extractable metabolites and unextractable residues, respectively. The mass balance for the extraction of worm tissues was >98%.

Factorial ANOVA testing of the mean percentages of the *L. variegatus* body burden fractions of parent FLU and TF and their extractable and unextractable metabolites identified numerous significant differences, primarily with respect to time. Two-factor ANOVA identified significant differences between sample mean percentages of unextractable residues of FLU in *L. variegatus* due to the individual main effects of dose and time ($F_{2,12}$ =4.29, p =0.039, and $F_{1,12}$ =316, p <0.0001, respectively). Post-hoc testing of these mean percentages of unextractable FLU residues with Tukey's HSD determined that the differences with respect to dose were between the 5 and 20 µg/L treatments (p =0.045). TF biotransformation data were significantly different due to the main effect of time for the mean percentages of parent compound ($F_{1,12}$ =131, p <0.0001), extractable metabolites ($F_{1,12}$ =55.8, p <0.0001) and unextractable residues ($F_{1,12}$ =181, p <0.0001).

The metabolism of FLU and TF by *H. azteca* after 19.5 h exposures to both compounds is summarized in Table 3.21. The amphipods readily metabolized FLU as the total body burden was 57-73% parent compound,

3.6-6.8% extractable metabolites and 23-36% unextractable metabolites. One-way ANOVA testing of the mean percentages of the body burden fractions identified significant differences with between doses for the parent compound ($F_{2,6}$ =27.6, p <0.001), the extractable metabolites ($F_{2,6}$ =11.8, p =0.008) and the unextractable residues ($F_{2,6}$ =18.6, p =0.003). Post-hoc testing of these mean percentages by Tukey's HSD test indicated that all three doses were different for parent compound (p =0.03), that there was no difference in the extractable residues between the 20 and 50 µg/L treated amphipods (p >0.05) and that there was no difference in the unextractable residues between the *H. azteca* exposed at 5 and 20 µg/L (p >0.05). The amphipods were capable of metabolizing TF as parent compound represented 65-66% of the total TF body burden while extractable metabolites and unextractable residues comprised 10-16 and 19-25% of the body burden, respectively. The mean percentages of FLU equivalents as unextractable residues were significantly different (one-way ANOVA, $F_{2,6}$ =5.65, p =0.042) between only the 5 and 50 µg/L treatments (Tukey's HSD, p =0.035).

DISCUSSION

Toxicity of waterborne FLU and TF

The treatment concentrations of FLU and TF used in the water-only exposures of *L. variegatus* and *H. azteca* were not expected to cause toxicity based on single-chemical exposure data obtained from the literature (Parrish et al., 1978; Mayer and Ellersieck, 1986; Suedel et al., 1993; Kane Driscoll et al., 1997b; Sheedy et al., 1998 Nowell et al., 1999). Survival was high during the

uptake exposures of both *L. variegatus* and *H. azteca* and during the elimination phase for the oligochaetes. However, when 12-h exposed *H. azteca* were transferred to the elimination beakers, the percentage survival of the amphipods based on the initial number of individuals placed into exposure beakers (20 each) was low (mean range 58-68%) at all sampling points for both control and treated organisms which would suggest toxicity, poor health, or handling stress. Control corrected mortality (<16%) indicated that the overall reduced survival of the *H. azteca* was an artifact of the handling stress placed on the organisms upon their transfer from the exposure beakers to the elimination beakers by sieving. During this process the organisms were exposed to air and, when rinsed, to mechanical stress, both of which are not recommended for the maintenance of healthy amphipods (USEPA, 2000a). A number of amphipods were observed to be floating on the water surface immediately after their transfer from the exposures, and since efforts to submerge them were unsuccessful, these individuals probably perished shortly thereafter. Therefore, it was concluded that the compounds were not toxic to either test species during exposures, to *L. variegatus* during elimination, and likely did not cause toxicity to the amphipods during the post-exposure elimination. However, confirmation of the latter portion of these conclusions would be provided by another test in which the *H. azteca* transfer is performed more gently by plastic pipet.

Tissue concentrations

The body residues of FLU and TF measured *H. azteca* and *L. variegatus* in the present water-only experiments compare well with other reported studies

of the body burdens of these chemicals in fish and aquatic invertebrates. The bioconcentration of FLU has recently been studied in water-only exposures of the marine amphipod *Leptocheirus plumulosus* and the freshwater species *Diporeia* sp. and *H. azteca* (Kane Driscoll et al., 1997b, 1998). *L. plumulosus*, exposed to nominal concentrations ranging from 8 to 128 µg FLU/L resulted in approximate body burdens of total FLU equivalents by 8-d that ranged from 0.074 to 2.98 µmol/g wet wt (Kane Driscoll et al., 1998). These authors also reported mean 8-d lethal body residues that produced 50% mortality (LR_{50}s) that ranged from 0.694 to 2.44 µmol/g wet wt, and these LR_{50} values were above the highest concentrations of total FLU equivalents measured in the *H. azteca* used in the present study (0.391 ± 0.053 µmol/g wet wt). *Diporeia*, which do not biotransform FLU, were exposed to nominal concentrations of FLU that ranged from 64.2 to 257 µg/L and the amphipods reached body burdens of 2.9 to 6.0 µmol/g wet wt (Kane Driscoll et al., 1997b) which were 1-2 orders of magnitude higher than the range observed in the *H. azteca* of the present study. In the same study (Kane Driscoll et al., 1997b), *H. azteca* were exposed to nominal doses of 16.2 to 127 µg/L and by day 10 their body burdens (0.2 ± 0.004 to 5.0 ± 1.1 µmol/g wet wt) encompassed the upper part of the range observed in the present study with this species. More specifically, the lowest exposure doses for *H. azteca* in the Kane Driscoll et al. (1997) 10-d study were 16.2 and 64.7 µg/L for which amphipods accumulated mean body burdens of 0.5 ± 0.5 to 2.1 ± 1.1 µmol/g, respectively in experiment 1 (highly variable, CVs 52-100%) and 0.2 ± 0.004 to 0.3 ± 006 wet wt in experiment 2 (more reliable data, CVs only

2%). The results of their second experiment seem to agree well with the mean body burdens observed at 12 h in the present study at 20 and 50 µg/L (0.133 ± 0.018 and 0.391 ± 0.053 µmol/g wet wt, respectively; Figure 3.10A). The LR_{50}s for mortality in water-only exposed *H. azteca* (3.6 and 5.6 µmol FLU/g wet wt) reported by Kane Driscoll et al. (1997b) were well above the levels measured for *H. azteca* in the present study, suggesting that stress to the animals during transfer was the more likely cause of their reduced survival during elimination.

The number of published studies of the uptake of FLU from water by *L. variegatus* is limited (only two papers), but the data that do exist compare favorable with the body burdens reported in the present study. In 96-h exposures of *L. variegatus* to nominal concentrations of FLU ranging from 3.7 to 60 µg/L, the approximate end-of-exposure tissue concentrations of FLU were from 0.074 to 0.640 µmol/g wet wt (Ankley et al. 1995). These values are close to the range of apparent C_{ss} values (0.032 ± 0.002 to 0.216 ± 0.036 µmol/g wet wt; Figure 3.7A) reported for FLU in the *L. variegatus* used in the present study at nominal exposure concentrations up to 50 µg/L. Sheedy et al. (1998) conducted time course studies of the uptake and elimination of four PAHs, including FLU, in water-only exposures of *L. variegatus*. By 6 h, the oligochaetes had accumulated approximately 0.010 and 0.012 µmol FLU/g wet wt at measured mean doses of 8 and 14 µg/L, respectively, and by 24 h the corresponding body burdens of FLU were 0.020 and 0.025 µmol/g wet wt. The concentrations for the 6 h samples of Sheedy et al., (1998) were about equal to the 5 h body residues of total FLU equivalents in the *L. variegatus* exposed to 5 µg/L (0.013 ± 0.0001

µmol/g wet wt) in the present study, and the 24 h body burdens reported in the citation were slightly less than the measured apparent C_{ss} of FLU (0.032 ± 0.002 µmol/g wet wt) in worms at the low dose of the present study.

Although the tissue data from previous studies with FLU generally compare to the body burdens of FLU reported for *H. azteca* and *L. variegatus* in this study, it should be noted, with the exception of the Sheedy et al. (1998) study, that the body burdens for amphipods and oligochaetes in the previous investigations were slightly higher. This difference may be due to the experimental design of the current study in which simultaneous exposures to two chemicals with similar lipophilicities were conducted (FLU, log Kow 5.2; TF, log Kow 5.3; Mackay et al., 1992, 1997) whereas FLU was the only chemical used in the studies cited above. However, other factors known to affect the toxicokinetics and accumulation of organic compounds include the exposure temperature (Dabrowska and Fisher, 1993; Koelmans and Jimenez, 1994), lipid content and size of the organisms (Landrum, 1988; Bruner et al., 1994; Landrum and Fisher, 1998; Hendriks et al., 2001), and biotransformation (Leversee et al., 1982; Lydy et al., 2000). These physiological and environmental factors may also have been responsible for this difference. On the whole, the tissue concentrations of FLU measured in the present study were similar to a number of previously reported body burdens for this compound.

Reports of controlled exposures of aquatic organisms to TF in the literature were primarily for fish with a few studies of invertebrate species. Parrish et al. (1978) exposed adult sheepshead minnows (*Cyprinodon*

variegatus), a saltwater fish, to TF in the water (range 0.5 to 17.7 µg/L) at 30 °C and measured TF residues in the fish at 166 and 189 d. The TF to body burdens in whole-fish samples from the long-term exposures at doses relevant to the present study were 0.101 µmol/g wet wt at 4.8 µg/L and 0.236 µmol/g wet wt at 17.7 µg/L. These steady state concentrations in the fish *C. variegatus* were higher than the apparent C_{ss} measured for *L. variegatus* exposed to 50 µg/L in the present study (0.074 ± 0.010 µmol/g ww) by factors of 1.4 and 3.2, respectively. However, the amphipods exposed for only 12 h to 50 µg/L of TF and FLU in the present study approached the levels of TF reported for *C. variegatus* at 4.8 µg/L (Figure 3.10B).Schultz and Hayton (1994, 1998) exposed a number of fish species (rainbow trout, *Oncorhynchus mykiss*; lake sturgeon, *Acipenser fulvescens*; bluegill sunfish, *Lepomis marchrochirus*; largemouth bass, *Micropterus salmoides*; channel catfish, *Ictalurus punctatus*; gizzard shad, *Dorosoma cepedianum*; and fathead minnow, *P. promelas*) of various weights and at various temperatures (12-23 °C) to 2 µg TF/L. Most species achieved C_{ss} levels between 24 and 96 h that ranged from approximately 1.5 to 3.1 nmol/g wet wt. The only exception was the fathead minnow, which accumulated only 0.081 nmol/g wet wt at the end of a 48 h exposure. The results of Schultz and Hayton (1994, 1998) were generally the same order of magnitude as the TF body burdens measured at the end-of-exposure for *L. variegatus* and *H. azteca* exposed to the lowest dose (5 µg/L) in the present study.

Yockim et al. (1980) conducted a study of the bioconcentration of TF by mosquitofish (*Gambusia affinis*), snails (*Helisoma* sp.), *Daphnia magna* and an algae (*Oedogonium cariacum*) in a flow-through mesocosm exposure system at verified concentrations that ranged from 0.1 to 9.3 µg/L after 24 h. *G. affinis* body burdens ranged from 0.003 to 0.033 µmol/g wet wt, *Helisoma* sp. tissue concentrations were from 0.0006 to 0.008 µmol/g wet wt and *D. magna* body burdens ranged from 0.001 to 0.059 µmol/g wet wt. Compared to the present study, the body burdens of TF in the fish and invertebrates reported by Yockim et al. (1980) range from below the lowest 24-h TF levels observed for *L. variegatus* in the present study study (<0.009 µmol/g wet wt; Figure 3.7) to levels that are between the apparent C_{ss} levels in *L. variegatus* and the end-of-exposure body burdens in *H. azteca* at the 20 and 50 µg/L treatments. Finally, in a recent study of the biotransformation of TF by invertebrates a body burden of 0.012 ± 0.002 µmol TF/g wet wt was reported for *L. variegatus* that were exposed to 16.8 µg TF/L for 48 h (Verrengia-Guerrero et al., 2002). This levels is between the apparent C_{ss} levels measured at 5 and 20 µg/L in the present study. Based on the review of TF accumulation by various species given above, it appears that *L. variegatus* and *H. azteca* reached body burdens that were within the range observed in other aquatic vertebrates and invertebrates exposed to comparable concentrations.

Kinetics and biotransformation

In the *L. variegatus* and *H. azteca* experiments, no apparent relationship was evident between exposure concentrations and kinetic rates (*i.e.,* k_u, k_e, $k_{e(m)}$).

The only exception was that the elimination of FLU by *L. variegatus* measured in the presence of sediments and water ($k_{e(m)}$) was significantly reduced at 50 µg/L compared to 20 µg/L (Table 3.9). This may have been an artifact of the clumping behavior observed at the early sample times during the accumulation exposure to 50 µg/L. This posed another problem to the analysis in that such behavior may have led to a change in the kinetics rates for these worms during the exposure (*e.g.,* enhancement of k_e; Table 3.8) and was likely the cause of the less-than-optimal fits of the model to the data (*i.e.,* relatively higher RSS and lower r^2 values for FLU and TF; Table 3.8). Unfortunately, there was no way to correct for this behavior in the modeling. Overall, the lack of a consistent relationship between concentration and kinetic rates, combined with the high survival observed for all treatments relative to controls, generally suggests that the physiological state of the organisms did not change during the experiments (Eadie et al., 1990; Landrum et al., 1991) which led to similar kinetics for each species across the doses.

There were numerous differences in the toxicokinetics of FLU and TF between the test organisms. The uptake clearance and elimination rates of total FLU equivalents by *L. variegatus* and *H. azteca*, as estimated from the accumulation data (*i.e.*, k_u and k_e), were not significantly different in any of the comparisons. However, in the presence of sediments, the measured elimination ($k_{e(m)}$) kinetics were faster for *H. azteca* at all doses tested; although, only the differences between species at 5 and 20 µg/L doses were significant. TF kinetics were different between the species in the present study, but for this compound

significantly faster rates of both uptake and elimination were measured for *L. variegatus*. It was also observed that *H. azteca* possessed a greater ability to biotransform both FLU and TF than *L. variegatus* during the accumulation exposure, but this was not sufficient to explain the differences in uptake and elimination rates outlined above. Therefore, a hypothesis that a higher rate of biotransformation would contribute to a more rapid elimination rate, as previously observed for exposures of amphipods to DDT (Lotufo et al., 2000), was not supported by the observations on FLU and TF. A number of alternative explanations to describe these kinetic differences were explored including the lipid content and body size of the organisms, sediment contact, and the nature of the metabolites of FLU and TF, and they are further discussed below.

Lipid content is known to be an important factor in the toxicokinetics of bioaccumulation and bioconcentration (Landrum and Fisher, 1998). For the *L. variegatus* and *H. azteca* exposed to FLU and TF, the lipid content on a dry wt basis was about twice as high in the worms (12.0 ± 0.03%) compared to the amphipods (5.36 ± 1.00%). The most often reported effect of lipids on chemical kinetics in organisms is an inverse relationship between elimination and lipid content (Landrum, 1988; Van den Huevel et al., 1991). This relationship may account for the observed slower elimination of FLU by *L. variegatus*, but it does not explain their more rapid elimination of TF compared to *H. azteca*. Recently, a direct relationship was found between uptake kinetics (k_u) and lipid contents for the zebra mussel, *Dreissena polymorpha*, exposed to organic contaminants (Bruner et al., 1994). The observations on TF in the present study supported this

relationship as the values of k_u for *L. variegatus* were higher than the uptake rates of this chemical by *H. azteca* by factors ranging from 1.3-1.7. However, there was no apparent difference in the FLU uptake kinetics between the test organisms.

Body size (*e.g.,* weight) has been shown to be an important determinant of toxicokinetic rates (Hendriks et al., 2001). Uptake rates and bioconcentration factors for organic chemicals generally are inversely related to the organism size (Weis et al., 1977; Tarr et al., 1990; Bruner et al., 1994; Hendricks et al., 2001). Elimination has also been reported to decrease with increasing body size (Hendriks et al., 2001), but others have concluded that k_e was not affected by body size (Bruner et al., 1994). In exposures to FLU and TF, the mean body weight of the *L. variegatus* (4.29 mg/individual) was greater than *H. azteca* (0.12 mg/individual) by a factor of 36. Although the elimination of FLU was faster for the smaller-bodied amphipods, overall, the kinetic rates of uptake and elimination of FLU and TF and their BCFs did not show a consistent relationship with respect to the body size difference between species.

Physical interaction with sediments may influence the elimination of organic chemicals by benthic invertebrates. Studies with deposit-feeding invertebrates have shown that actively feeding on sediments resulted in faster elimination rates for organic contaminants such as pyrene (*L. variegatus*), anthracene (*H. azteca*) and FLU (*Coullana* sp.) when compared to elimination in water-only elimination (Landrum and Scavia, 1983; Kukkonen and Landrum, 1994; Lotufo, 1998b). However, a more recent study of the elimination of PAHs

by amphipods concluded that passive diffusion of organic chemicals from the organism to sorptive sediments, even in the absence of feeding on sedimentary materials, was sufficient to enhance elimination compared to a water-only system (Lotufo and Landrum, 2002). The *L. variegatus* exposed to FLU and TF were observed to burrow into the sediments and feed during the post-exposure elimination phase. The worms contained gut material at each sampling point during elimination, although this was not quantified. The *H. azteca* appeared to graze on the additional YCT (food) provided to their elimination beakers; however, they were not observed to be in intimate contact with the sediments which was characteristic of their epibenthic nature (USEPA, 2000a). Therefore, reduced contact with the sediments by *H. azteca* compared to the constant contact by *L. variegatus* may explain the higher rate of TF elimination by the worms. However, this hypothesis that contact time and interaction with the sediments would be directly related to k_e did not explain the results for FLU.

The characteristics of the specific metabolites formed from parent FLU and TF by the *L. variegatus* and *H. azteca* may have led to the observed differences between elimination rates. Slower release of metabolites compared to their formation or to the elimination of the parent compound has been reported for PAHs in numerous invertebrate species (James, 1989). For example, Kane Driscoll et al. (1998) observed that after 4 d of exposure to FLU, the body burden in the marine amphipod, *L. plumulosus,* was about 60% parent compound which is similar to the amount of parent compound measured in amphipods at the end of 19.5 h exposure to FLU and TF in the present study. The elimination rate for

total equivalents (*i.e.,* parent compound, polar and aqueous metabolites) reported for *L. plumulosus* was 0.0061/h whereas the k_e for only the parent FLU was faster, at 0.0092/h (Kane Driscoll et al., 1998). In the marine polychaete *Marenzellaria viridis* the build-up and slow release of aqueous-soluble metabolites of benzo[a]pyrene was observed (Kane Driscoll and McElroy, 1997). Freshwater species such as *Chironomus riparius* eliminated parent anthracene more rapidly than polar metabolites (Gerould et al., 1983). In a study of the kinetics of pyrene in *L. variegatus,* Leppänen and Kukkonen (2000) observed a rapid decrease in the body burden of parent pyrene and an increase in metabolites of the compound in the worm tissues during the post-exposure elimination phase. For non-PAHs, parent compound was also eliminated more rapidly than polar metabolites by the sea urchin, *Strongylocentrotus purpuratus* exposed to *p*-nitroanisole (Landrum and Crosby, 1981), and by *Chironomus tentans* exposed to 2-chlorobiphenyl (Lydy et al., 2000). By the end of the present exposures, *H. azteca* had more extensively metabolized both FLU (26.9-42.5%) and TF (≈34%) compared to *L. variegatus* (FLU, negligible; TF, ≈6.5%). Thus, the trends observed in the investigations cited above may explain the higher elimination rates of TF equivalents by *L. variegatus* but they were not supported by the observed faster elimination of FLU by *H. azteca*.

Lastly, chemical characteristics may have been responsible for some of the observed species differences in the kinetics of FLU and TF. A possible explanation for the slower rate of uptake of TF by the amphipods is that due to the slight polarity of this herbicide (Albanis et al., 1998; Liskra and Bilikova), even

though it has a similar log K_{ow} to FLU, it may cross the respiratory membranes (*i.e., gills*) more slowly than the integument of *L. variegatus* (Gobas et al., 1986). The respiratory membranes and integument of amphipods are likely the primary sites of uptake of hydrophobic compounds by amphipods (Landrum and Stubblefield, 1991), and this is especially so for charged or polar compounds (Pantani et al., 1995). However, this explanation is highly speculative since no data was collected that can support such a hypothesis.

Overall, no single explanation given in the above discussion was sufficient to explain the differences in kinetic rates observed between species. In all cases, a given explanation supported the observations of one of the test chemicals, but not the other. The higher lipid content of the *L. variegatus* supported the higher k_u and slower $k_{e(m)}$ for FLU in *L. variegatus* compared to the amphipods, but did not explain the more rapid k_e and $k_{e(m)}$ values for TF in the worms. The smaller body size of the *H. azteca* supported only the observed higher elimination rate of FLU by the amphipods compared to the worms. The hypothesis that physical interaction with the sediments would enhance elimination ($k_{e(m)}$) was supported by TF elimination by infaunal oligochaetes, but not by their elimination of FLU. And finally, the hypothesis that invertebrates generally eliminate parent compounds more rapidly than polar and aqueous metabolites appeared to apply to the elimination of TF equivalents by the amphipods, but not FLU equivalents. Therefore, future studies of these compounds should measure accumulation and biotransformation in order to more fully describe the toxicokinetics of the parent compound and metabolites.

Biotransformation of both test compounds was observed in *H. azteca* and *L. variegatus*, although the evidence was much stronger for *H. azteca*. The biotransformation of PAHs, such as FLU, is well described in the literature and begins via oxidation by cyctochrome P450 enzymes which forms diols, quinones and phenolic compounds (Varanasi, et al., 1989; Di Giulio et al., 1995). These primary metabolites can be acted upon by other oxidative enzymes to form more toxic or carcinogenic moieties, such as diol-epoxides, that are capable of covalently binding to cellular macromolecules (*i.e.,* DNA, RNA, proteins) (Ahokas, 1979; Shugart et al., 1987). Or, the primary PAH metabolites can be conjugated by Phase II enzymes, such as transferases, that often lead to more excretable, less toxic metabolites including glucuronide, glutathione and sulfate conjugates (Van Hofe et al., 1979; George and Young, 1988; Zaleski et al., 1991). TF metabolism in fish (Schultz and Hayton, 1993; 1994; 1999) and rats (Erkog and Menzer, 1985) has been investigated and characterized. These studies report that the primary biotransformation step of TF is the cytochrome P450-mediated aliphatic oxidation of one of the *N*-propyl groups followed by dealkylation. Following this initial step, secondary transformations can occur including further depropylation, conjugations with amino acids (*e.g.,* -GSH) and nitroreduction (Erkog and Menzer, 1985). Cytochrome P450s or P450-like enzymes have been identified in a number of marine and freshwater invertebrates that were capable of metabolizing various hydrocarbons and these organisms include polychaetes (*e.g., Neireis* sp., *Capitella* sp.), oligochaetes, crustaceans (*e.g., D. magna, H. azteca*) and dipterans (*e.g., C. riparius*) (James

and Boyle, 1998; Lee, 1998; Snyder, 2000; Forbes et al., 2001; Verrengia Guerrero et al., 2002).

The extent to which *H. azteca* appeared to metabolize of FLU was a surprise. A previous study of the biotransformation of FLU by *H. azteca* exposed to the compound in the water for 24 h resulted in body burdens of 83.2% parent compound, 9.9% extractable metabolites and 7.0% residual or unextractable (Kane Driscoll et al., 1997). In the present study, in which the experimental conditions were similar (*e.g.,* temperature, photoperiod, wavelength of ambient lighting) after 19.5-h exposures of the *H. azteca* to both FLU and TF, parent FLU was 18% lower and residual activity was 23% higher than the levels reported by Kane Driscoll et al. (1997). A hypothesis to explain this would be that there was an enhancement of the metabolism of FLU by the co-exposure to TF. This apparent increase in contaminant metabolism could be due to induction of biotransformation enzymes. Herbicides such as atrazine have been shown to induce cytochrome P450s in insects (Kao et al., 1998) so based on this and the discussion above on the biotransformation of TF by vertebrates, TF may have activated P450s or a similar mixed function oxidase (MFO) enzyme system in *H. azteca*. Levine and Oris (1999) using both *in vivo* and *in vitro* methods demonstrated that pre-exposure of fathead minnows, *P. promelas*, to the antifungal triazole compound propiconazole enhanced the acute toxicity of the organophosphate parathion. These authors suggested, based on microsomal assays, that the increase in toxicity of parathion was due an increase in its rate of activation to paraoxon by pre-treatment with propiconazole which induced higher

levels of cytochrome P450s (Levine and Oris, 1999). Recent studies of the synergistic toxicity between pesticides of different classes to the midge, *Chironomus tentans,* indicated that the triazine herbicide atrazine enhanced the toxicity of the organophosphate insecticide chlorpyrifos, by increasing the biotransformation rate of the organophosphate to its toxic *O*-analog through the induction of MFOs (Belden and Lydy, 2000). These results were also observed with another triazine herbicide (cyanazine) and other organophosphates including methyl-parathion and diazinon (Pape-Lindstrom and Lydy, 1997; Jin-Clark et al., 2002). Although acute toxicity was not observed in the *H. azteca* that were exposed to a mixture of the herbicide TF and FLU, it was possible that TF enhanced the metabolism of FLU.

L. variegatus metabolized negligible (FLU, =3.5%, equivalent to impurities in starting material) and small (TF, =6.8%) amounts of the test compounds by the end of the exposure period. However, biotransformation products were a large proportion of their body burdens after 48 h of elimination. Although evidence that *L. variegatus* is capable of metabolizing PAHs has been recently reported for FLU (about 9% non-parent residues; Landrum et al., 2002), benzo[a]pyrene and pyrene (approx. 2-15% and 15-35% non-parent compound, respectively; Harkey et al., 1994; Leppänen and Kukkonnen, 2000) this is the first report of the biotransformation of TF by this species. Extensive metabolism of TF has been measured in fish (Schultz and Hayton, 1999), but there are few comparative data on the biotransformation of TF by invertebrates. The snail, *Helisoma* sp., contained 26 and 36% of its body burden as metabolites of TF at days 15 and 30

of 50-d exposures to parent TF (Yockim et al., 1980). More recently, Verrengia Guerrero et al. (2002) reported that *L. variegatus* did not produce metabolites of TF after 48-h water-only exposures to the compound, whereas *C. riparius* metabolized TF by about 60%. Since the 2-d exposures were conducted at 8 ºC (Verrengia Guerrero et al., 2002), it is likely that the higher temperature (22 ºC) over a shorter (24 h) exposure duration in the present study allowed biotransformation of TF.

There were large differences in the percentages of FLU and TF parent, extractable, and unextractable residues between the *L. variegatus* samples taken at the end-of-exposure and after 48 h of elimination. These differences were due to the elimination of primarily parent compound between the two samples. Evidence to support this conclusion is provided by examining the total disingegrations per minute (dpm) present in the tissues at each sample time. Mean total dpms for ^{3}H and ^{14}C at the end-of-exposure ranged between 74712-77556 and 6622-8164, respectively. By 48 h of post-exposure elimination, these levels had dropped to 1784-3120 dpm for tritium and 223-461 dpm for carbon-14. Interestingly, these 48-h values were very similar to the dpm associated with the extractable metabolites and non-extractable residues measured at the 24 h end-of-exposure sample (^{3}H, 2400; ^{14}C, 475 dpms). These results suggest that a small amount of radioactivity at both samples times was associated with metabolites and, based on the consistency in the dpms associated with non-parent material, that these metabolites may have been formed and eliminated at similar rates (Kane Driscoll et al., 1998; Lydy et al., 2000).

Bioconcentration Factors (BCFs)

The BCFs for FLU and TF in *L. variegatus* and *H. azteca* were estimated from the kinetic rate constants (k_u, k_e) and the BCF values indicated that bioconcentration of the test compounds would occur upon exposure in the aquatic environment. For *L. variegatus*, it was possible to calculate an empirical BCF (*i.e.,* BCF = C_{ss}/C_w) since the worms accumulated FLU and TF equivalents to an apparent steady state. These empirical BCFs were in very close agreement with the estimates obtained using the kinetic rates. However, the BCFs should be viewed with caution as they are based on kinetic rate estimates or matrix concentrations that were derived from measurements of the total equivalents of FLU and TF in samples of tissue and water. Thus, since the body burden data was not adjusted for biotransformation the BCFs for the parent compounds are likely overestimated in this study (Franke et al., 1994; Franke, 1996; Lydy et al., 2000). The BCFs for FLU and TF obtained for *H. azteca* and *L. variegatus* were near the lower estimates obtained from regression equations based on log K_{ow} (Vieth and Kosian, 1983; Meylan et al., 1999) and were near other reported or calculated BCFs for these compounds. The regression equations for estimating BCFs took the form of:

$$\log \text{BCF} = a \log K_{ow} + b \qquad (3.12)$$

where *a* and *b* are empirically determined constants, and have been commonly used with values of a =0.79 and b =-0.40 (Veith and Kosian, 1983) or a =0.77 and b =-0.70 (Meylan et al., 1999). From these two verisions of Equation 3.12, BCFs were predicted to be 2014 to 5105 for FLU and 2404 to 6124 for TF. The

values obtained in the present study were at or below estimates based on low K_{ow}.

The BCFs of FLU and TF that were estimated for *L. variegatus* and *H. azteca* in the present study compare well with other BCFs reported for these and other benthic species. Sheedy et al. (1998) reported a BCF of 2390 for FLU following 96-h water-only exposures of *L. variegatus* and Ciarelli et al. (2000) reported BCFs ranging from 1145 to 1237 for dissolved FLU in exposures of marine polychaetes. BCFs for other PAHs accumulated by *L. variegatus* were similar to those for FLU in the present study and these included anthracene (log Kow, 4.54; BCF, 1370) and pyrene (log Kow, 5.18; BCF 1720) (Ankley et al., 1997). The 10-d BCFs for FLU in *H. azteca* were calculated from body burden and aqueous concentration data obtained from Kane Driscoll et al., (1997b) and the mean (± SD) BCF was 5705 ± 2786 with a range of values from 1382 to 8432. BCFs for *H. azteca* exposed to FLU for 12 h in the present study were below or at the lower end of this range. There are few data on TF BCFs for comparison to the estimated BCF values of the present study. Invertebrate species for which BCFs for TF have been reported include snails (*Helisoma* sp.; BCF, 130-2360) and daphnids (*D. magna*; BCF, 20-1080) (Yockim et al., 1980). BCFs from various marine and freshwater fish species ranged from 1333 to 21,964 (Macek et al., 1976; Parrish et al., 1978; Spacie and Hamelink, 1979; Graper and Rainey, 1988; Schultz and Hayton, 1993, 1994). Therefore, even with the minimal (*L. variegatus*) to moderate (*H. azteca*) amounts of biotransformation observed in the present study, the comparisons of the BCFs of

FLU and TF reported here to those obtained by regression or empirical data indicated that present BCFs should provide good estimates of expected levels of these compounds in environmental exposures of benthic organisms.

Species differences

The exposures of *L. variegatus* and *H. azteca* to waterborne FLU and TF led to dose-dependent accumulations of the chemicals that, in terms of their end-of-exposure body burdens, were in most cases similar (Figures 3.7 and 3.10). This point, and the following comparisons of C_{ss} levels in *L. variegatus* and non-C_{ss}, 12-h body burdens in *H. azteca* are necessary for the discussion of the predicted *H. azteca* C_{ss} values presented below. For the 5 and 20 µg/L treatments, TF body burdens in *H. azteca* after a 12-h exposure were nearly identical to the apparent C_{ss} levels measured in *L. variegatus* and at the highest dose the mean body burden in the amphipods at 12 h was only slightly higher than the apparent C_{ss} in the worms. Although FLU accumulation in the 5 µg/L and 20 µg/L treatment was similar at the end-of-exposure between the test species, the tissue concentrations of FLU in the 50 µg/L dose was higher, by a factor of 1.8, for the *H. azteca* than the apparent C_{ss} values measured in the *L. variegatus*. This may be explained by the observations of the clumping behavior of the *L. variegatus* at 50 µg/L during the earlier (0-5 h) sampling times of the uptake exposure. When in such a tight formation, less of the worms' total surface area was exposed to the dissolved compounds and this stress response appeared to enhance elimination during the accumulation phase (Table 3.8); although, this was not statistically significant (Table 3.9).

Although the observed difference in FLU accumulation between species at the highest dose appeared to be driven by a behavioral response in the worms to the test conditions, toxicokinetic differences between *L. variegatus* and *H. azteca* suggest that the C_{ss} levels of TF would generally be higher for amphipods. The 12-h exposures of the amphipods were not of sufficient length to allow them to reach steady state for TF, but based on the estimations of Tss_{95} (43.3-56.6 h), the *H. azteca* would be expected to accumulate TF to C_{ss} levels above those observed in *L. variegatus* who were already at or very near their predicted Tss_{95} values for both FLU (26.1-64.8 h) and TF (21.0-31.8 h). This potentially higher steady state body burden of TF in the tissues of *H. azteca* can be estimated by the product of the mean BCF for TF in *H. azteca* (1450) and the initial aqueous concentrations (*i.e.,* C_w^0; Table 3.4). Use of the mean BCF for such predictions is justified because for *H. azteca* the BCFs were not significantly different across the tested doses (Table 19). For 5, 20 and 50 µg/L this would lead to an average body burden of TF in *H. azteca* of 0.017, 0.064 and 0.184 µmol/g wet wt, or about a factor of 1.1-2.5 fold greater than either the measured (0.009, 0.037, 0.074 µmol/g wet wt, respective of dose; Figure 3.7) or individual BCF-estimated C_{ss} values for TF in *L. variegatus* (0.013, 0.058, 0.104 µmol/g wet wt respective of dose). Much of this species difference was likely driven by the differences in the kinetic rates between species for TF, as *L. variegatus* generally had slightly higher k_u and, more importantly, higher k_e and $k_{e(m)}$ values than *H. azteca* (Tables 3.8, 3.9, 3.12, 3.13 and 3.16), as discussed earlier. Thus it appears that *H. azteca* had a greater ability to accumulate TF than *L. variegatus* even though

the amphipods exhibited a greater ability to metabolize TF during the exposures (Tables 3.20 and 3.21).

Conclusions

The original hypothesis stated that the levels of FLU and TF would not be toxic to *L. variegatus* and *H. azteca*, that the toxicokinetic parameters would not differ between doses with respect to species and that both species would be able to metabolize the test compounds. These hypotheses were supported by the results of the present study. Water-borne FLU and TF exposures were not toxic to *L. variegatus* and *H. azteca* and the compounds were rapidly accumulated over a short period. The conditional rates of uptake (k_u) and elimination ($k_{e(m)}$) were generally not significantly different between doses for *L. variegatus* and *H. azteca* except in the case of the $k_{e(m)}$ value for FLU in *L. variegatus* at 50 µg/L which was found to be significantly lower than elimination at 20 µg/L. This difference was likely driven by the clumping behavior that was exhibited by the worms during the first 5-10 hours of exposure. *L. variegatus* was able to biotransform a small percentage (3-6%) of the parent TF to metabolites, while *H. azteca* was shown to moderately (=27% of parent) metabolize both compounds. The presence of FLU and TF in a mixture appeared to enhance the metabolism of FLU by the amphipods, but not by *L. variegatus* which suggests that the oligochaetes may not possess large quantities of inducible enzymes capable of metabolizing organic chemicals. Future studies should also investigate the effects of mixtures of different classes of organic contaminants on biotransformation by benthic invertebrates.

There were a number of differences in the toxicokinetics between species. The uptake of FLU was similar between the test species, but elimination was faster for the amphipods. TF uptake and elimination were generally faster for *L. variegatus*. No single explanation for these discrepancies was satisfactory and these included: 1) lipid status of the organisms, 2) body size, 3) physical interaction with sediments, and 4) characteristics of the metabolites. BCFs for the compounds, however, were generally similar between species and the values (range 735-4011) were in good agreement with previously reported BCFs.

Overall, the experimental design provided estimates of k_u and $k_{e(m)}$ that will be useful to exposure and fate modeling for these contaminants in benthic species exposed in the field. It was important that FLU and TF were administered in a mixture, as this is a more realistic exposure model of natural environments. This was the first study to report toxicokinetic parameters for TF in non-vertebrate species and biotransformation of TF by *L. variegatus* and *H. azteca*. However, a weakness of the current study that leads to some uncertainty regarding the estimates of the rate constants was that the toxicokinetics of both FLU and TF were estimated for the total equivalents of these compounds that were measured in tissues. Therefore, future studies should include measurements of the metabolites of FLU and TF at each time point in order to obtain estimates of the rates of formation and excretion of the metabolites and to improve the current estimates of the uptake and elimination rates and BCFs of the parent compounds.

Table 3.1. Toxicokinetic studies conducted with two benthic invertebrates under water-only exposures to fluoranthene and trifluralin.

Experiment/ organism	Conc. of FLU and TF (µg/L)[a]	Test duration (h)	Study Component	No. of sampling points	Initial no. animals per beaker	No. of replicates per conc. x time point
L. variegatus	0, 5, 20, 50	96	Uptake kinetics	6	10	3
			Elimination kinetics[b]	6	10	3
			Biotransformation[c]	2	10	3
H. azteca	0, 5, 20, 50	38	Uptake kinetics	6	20	3
			Elimination kinetics	5	20	3
			Biotransformation	1	100	3

[a]Control beakers (0 µg/L) only for the end of the uptake and elimination phase samples

[b]Organisms transferred to uncontaminated sediments and water at 24 h (*L. variegatus*) or 12 h (*H. azteca*)

[c]Organisms sampled for metabolite analysis at 24 and 72 h (*L. variegatus*) or 19.5 h (*H. azteca*)

Abbreviations:
FLU = fluoranthene
TF = trifluralin
Conc. = concentrations
No. = number

Table 3.2. Experiment set up, initiation and sampling (termination) schedule.

		Set-up		Initiation		Termination	
Experiment/ organism	Test Component	Date	Amount of water added beakers (mL)	Date	Time points (h)	Date	Time points (h)
L. variegatus	Uptake	29-Nov-01	185	29-Nov-01	1, 2, 5, 10, 18, 24, 27	29-Nov-01	1, 2, 5
						30-Nov-01	10, 17, 24
	Elimination[a]	27-Nov-01	185	29-Nov-01	33, 43, 51, 72, 96	30-Nov-01	27, 33
						01-Dec-01	43, 51
						02-Dec-01	72
						03-Dec-01	96
	Biotransformation	29-Nov-01	185	29-Nov-01	24, 72	30-Nov-01	24
						02-Dec-01	72

H. azteca	Uptake	17-Dec-01	185	17-Dec-01	1, 2, 4, 6, 9, 12	17-Dec-01	1, 2, 4, 6, 9, 12
	Elimination	20-Dec-01	185	20-Dec-01	13, 15, 18, 24.5, 38	21-Dec-01	13, 15, 18, 24.5
						22-Dec-01	38
	Biotransformation	20-Dec-01	185	20-Dec-01	19.5	22-Dec-01	19.5

[a]Organisms transferred to uncontaminated sediments and water at 24 h (*L. variegatus*) or 12 h (*H. azteca*)

Table 3.3. Physical and chemical characteristics of water used in the water-only exposures and elimination studies with *Lumbriculus variegatus* and *Hyalella azteca.* The organisms were exposed to 5, 20 and 50 µg/L of fluoranthene and trifluralin in equal-mass mixtures. Data are presented as means ± 1 SD.

	L. variegatus 24-h uptake		*L. variegatus* 72-h elimination		*H. azteca 12-h* uptake		*H. azteca* 26-h elimination	
Characteristics	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n	Mean ± 1 SD	n
Temperature (°C)	21.33 ± 0.58	3	21.50 ± 0.50	3	22.00 ± 0.00	2	21.00 ± 0.00	2
Dissolved oxygen (mg/L)	7.45 ± 0.41	2	6.64 ± 1.43	3	7.05 ± 0.12	2	6.90 ± 0.42	2
pH	8.45 ± 0.12	2	8.23 ± 0.35	2	8.39 ± 0.04	2	8.35 ± 0.01	2
Hardness (mg/L)	165 ± 2.83	2	178 ± 43.1	2	159 ± 0.71	2	163 ± 0.00	2
Alkalinity (mg/L)	152 ± 5.66	2	166 ± 19.8	2	146 ± 2.83	2	154 ± 8.49	2
Conductivity (µS/cm)	310 ± 0.00	2	375 ± 77.8	2	305 ± 7.07	2	310 ± 0.00	2
Total Ammonia (mg/L)	0.53 ± 0.23	2	0.70 ± 0.35	2	0.46 ± 0.35	2	0.27 ± 0.13	2

Table 3.4. Summary of loss rates (λ) and initial concentrations (C_w^0) of fluoranthene and trifluralin estimated[a] from water samples taken from the uptake exposure jars during the 24-h exposure of *Lumbriculus variegatus* to the test compounds. Also shown are the asymptotic standard errors (± A.S.E.), the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Dose (ug/L)	l ± A.S.E.	C_w^0 ± A.S.E.	RSS	R^2
Fluoranthene	5	0.017 ± 0.001	2.11×10^{-5} ± 2.70×10^{-7}	0.00001	0.937
	20	0.018 ± 0.001	8.45×10^{-5} ± 9.70×10^{-7}	0.00010	0.954
	50	0.011 ± 0.001	1.98×10^{-4} ± 3.05×10^{-6}	0.00102	0.821
Trifluralin	5	0.022 ± 0.001	1.14×10^{-5} ± 1.70×10^{-7}	0.00000	0.944
	20	0.024 ± 0.001	4.88×10^{-5} ± 6.60×10^{-7}	0.00004	0.958
	50	0.021 ± 0.002	1.23×10^{-4} ± 2.38×10^{-6}	0.00057	0.901

[a] Values were obtained by fitting Equation 3.1, $C_w^t = C_w^0 e^{-lt}$, where C_w^t is the concentration of the contaminant in the water (µmol/mL) at time *t*, and t is time (h). The units λand C_w^0 are h^{-1} and µmol/mL, respectively.

Table 3.5. Coefficients calculated for the measured concentrations of fluoranthene and trifluralin in water from the elimination beakers following a 24-h exposure of *L. variegatus* to 5, 20 and 50 µg/L of the test chemicals[a].

Chemical	Dose (µg/L)	*a*	*b*	r^2	n
Fluoranthene	5	8.33×10^{-8}	-3.66×10^{-8}	0.87	11
	20	2.66×10^{-7}	$+1.05 \times 10^{-7}$	0.82	11
	50	4.50×10^{-7}	$+8.45 \times 10^{-7}$	0.32	11
Trifluralin	5	4.30×10^{-8}	-2.92×10^{-8}	0.64	11
	20	2.41×10^{-7}	-3.15×10^{-7}	0.78	11
	50	3.69×10^{-7}	-2.55×10^{-7}	0.50	11

[a] Data were fit to Equation 3.6: $C_w = a \bullet \ln(t) + b$, where C_w is the concentration in the water (µmol/mL) and t is time (h).

Table 3.6. Summary of loss rates (λ) and initial concentrations (C_w^0) of fluoranthene and trifluralin estimated[a] from water samples taken from the uptake exposure jars during the 24-h exposure of *Hyalella azteca* to the test compounds. Also shown are the asymptotic standard errors (± A.S.E.), the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Dose (ug/L)	l ± A.S.E.	C_w^0 ± A.S.E.	RSS	R^2
Fluoranthene	5	0.003 ± 0.001	2.22×10^{-5} ± 1.10×10^{-7}	0.00000	0.429
	20	0.009 ± 0.001	9.44×10^{-5} ± 6.40×10^{-7}	0.00004	0.814
	50	0.005 ± 0.002	2.34×10^{-4} ± 2.36×10^{-6}	0.00049	0.439
Trifluralin	5	0.014 ± 0.002	1.17×10^{-5} ± 1.50×10^{-7}	0.00000	0.747
	20	0.022 ± 0.003	4.44×10^{-5} ± 7.40×10^{-7}	0.00004	0.815
	50	0.015 ± 0.002	1.27×10^{-4} ± 1.74×10^{-6}	0.00025	0.761

[a] Values were obtained by fitting Equation 3.1, $C_w^t = C_w^0 e^{-lt}$, where C_w^t is the concentration of the contaminant in the water (µmol/mL) at time *t*, and t is time (h). The units λand C_w^0 are h^{-1} and µmol/mL, respectively.

Table 3.7. Coefficients calculated for the measured concentrations of fluoranthene and trifluralin in water from the elimination beakers following a 24-h exposure of *H. azteca* to 5, 20 and 50 µg/L of the test chemicals[a]

Chemical	Dose (µg/L)	*a*	*b*	r^2	n
Fluoranthene	5	2.54×10^{-8}	$+3.10 \times 10^{-7}$	0.05	12
	20	3.43×10^{-7}	$+7.10 \times 10^{-7}$	0.36	12
	50	1.00×10^{-6}	$+1.68 \times 10^{-6}$	0.57	12
Trifluralin	5	6.35×10^{-9}	$+1.09 \times 10^{-7}$	0.25	12
	20	9.75×10^{-8}	$+2.03 \times 10^{-7}$	0.65	12
	50	1.98×10^{-7}	$+7.33 \times 10^{-7}$	0.72	12

[a] Data were fit to Equation 3.6: $C_w = a \bullet \ln(t) + b$, where C_w is the concentration in the water (µmol/mL) and t is time (h).

Table 3.8. Summary of the accumulation kinetics of fluoranthene and trifluralin for *Lumbriculus variegatus* in 24-h water-only exposures to the test chemicals. The uptake (k_u) and elimination (k_e) clearance constants (± 1 asymptotic standard errors; A.S.E.) were estimated[a]. Also shown are the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Dose (ug/L)	k_u	±	A.S.E.	k_e	±	A.S.E.	RSS	R^2
Fluoranthene	5	165	±	10.3	0.067	±	0.008	0.00006	0.973
	20	138	±	12.0	0.046	±	0.010	0.00165	0.950
	50	158	±	24.9	0.115	±	0.026	0.01849	0.675
Trifluralin	5	129	±	6.73	0.114	±	0.009	0.00000	0.967
	20	112	±	7.56	0.094	±	0.010	0.00012	0.942
	50	121	±	17.0	0.142	±	0.027	0.00233	0.494

[a] Values were obtained by fitting Equation 3.3 to the organism wet-weight-normalized data. Units for ku are in mL/g wet organism/h. Units for ke are h^{-1}.

Table 3.9. Summary of statistical comparisons of the uptake clearance (k_u) and elimination (k_e and $k_{e(m)}$) rate constants[a] for fluoranthene and trifluralin by *Lumbriculus variegatus* and *Hyalella azteca*. Student's t-test was used to compare estimated values of the rate constants (± asymptotic standard errors) determined by toxicokinetic modeling. Separate hypothesis tests of kinetic rate equality with respect to species were performed to test for significant differences across the three treatment concentrations. Pairwise test of the kinetic rates between species at a given dose were also performed. All comparisons were performed at α =0.05 with a two-tailed test.

Compound	Statistical comparison	Kinetic constant	*df*[b]	t_{crit}[c]	t_{obs}[d]	Signficance[e]
		Across dose for *Lumbriculus variegatus*[f]				
Fluoranthene	5 vs. 20 µg/L	k_u	32	2.74	1.71	NS
		k_e	32	2.74	1.65	NS
		$k_{e(m)}$	31	2.74	-1.99	NS
	5 vs. 50 µg/L	k_u	32	2.74	0.28	NS
		k_e	32	2.74	-1.73	NS
		$k_{e(m)}$	32	2.74	1.74	NS
	20 vs. 50 µg/L	k_u	32	2.74	-0.70	NS
		k_e	32	2.74	-2.44	NS
		$k_{e(m)}$	31	2.74	2.99	*
Trifluralin	5 vs. 20 µg/L	k_u	32	2.74	1.62	NS
		k_e	32	2.74	1.52	NS
		$k_{e(m)}$	31	2.74	-1.00	NS

	5 vs. 50 µg/L	k_u	32	2.74	0.42	NS
		k_e	32	2.74	-1.01	NS
		$k_{e(m)}$	32	2.74	-1.36	NS
	20 vs. 50 µg/L	k_u	32	2.74	-0.47	NS
		k_e	32	2.74	-1.70	NS
		$k_{e(m)}$	31	2.74	-0.70	NS
	Across dose for *Hyalella azteca*					
Fluoranthene	5 vs. 20 µg/L	k_u	32	2.74	-0.32	NS
		k_e	32	2.74	0.61	NS
		$k_{e(m)}$	26	2.78	0.80	NS
	5 vs. 50 µg/L	k_u	32	2.74	-0.75	NS
		k_e	32	2.74	0.62	NS
		$k_{e(m)}$	26	2.78	2.05	NS
	20 vs. 50 µg/L	k_u	32	2.74	-0.47	NS
		k_e	32	2.74	0.02	NS
		$k_{e(m)}$	26	2.78	2.66	NS
Trifluralin	5 vs. 20 µg/L	k_u	32	2.74	-0.61	NS
		k_e	32	2.74	0.00	NS
		$k_{e(m)}$	26	2.78	1.12	NS
	5 vs. 50 µg/L	k_u	32	2.74	-1.58	NS
		k_e	32	2.74	-0.54	NS

		$k_{e(m)}$	26	2.78	1.21	NS
	20 vs. 50 µg/L	k_u	32	2.74	-0.92	NS
		k_e	32	2.74	-0.54	NS
		$k_{e(m)}$	26	2.78	0.26	NS
Between species at each dose						
Fluoranthene	Lv vs. Ha 5 µg/L[g]	k_u	32	2.04	-0.09	NS
		k_e	32	2.04	-1.16	NS
		$k_{e(m)}$	29	2.05	-2.30	*
	Lv vs. Ha 20 µg/L	k_u	32	2.04	-1.45	NS
		k_e	32	2.04	-1.09	NS
		$k_{e(m)}$	28	2.05	-4.07	***
	Lv vs. Ha 50 µg/L	k_u	32	2.04	-1.05	NS
		k_e	32	2.04	0.71	NS
		$k_{e(m)}$	29	2.05	-0.63	NS
Trifluralin	Lv vs. Ha 5 µg/L	k_u	32	2.04	5.25	***
		k_e	32	2.04	2.59	*
		$k_{e(m)}$	29	2.05	-0.20	NS
	Lv vs. Ha 20 µg/L	k_u	32	2.04	2.56	*
		k_e	32	2.04	1.68	NS
		$k_{e(m)}$	28	2.05	3.33	**

Lv vs. Ha 50 µg/L	k_u	32	2.04	1.40	NS
	k_e	32	2.04	2.18	*
	$k_{e(m)}$	29	2.05	2.93	**

[a] Kinetic constants, k_u (conditional uptake clearance rate from water; estimated from accumulation data; mL/g wet organism/h), k_e (conditional elimination rate; estimated from accumulation data; 1/h), and $k_{e(m)}$ (experimentally measured conditional elimination rate constant; 1/h). Estimates of k_u, k_e and $k_{e(m)}$ for *L. variegatus* are given in Tables 3.8 and 3.12 and the values for *H. azteca* are given in Tables 3.13 and 3.16.

[b] *df* = degrees of freedom.

[c] t_{crit} = critical value of *t* at α =0.05.

[d] t_{obs} = observed value of t.

[e] Differences were significant only if $|t_{obs}| = t_{crit}$ and $p < \alpha$ =0.05. NS = no significant difference; * = significant (p <0.05); ** = strongly significant (p <0.01); *** = highly significant (p <0.001).

[f] A Bonferroni-adjusted t_{crit} was used to control the experiment-wise error (α) for the three comparisons (*i.e.,* across three concentrations) for each species.

[g] Lv = *Lumbriculus variegatus*; Ha = *Hyalella azteca*.

Table 3.10. Starting parameter values[a] of the initial concentrations in the tissues (C_a^0) and the experimentally measured elimination rate constants ($k_{e(m)}$) for use in the primary iterations[b] of the numeric integration modeling of the elimination of fluoranthene and trifluralin with re-uptake by *Lumbriculus variegatus* following 24-h water-only exposures to the test chemicals. Also shown are the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Dose (ug/L)	C_a^0	±	A.S.E.	$k_{e(m)}$	±	A.S.E.	RSS	R^2
Fluoranthene	5	0.028	+	0.001	0.116	+	0.006	0.00001	0.988
	20	0.123	+	0.005	0.124	+	0.007	0.00018	0.988
	50	0.194	+	0.008	0.107	+	0.007	0.00072	0.983
Trifluralin	5	0.007	+	0.0003	0.095	+	0.006	0.00000	0.984
	20	0.032	+	0.001	0.100	+	0.005	0.00001	0.988
	50	0.062	+	0.003	0.102	+	0.008	0.00013	0.972

[a] The starting values of C_a^0 and $k_{e(m)}$ were estimated by least squares nonlinear regression of Equation 3.4 using *SYSTAT for Windows* software. Units of C_a are µmol/g wet wt. Units for $k_{e(m)}$ are h^{-1}.

[b] Primary iterations of Equation 3.5 were performed in the *Scientist*® software package using the Bulirsch-Stoer method.

Table 3.11. Starting parameter values[a] of the uptake clearance (k_u) and the experimentally measured elimination rate constants ($k_{e(m)}$) for use in the final iterations[b] of the numeric integration modeling of the elimination of fluoranthene and trifluralin with re-uptake by *Lumbriculus variegatus* following 24-h water-only exposures to the test chemicals.

Compound	Dose (ug/L)	k_u	$k_{e(m)}$
Fluoranthene	5	402	0.120
	20	355	0.128
	50	225	0.112
Trifluralin	5	190	0.099
	20	173	0.102
	50	220	0.106

[a] The starting values of k_u and $k_{e(m)}$ were estimated by least squares nonlinear regression of Equation 3.5 with *Scientist*® software using the Bulirsch-Stoer method. Units of C_a are µmol/g wet wt. Units for k_u are mL/g wet organism/h. Units for $k_{e(m)}$ are h^{-1}.

[b] Final iterations of Equation 3.5 were performed in the *Scientist*® software package using the fourth-order Runga-Kutta method.

Table 3.12. Summary of the elimination kinetics of fluoranthene and trifluralin for *Lumbriculus variegatus* in 24-h water-only exposures to the test chemicals. The experimentally measured elimination ($k_{e(m)}$) rate constants (± 1 asymptotic standard errors; A.S.E.) were estimated using a model that accounted for the re-uptake of eliminated parent fluoranthene and trifluralin[a]. Also shown are the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Dose (ug/L)	$k_{e(m)}$	±	A.S.E.	RSS	R^2
Fluoranthene	5	0.120	±	0.002	0.000003	0.997
	20	0.128	±	0.003	0.00011	0.993
	50	0.112	±	0.004	0.00052	0.988
Trifluralin	5	0.099	±	0.003	0.0000004	0.992
	20	0.102	±	0.002	0.00001	0.994
	50	0.106	±	0.005	0.00009	0.981

[a] Values were obtained by fitting Equation 3.5 with *Scientist*® software using the fourth-order Runga-Kutta method. Units for $k_{e(m)}$ are h^{-1}.

Table 3.13. Summary of the accumulation kinetics of fluoranthene and trifluralin for *Hyalella azteca* in 12-h water-only exposures to the test chemicals. The uptake (k_u) and elimination (k_e) clearance constants (± 1 asymptotic standard errors; A.S.E.) were estimated[a]. Also shown are the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Dose (ug/L)	k_u	±	A.S.E.	k_e	±	A.S.E.	RSS	R^2
Fluoranthene	5	168	±	27.7	0.118	±	0.043	0.00017	0.784
	20	180	±	26.4	0.085	±	0.034	0.00407	0.802
	50	199	±	30.3	0.084	±	0.034	0.03789	0.758
Trifluralin	5	75.4	±	7.59	0.053	±	0.022	0.00001	0.928
	20	82.5	±	8.84	0.053	±	0.022	0.00012	0.891
	50	94.0	±	9.03	0.069	±	0.020	0.00108	0.890

[a] Values were obtained by fitting Equation 3.3 to the organism wet-weight-normalized data. Units for k_u are in mL/g wet organism/h. Units for k_e are h^{-1}.

Table 3.14. Starting parameter values[a] of the initial concentrations in the tissues (C_a^0) and the experimentally measured elimination rate constants ($k_{e(m)}$) for use in the primary iterations[b] of the numeric integration modeling of the elimination of fluoranthene and trifluralin with re-uptake by *Hyalella azteca* following 12-h water-only exposures to the test chemicals. Also shown are the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Dose (ug/L)	C_a^0	±	A.S.E.	$k_{e(m)}$	±	A.S.E.	RSS	R^2
Fluoranthene	5	0.042	+	0.006	0.188	+	0.044	0.00046	0.799
	20	0.165	+	0.014	0.159	+	0.022	0.00214	0.916
	50	0.329	+	0.034	0.096	+	0.020	0.02277	0.796
Trifluralin	5	0.010	+	0.001	0.040	+	0.011	0.00004	0.563
	20	0.038	+	0.002	0.033	+	0.006	0.00025	0.754
	50	0.089	+	0.005	0.024	+	0.006	0.00169	0.618

[a] The starting values of C_a^0 and $k_{e(m)}$ were estimated by least squares nonlinear regression of Equation 3.4 using *SYSTAT for Windows* software. Units of C_a are µmol/g wet wt. Units for $k_{e(m)}$ are h^{-1}.

[b] Primary iterations of Equation 3.5 were performed in the *Scientist*® software package using the Bulirsch-Stoer method.

Table 3.15. Starting parameter values[a] of the uptake (k_u) and the experimentally measured elimination rate constants ($k_{e(m)}$) for use in the final iterations[b] of the numeric integration modeling of the elimination of fluoranthene and trifluralin with re-uptake by *Hyalella azteca* following 12-h water-only exposures to the test chemicals.

Compound	Dose (ug/L)	k_u	$k_{e(m)}$
Fluoranthene	5	1925	0.220
	20	1767	0.184
	50	1453	0.124
Trifluralin	5	3580	0.109
	20	1100	0.050
	50	1097	0.043

[a] The starting values of k_u and $k_{e(m)}$ were estimated by numeric integration of Equation 3.5 with *Scientist*® software using the Bulirsch-Stoer method. Units of C_a are µmol/g wet wt. Units for k_u are mL/g wet organism/h. Units for $k_{e(m)}$ are h^{-1}.

[b] Final iterations of Equation 3.5 were performed in the *Scientist*® software package using the fourth-order Runga-Kutta method.

Table 3.16. Summary of the elimination kinetics of fluoranthene and trifluralin for *Hyalella azteca* in 12-h water-only exposures to the test chemicals. The experimentally measured elimination ($k_{e(m)}$) rate constants (± 1 asymptotic standard errors; A.S.E.) were estimated using a model that accounted for the re-uptake of eliminated parent fluoranthene and trifluralin[a]. Also shown are the residual sum-of-squares (RSS) and the correlation coefficient (R^2) from the model fit to the data.

Compound	Dose (ug/L)	$k_{e(m)}$	±	A.S.E.	RSS	R^2
Fluoranthene	5	0.220	±	0.043	0.00041	0.819
	20	0.184	±	0.013	0.00115	0.955
	50	0.124	±	0.018	0.01595	0.857
Trifluralin	5	0.109	±	0.051	0.00003	0.652
	20	0.050	±	0.016	0.00022	0.778
	50	0.043	±	0.021	0.00157	0.645

[a] Values were obtained by fitting Equation 3.5 with *Scientist*® software using the fourth-order Runga-Kutta method. Units for $k_{e(m)}$ are h^{-1}.

Table 3.17. Bioconcentration factors (BCFs) and their 95% confidence intervals for fluoranthene and trifluralin for *Lumbriculus variegatus* in water-only exposures to the test chemicals. The BCFs were estimated from the steady state tissue and water concentration data and from the rate constants for the uptake (k_u) and elimination (k_e) of fluoranthene and trifluralin[a].

		Empirical BCF[b]	Kinetics-based BCFs			
					95% Confidence Interval[e]	
Compound	Dose (ug/L)	mean ± SD	kinetic BCF[c]	log BCF ± SE[d]	Lower BCF	Upper BCF
Fluoranthene	5	2149 ± 143	2459	7.81 ± 0.065	2166	2791
	20	2198 ± 324	2995	8.00 ± 0.135	2297	3904
	50	1359 ± 256	1375	7.23 ± 0.094	1143	1654
Trifluralin	5	1175 ± 66.9	1129	7.03 ± 0.032	1059	1202
	20	1222 ± 118	1194	7.09 ± 0.049	1085	1314
	50	934 ± 144	850	6.74 ± 0.074	735	982

[a] The values of the rate constants k_u and k_e and their associated standard errors are given in Table 3.8.
[b] BCF as the steady state concentrations of fluoranthene and trifluralin in *L. variegatus* relative to the aqueous concentrations (Rand et al., 1995).
[c] Calculated by Equation 3.7.
[d] Calculated by Equations 3.8 and 3.9; log represents the natural logarithm (*ln*).
[e] Calculated by Equation 3.10.

Table 3.18. Bioconcentration factors (BCFs) and their 95% confidence intervals for fluoranthene and trifluralin for *Hyalella azteca* in water-only exposures to the test chemicals. The BCFs were estimated from the rate constants for the uptake (k_u) and elimination (k_e) of fluoranthene and trifluralin[a].

Compound	Dose (ug/L)	BCF[b]	log BCF ± SE[c]	95% Confidence Interval[d]	
				Lower BCF	Upper BCF
Fluoranthene	5	1418	7.26 ± 0.214	932	2158
	20	2127	7.66 ± 0.265	1265	3576
	50	2370	7.77 ± 0.268	1400	4011
Trifluralin	5	1426	7.26 ± 0.321	760	2675
	20	1562	7.35 ± 0.323	829	2944
	50	1361	7.22 ± 0.208	906	2044

[a] The values of the rate constants k_u and k_e and their associated standard errors are given in Table 3.13.
[b] Calculated by Equation 3.7.
[c] Calculated by Equations 3.8 and 3.9; log represents the natural logarithm (*ln*)..
[d] Calculated by Equation 3.10.

Table 3.19. Summary of statistical comparisons of the bioconcentration factors (BCFs) for fluoranthene and trifluralin in *Lumbriculus variegatus* and *Hyalella azteca* [a]. A Z statistic was used to compare estimated values of the BCFs that were calculated from independently determined rate constants for the uptake and elimination of fluoranthene and trifluralin. Pairwise tests of the BCFs with respect to species were performed to test for significant differences across the three treatment concentrations. Pairwise test of the BCFs between species at a given dose were also performed. All comparisons were performed at α =0.05 with a two-tailed test.

Compound	Statistical comparison	Z_{crit} [b]	Z_{obs} [c]	Signficance [d]
	Across dose for *Lumbriculus variegatus* [e]			
Fluoranthene	5 vs. 20 µg/L	2.40	-1.31	NS
	5 vs. 50 µg/L	2.40	5.08	*
	20 vs. 50 µg/L	2.40	4.72	*
Trifluralin	5 vs. 20 µg/L	2.40	-0.96	NS
	5 vs. 50 µg/L	2.40	3.52	*
	20 vs. 50 µg/L	2.40	3.84	*
	Across dose for *Hyalella azteca*			
Fluoranthene	5 vs. 20 µg/L	2.40	-1.19	NS
	5 vs. 50 µg/L	2.40	-1.49	NS
	20 vs. 50 µg/L	2.40	-0.29	NS

Trifluralin	5 vs. 20 µg/L	2.40	-0.20	NS
	5 vs. 50 µg/L	2.40	0.12	NS
	20 vs. 50 µg/L	2.40	0.36	NS
	Between species at each dose			
Fluoranthene	Lv 5 vs. Ha 5 µg/L[f]	1.96	2.46	*
	Lv 20 vs. Ha 20 µg/L	1.96	1.15	NS
	Lv 50 vs. Ha 50 µg/L	1.96	-1.91	NS[g]
Trifluralin	Lv 5 vs. Ha 5 µg/L	1.96	-0.73	NS
	Lv 20 vs. Ha 20 µg/L	1.96	-0.82	NS
	Lv 50 vs. Ha 50 µg/L	1.96	-2.14	*

[a] BCFs and their associated standard errors are given in Tables 3.17 and 3.18.
[b] Z_{crit} = critical value of Z at α =0.05.
[c] Z_{obs} = observed value of Z.
[d] Differences were significant only if $|Z_{obs}| = Z_{crit}$ and $p < \alpha$ =0.05. NS = no significant difference, * = significant (p <0.05).
[e] A Bonferroni-adjusted critical value (Z_{crit} =2.40) was used to control the experiment-wise error (α) for the three comparisons (*i.e.,* across three concentrations) for each species.
[f] Lv = *Lumbriculus variegatus*; Ha = *Hyalella azteca*.
[g] Nearly significant, p =0.0561.

Table 3.20. Biotransformation of radiolabeled fluoranthene and trifluralin by *Lumbriculus variegatus* at the end of 24-h water-only exposures to the test chemicals and at 48-h of the post-exposure elimination phase (*i.e.,* 72 h from the start of exposure). The mean percentages (± 1 SD; n =3) of parent compound, extractable metabolites and unextractable residues are shown. Unextractable radioactivity was assumed to represent metabolites of fluoranthene and trifluralin that were covalently bound to cellular macromolecules.

			% Parent			% Extractable metabolites			% Unextractable		
Compound	**Time**	**Dose (ug/L)**	**mean**	**±**	**SD**	**mean**	**±**	**SD**	**mean**	**±**	**SD**
Fluoranthene	24-h end of exposure	5	96.51	±	0.12	1.24	±	0.12	2.24	±	0.18
		20	97.00	±	0.05	1.23	±	0.05	1.78	±	0.01
		50	97.06	±	0.11	1.08	±	0.09	1.86	±	0.08
	48-h post-exposure elimination	5	31.20	±	2.11	13.75	±	0.22	55.05	±	2.02
		20	50.11	±	15.73	10.64	±	3.41	39.25	±	12.33
		50	43.20	±	2.47	15.09	±	2.48	41.71	±	2.10
Trifluralin	24-h end of exposure	5	93.32	±	0.24	4.08	±	0.42	2.60	±	0.21
		20	93.67	±	0.59	4.06	±	0.24	2.27	±	0.36
		50	93.25	±	0.40	4.23	±	0.20	2.53	±	0.23
	48-h post-exposure elimination	5	62.30	±	2.95	11.95	±	1.40	25.75	±	2.78
		20	67.94	±	13.64	11.89	±	6.16	20.17	±	7.97
		50	52.39	±	4.80	17.59	±	2.30	30.01	±	2.51

Table 3.21. Biotransformation of radiolabeled fluoranthene and trifluralin by *Hyalella azteca* at the end of 19.5-h water-only exposures to the test chemicals. The mean percentages (± 1 SD; n =3) of parent compound, extractable metabolites and unextractable residues are shown. Unextractable radioactivity was assumed to represent metabolites of fluoranthene and trifluralin that were covalently bound to cellular macromolecules.

			% Parent			% Extractable metabolites			% Unextractable		
Compound	**Time**	**Dose (ug/L)**	**mean**	**±**	**SD**	**mean**	**±**	**SD**	**mean**	**±**	**SD**
Fluoranthene	19.5-h end of exposure	5	57.48	±	3.24	6.83	±	0.52	35.69	±	3.37
		20	65.45	±	2.01	4.74	±	1.28	29.82	±	1.20
		50	73.08	±	2.31	3.60	±	0.38	23.31	±	2.41
Trifluralin	19.5-h end of exposure	5	64.84	±	6.46	16.09	±	3.06	19.08	±	3.49
		20	66.10	±	0.55	11.42	±	1.76	22.47	±	1.26
		50	64.59	±	2.68	10.10	±	2.23	25.30	±	1.32

Figure 3.1. Concentrations of **(A)** fluoranthene and **(B)** trifluralin in water samples taken from the exposure jars at each time point during the 24-h water-only exposures of *Lumbriculus variegatus*. Error bars represent standard deviations of three samples. Lines represent best-fit results to the single-exponential model of the loss of the compounds from water (Equation 3.1; see text).

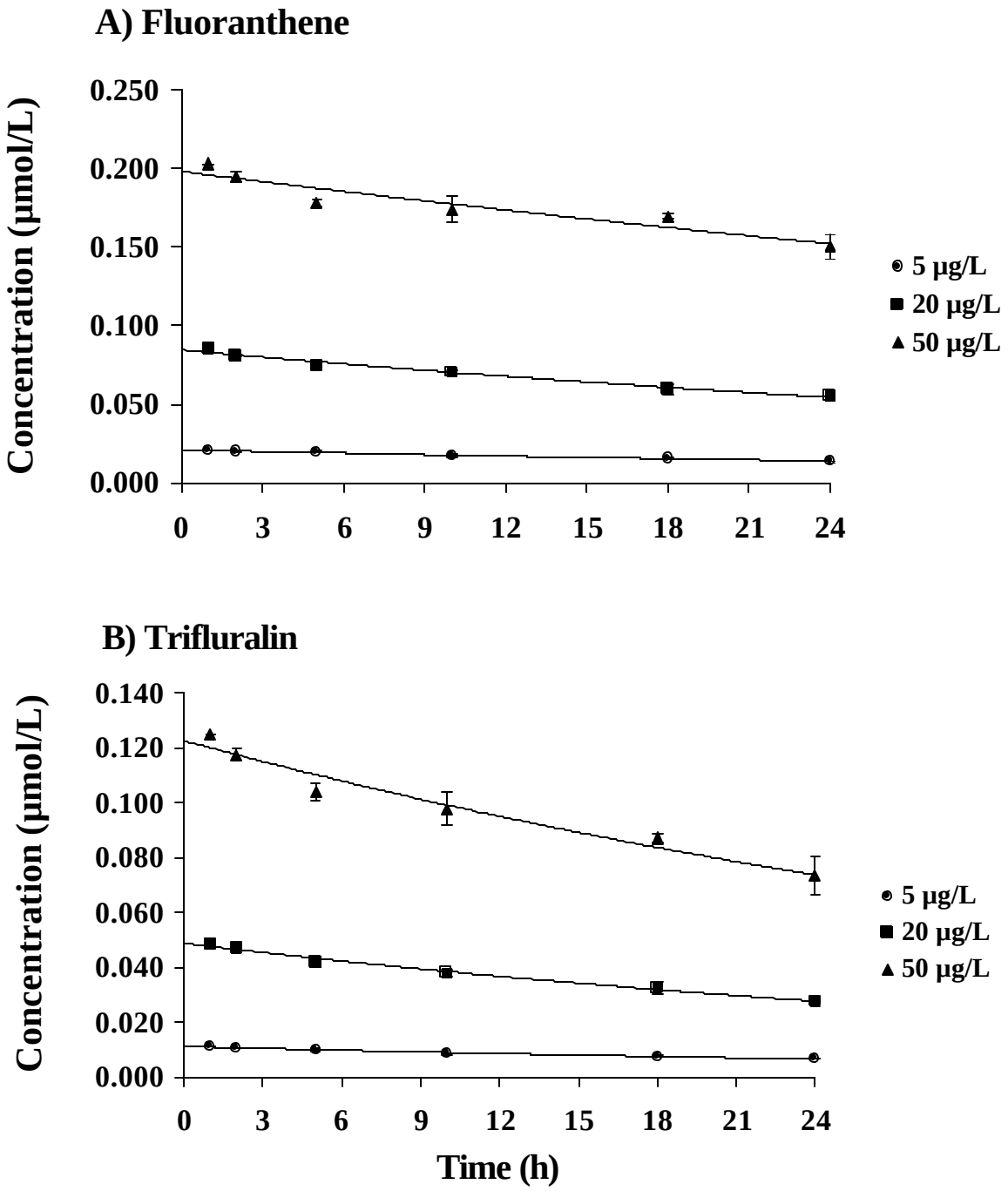


Figure 3.2. Concentrations of fluoranthene in water samples collected at each time point during the elimination of the test chemicals by *Lumbriculus variegatus* following a 24-h exposure to **(A)** 5 µg/L, **(B)** 20 µg/L and **(C)** 50 µg/L. Symbols indicate experimentally determined values. Lines represent the fit to Equation 3.6 (see text).

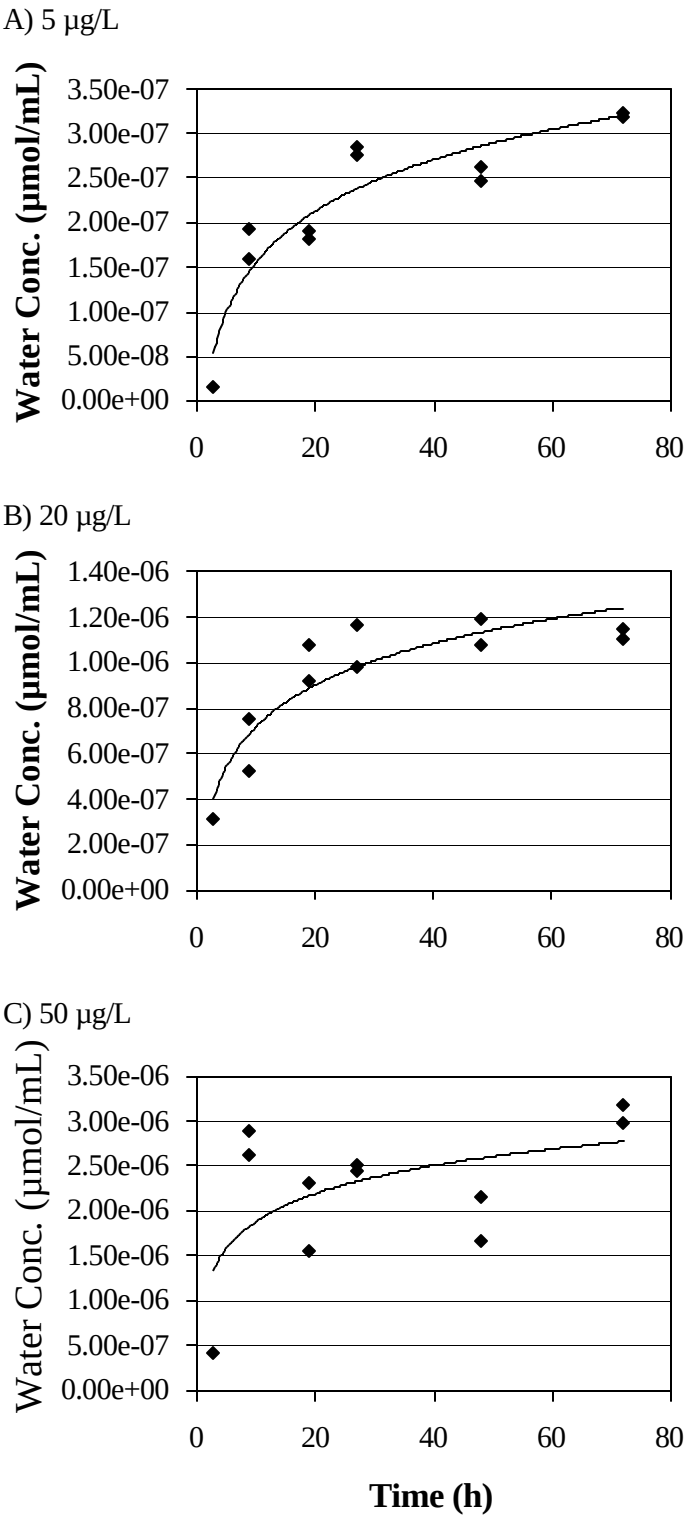


Figure 3.3. Concentrations of trifluralin in water samples collected at each time point during the elimination of the test chemicals by *Lumbriculus variegatus* following a 24-h exposure to **(A)** 5 µg/L, **(B)** 20 µg/L and **(C)** 50 µg/L. Symbols indicate experimentally determined values. Lines represent the fit to Equation 3.6 (see text).

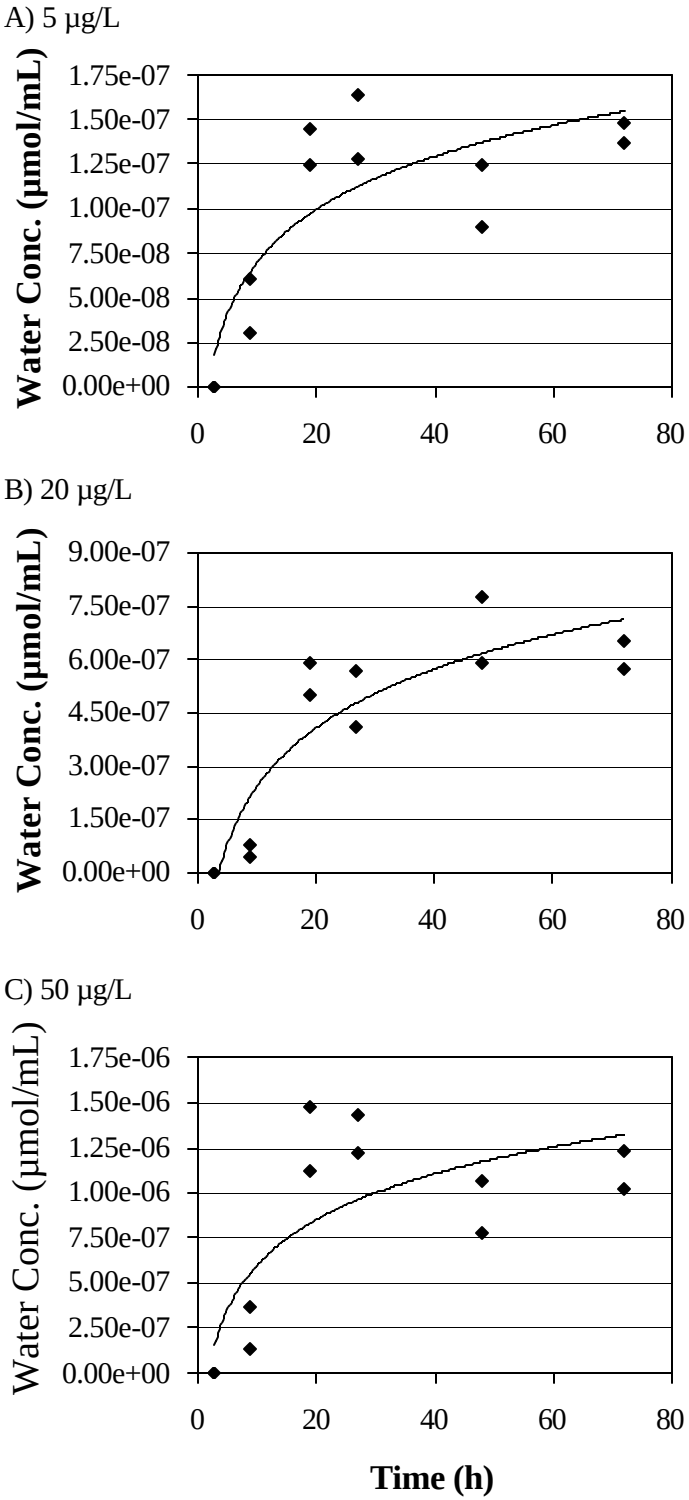


Figure 3.4. Concentrations of **(A)** fluoranthene and **(B)** trifluralin in water samples taken from the exposure jars at each time point during the 12-h water-only exposures of *Hyalella azteca*. Error bars represent standard deviations of three samples. Lines represent best-fit results to the single-exponential model of the loss of the compounds from water (Equation 3.4; see text).

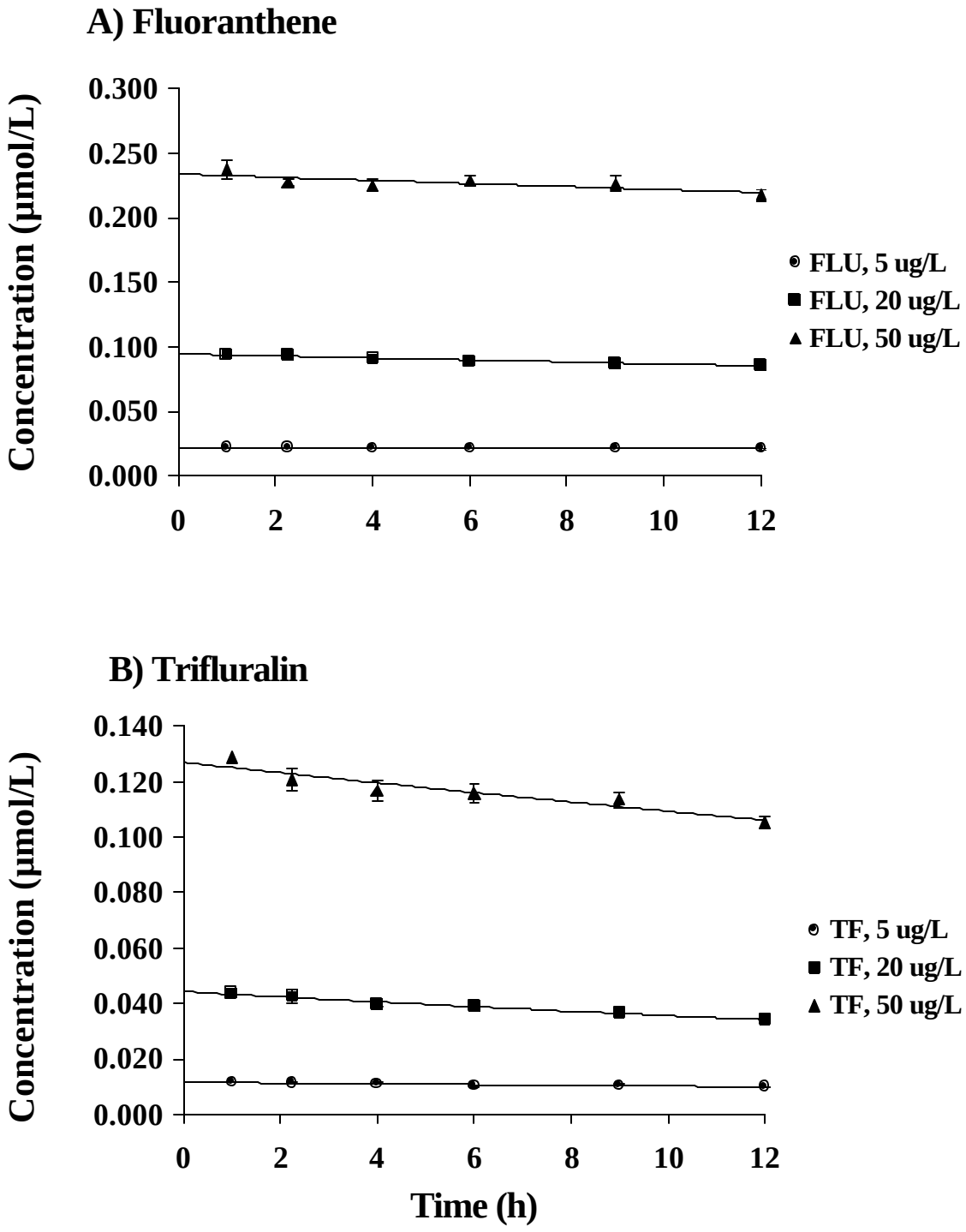


Figure 3.5. Concentrations of fluoranthene in water samples collected at each time point during the elimination of the test chemicals by *Hyalella azteca* following a 12-h exposure to **(A)** 5 µg/L, **(B)** 20 µg/L and **(C)** 50 µg/L. Symbols indicate experimentally determined values. Lines represent the fit to Equation 3.6 (see text).

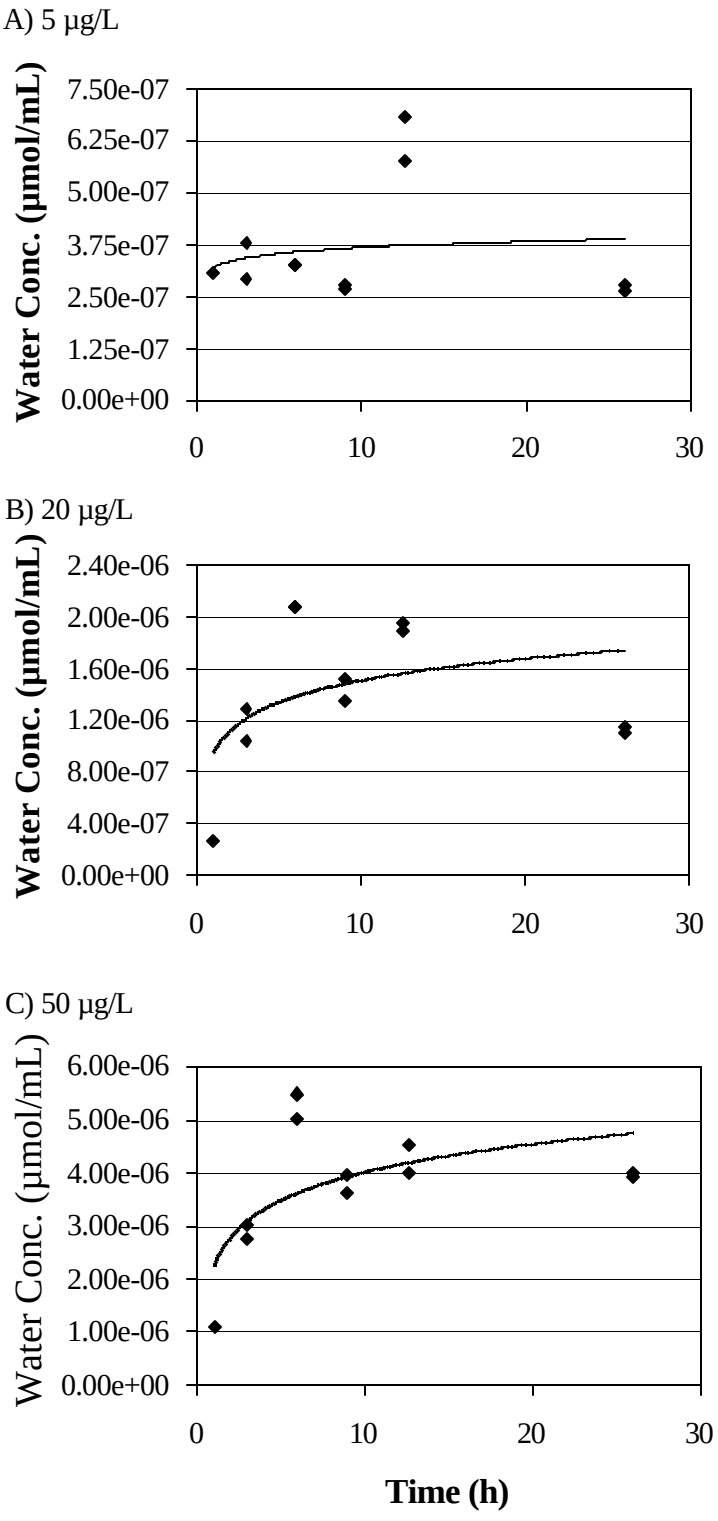


Figure 3.6. Concentrations of trifluralin in water samples collected at each time point during the elimination of the test chemicals by *Hyalella azteca* following a 12-h exposure to **(A)** 5 µg/L, **(B)** 20 µg/L and **(C)** 50 µg/L. Symbols indicate experimentally determined values. Lines represent the fit to Equation 3.6 (see text).

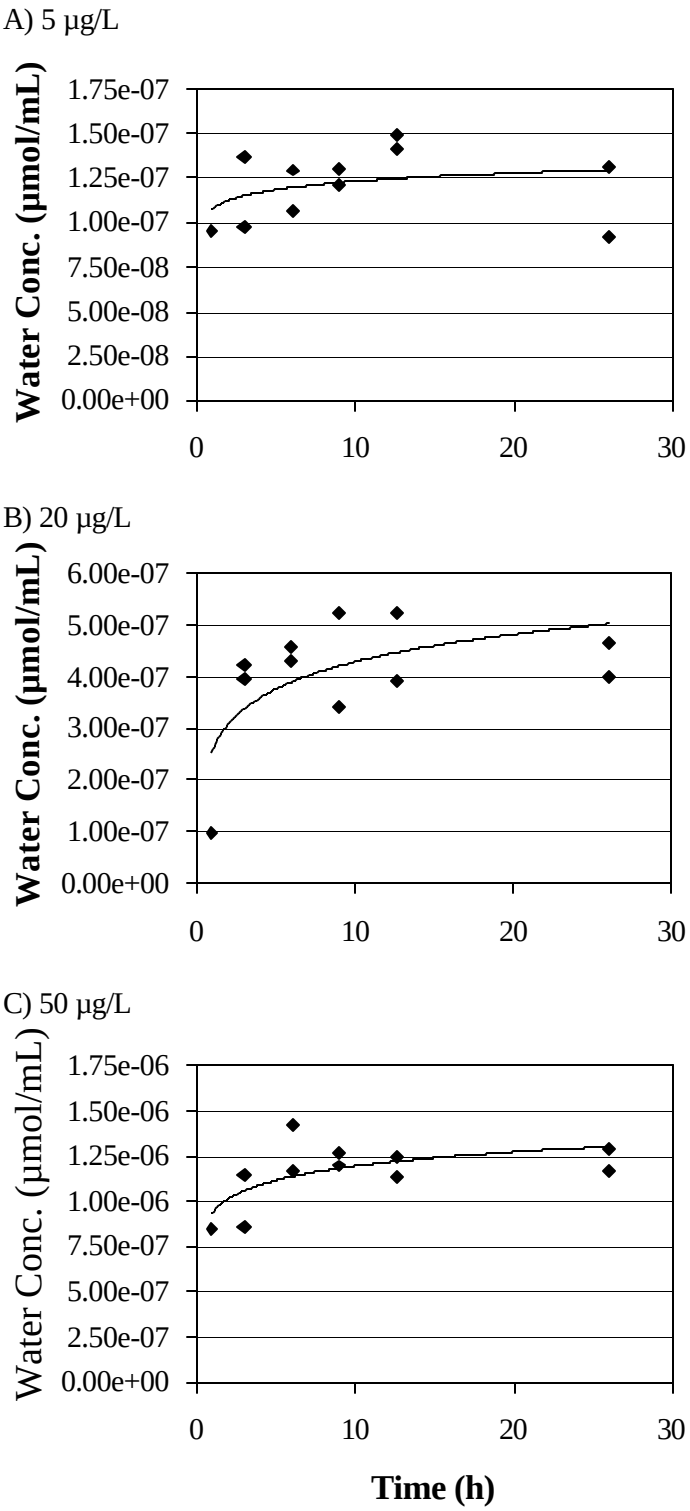


Figure 3.7. Body burdens of (**A**) fluoranthene and (**B**) trifluralin in *Lumbriculus variegatus* over a 24 h exposure to nominal water concentrations of 5, 20 and 50 µg/L. Error bars represent standard deviations of three samples. Lines represent best-fit model results.

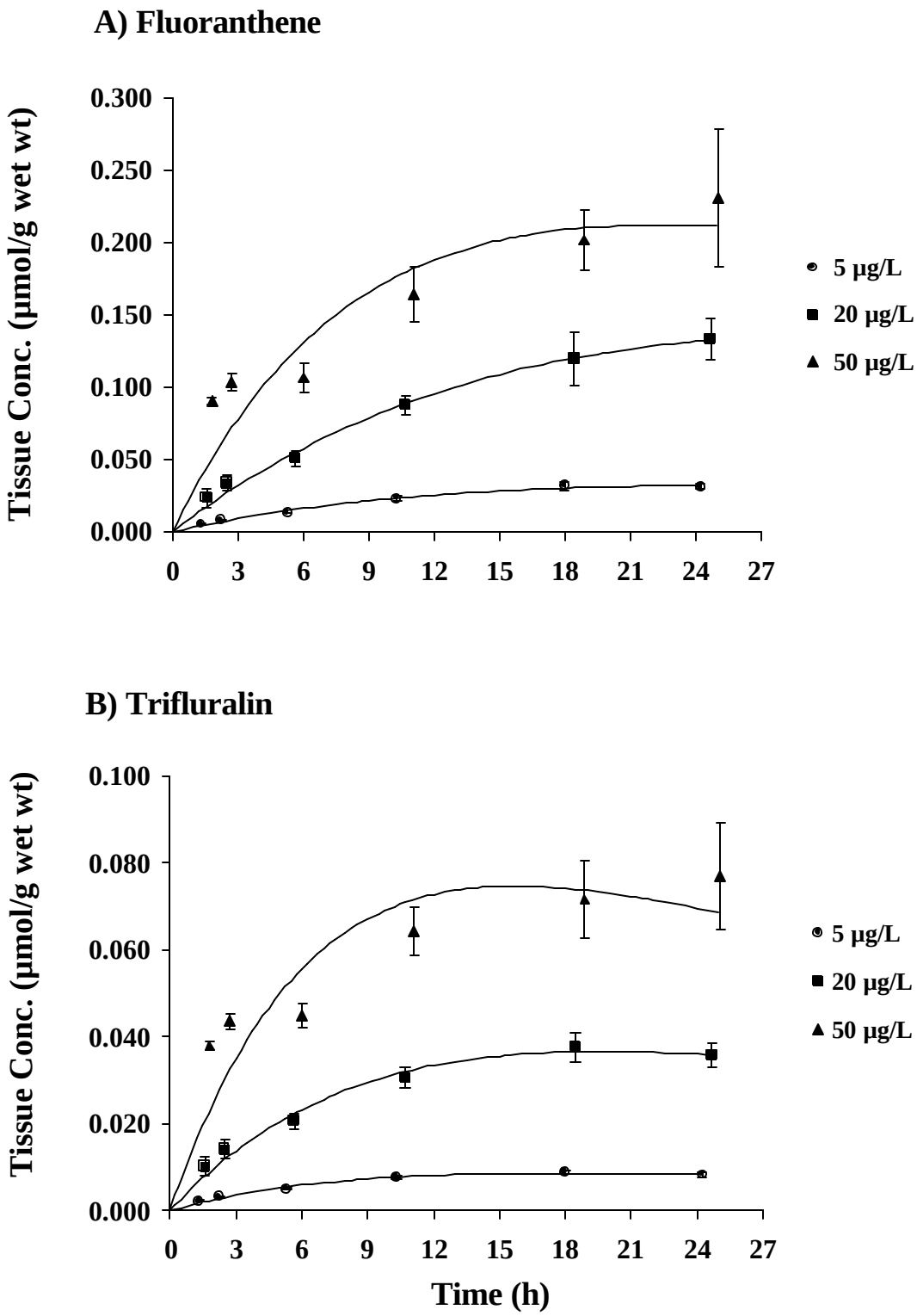


Figure 3.8. Elimination of fluoranthene by *Lumbriculus variegatus* following 24-h exposures to both fluoranthene and trifluralin at **(A)** 5 µg/L, **(B)** 20 µg/L and **(C)** 50 µg/L. Symbols indicate experimentally determined values. Lines represent best-fit model results.

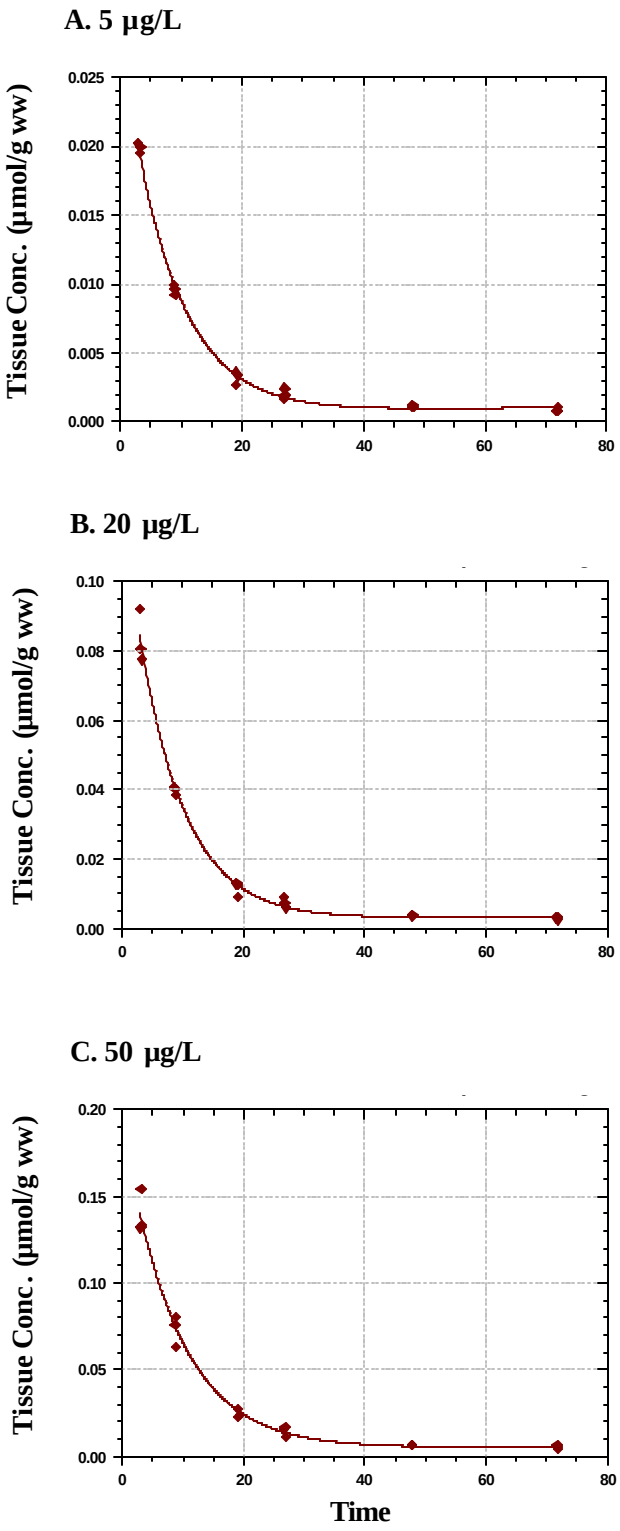


Figure 3.9. Elimination of trifluralin by *Lumbriculus variegatus* following 24-h exposures to both fluoranthene and trifluralin at **(A)** 5 µg/L, **(B)** 20 µg/L and **(C)** 50 µg/L. Symbols indicate experimentally determined values. Lines represent best-fit model results.

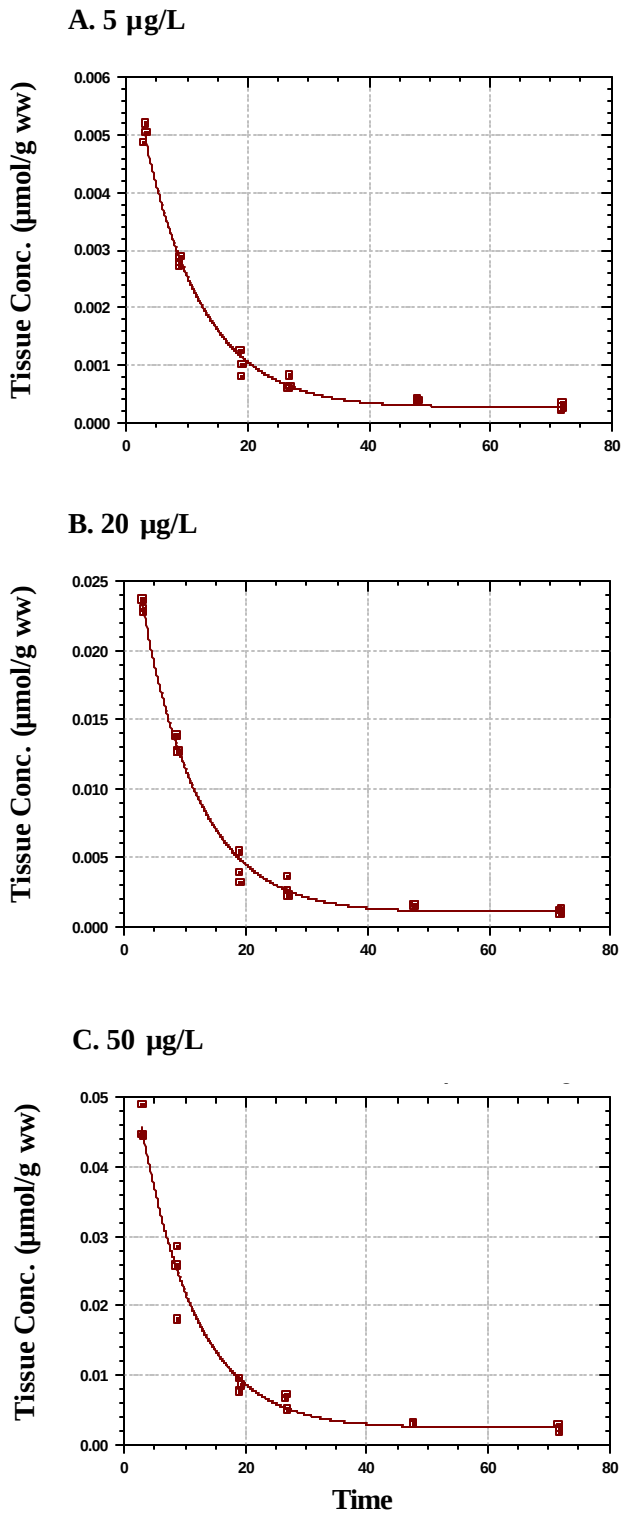


Figure 3.10. Body burdens of (**A**) fluoranthene and (**B**) trifluralin in *Hyalella azteca* over a 12-h exposure to nominal water concentrations of 5, 20 and 50 µg/L. Error bars represent standard deviations of three samples. Lines represent best-fit model results.

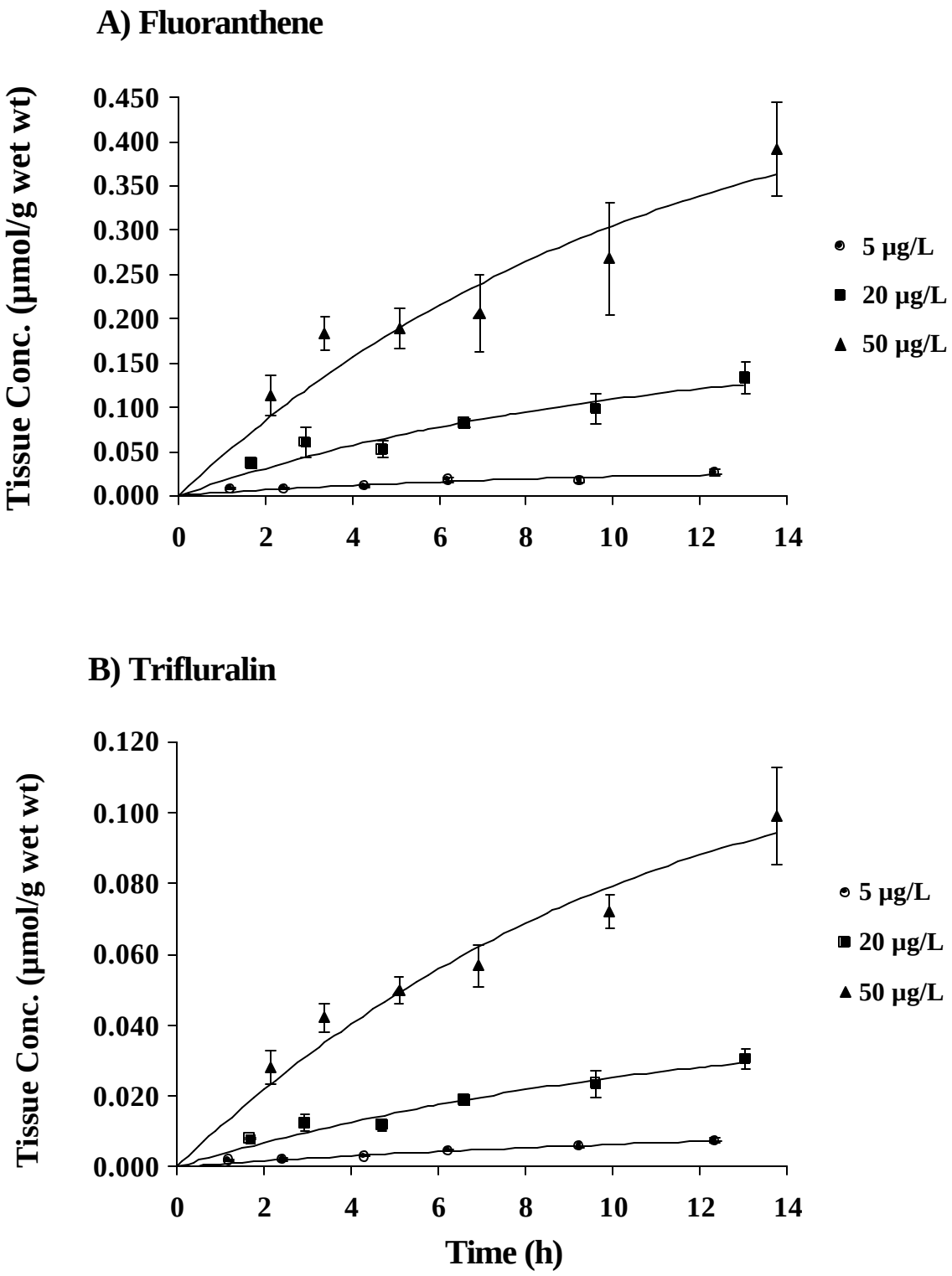


Figure 3.11. Elimination of fluoranthene by *Hyalella azteca* following 12-h exposures to both fluoranthene and trifluralin at **(A)** 5 µg/L, **(B)** 20 µg/L and **(C)** 50 µg/L. Symbols indicate experimentally determined values. Lines represent best-fit model results.

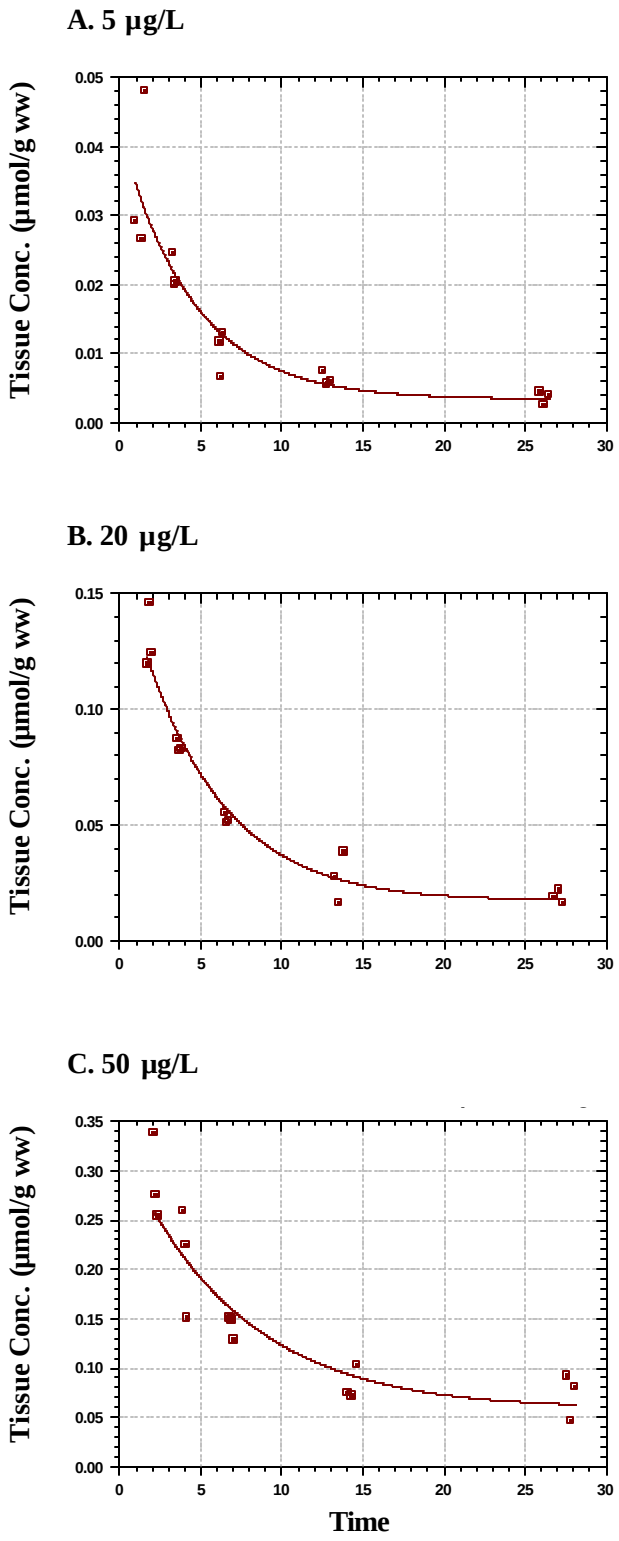
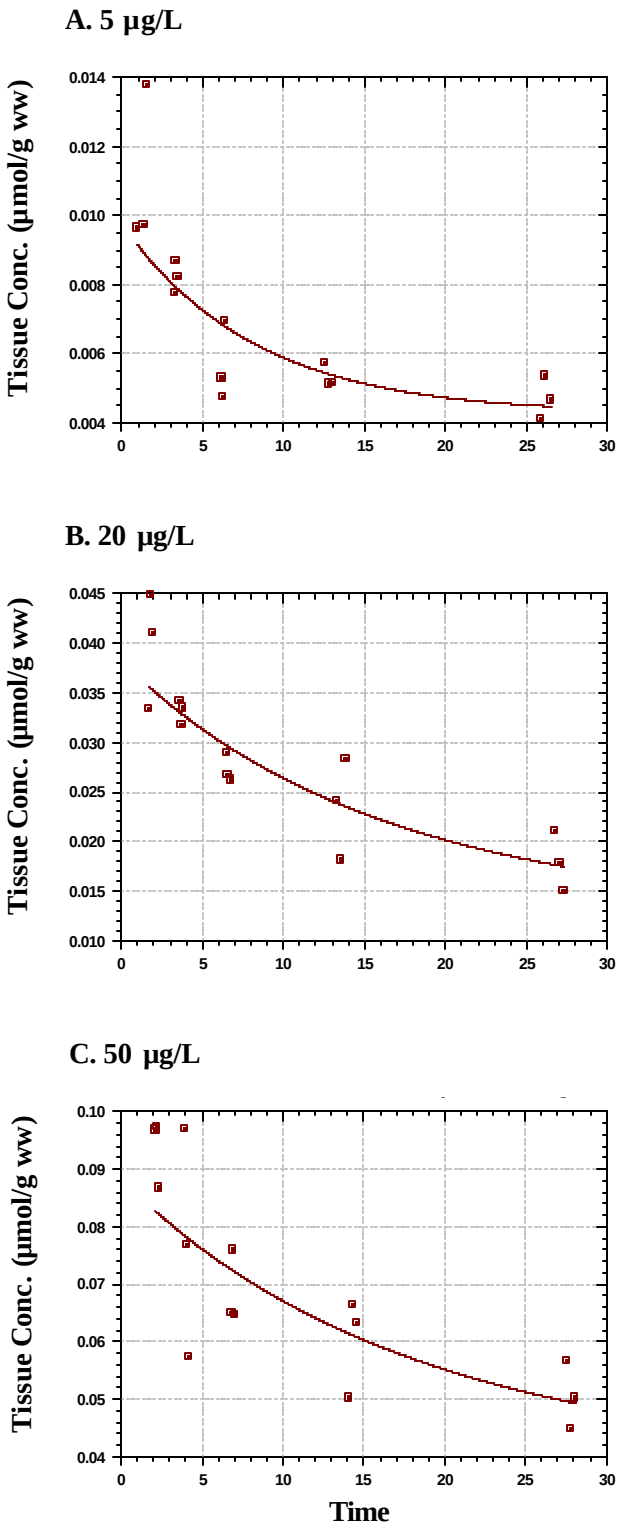


Figure 3.12. Elimination of trifluralin by *Hyalella azteca* following 12-h exposures to both fluoranthene and trifluralin at **(A)** 5 µg/L, **(B)** 20 µg/L and **(C)** 50 µg/L. Symbols indicate experimentally determined values. Lines represent best -fit model results .



CHAPTER 4

Desorption Kinetics of Fluoranthene and Trifluralin from Lake Huron and Lake Erie Sediments

INTRODUCTION

The sorption of organic contaminants in sediments is an important environmental fate process because it can greatly influence the bioavailability and hence the effects and/or biodegradation of these pollutants (Karickhoff, 1981; Mihelcic et al., 1993). Ecological risk assessment of contaminated sediments is often based on whole sediment concentrations of hydrophobic organic chemicals (HOCs), but a number of studies have shown that bioavailability, biodegradation and toxic effects decrease with increasing contact time between these contaminants and sediment particles (Landrum et al., 1992b; Hatzinger and Alexander, 1995). This is thought to occur due to the process of sequestration, or the formation of contaminant fractions that are resistant to desorption (Luthy et al., 1997). Thus, using bulk sediment concentrations in environmental assessments may overestimate risk to aquatic species (Alexander, 2000).

Although the importance of sediment aging and contaminant sequestration has been identified, the processes behind the formation resistant desorption compartments are not well understood. Some proposed mechanisms include:

1) chemical nonequilibrium reactions between functional groups on the sorbent and sorbate, 2) slow diffusion through intraparticle micropores, 3) diffusion in the organic matter matrix, and 4) entrapment (Farrell and Reinhard, 1994; Pignatello and Xing, 1996; Cornelissen, 1999b; Johnson et al., 1999). Regardless of the exact mechanism, sequestration of contaminants has been shown to result in slowly desorbing fractions within the sediments that can persist for years (Pignatello and Xing, 1996). Current kinetic models of contaminant desorption include triphasic models which describe rapidly, slowly and very slowly desorbing fractions (ten Hulscher et al., 1999).

In this study, the desorption kinetics of sediment-associated fluoranthene (FLU) and trifluralin (TF) were measured over a 34-d period. Then, a three-phase model was used to estimate the rapidly, slowly and very slowly desorbing fractions of the test chemicals from the sediments and their respective first-order rate constants. The objective of this investigation was to obtain estimates of the fractions of FLU and TF in the rapidly, slowly and very slowly desorbing sediment compartments and their associated first-order rate constants. Obtaining these estimates was important because: 1) they are indicators of the bioavailability of the contaminants to benthic invertebrates, and 2) they were needed as input parameters in the bioaccumulation model (Chapter 6). Since the organic contents of the sediments were different, it was hypothesized that the desorption rates of FLU and TF would be inversely related to the sediment organic carbon.

MATERIALS AND METHODS

Experimental design

Lake Huron and Lake Erie sediments were spiked with FLU and TF, aged for 4 months and an experiment was conducted to determine the desorption kinetics of the compounds from the sediment matrix. Desorption of FLU and TF from the sediments was measured in triplicate for each of 4 concentrations (10, 40, 100 and 200 mg/kg dry wt) over 34 days. Vials containing the spiked sediments, culture water and Tenax®-TA beads, which acted as a sink for the desorbing chemicals, were continuously mixed on a rolling mill. The Tenax was removed and replaced at 12 scheduled sample times (2, 5, 9, 13, 24, 48, 96, 168, 288, 456, 672 and 816 h) for each of the test vials and the amount of contaminant sorbed to the Tenax at each sample was measured. Cumulative desorption curves were constructed from the data and the kinetic parameters were estimated with a triphasic model of desorption. It was assumed that the results of this experiment would represent the maximum apparent rates of desorption of the contaminants from the sediment particles to the pore water.

Rationale for mass of Tenax-TA beads. Prior to the start of the experiment, the mass of Tenax beads (150 mg) added to the vials for each sample time was determined based on previous studies by Pignatello (1990) and Cornelissen et al. (1997a). These authors demonstrated that the high sorption capacity of Tenax serves as a sink for desorbing organic contaminants (Pignatello, 1990) and that using 10x more Tenax than organic carbon present in the sediments provides sufficient adsorption capacity to extract chemicals from

the sediment organic matter (Cornelissen *et al.*, 1997a). In addition, contaminant concentrations on Tenax beads remain low compared to sediment concentrations because fresh Tenax is added after each sample time in the experiment. Therefore, in the present study, 150 mg of Tenax was greater than 10x the dry mass of organic carbon in 2 g wet mass of Lake Erie (~10 mg OC) and Lake Huron (~14 mg OC) sediments.

Chemicals

Radiolabeled [G-^{3}H]fluoranthene (FLU) and [Ring-UL-^{14}C]trifluralin (TF) and unlabelled FLU and TF were obtained from suppliers as described in Chapter 2, Chemicals. The purity of the radiolabeled TF was determined to be >98% by the manufacturer (January, 2001) and was used without further purification and the radiolabeled FLU was determined to be >96% pure by TLC prior to the spiking of sediments. Tenax®-TA (60-80 mesh; 177-250 µm), a porous polymer based on 2,6-dipheyl-*p*-phenylene oxide, was purchased from Alltech Associates (Deerfield, IL, USA). Before use, the Tenax beads were washed with deionized water, acetone, and hexane (three times each; 10 mL/g) and dried overnight at 75 °C. All reagents used for rinsing and analyses, and the scintillation cocktail and solubilizer used for radionuclide analysis were as described in Chapter 2, Chemicals.

Sediment spiking

Solutions of radiolabeled and unlabeled FLU and TF were spiked onto Lake Huron and Lake Erie sediments at nominal concentrations of 10, 40, 100

and 200 mg/kg dry wt of each test compound as previously described (see Chapter 2, Sediment spiking). These nominal mass-based concentrations equated to 0.049, 0.198, 0.494 and 0.989 µmol/g dry wt for FLU and 0.030, 0.119, 0.298 and 0.596 µmol/g dry wt for TF. Lake Huron sediments were spiked on May 15, 2001 and Lake Erie sediments were spiked on May 18, 2001. After spiking, the sediments were stored in the dark at 4 ºC. The 200 mg/kg sediments used in the desorption studies came from the same batch of spiked sediments that were used in the bioaccumulation studies of sediment-associated FLU and TF by *H. azteca*. Therefore, the 10, 40 and 100 mg/kg treatments used for these studies were allowed to equilibrate undisturbed for approximately 4 months, whereas the sediments at 200 mg/kg of the test chemicals were manipulated on July 24, 2001 (Lake Huron sediments) and August 7, 2001 (Lake Erie sediments) by thoroughly mixing the sediments prior to their distribution to the *H. azteca* test beakers. After these manipulations of the spiked sediments at 200 mg/kg, the sediments were again stored until the start of the desorption experiment (September 13, 2001). The potential degradation of the test compounds in the sediments was estimated from a first order decay model using measured degradation data from Chapter 2 (see Equation 2.6). The purity of the test compounds in the sediments at the start of the desorption experiments was estimated to be >95% for FLU and 68-78% for TF.

Desorption experiment using Tenax beads

Prior to test initiation, triplicate samples of each sediment treatment were taken for dry weight determination and for measurement of FLU and TF

concentrations by LSC using methods described in Chapter 2, Analytical methods. Briefly, wet sediment samples (100 mg) were placed into 20-ml borosilicate glass scintillation vials, 1.0 ml solubilizer was added and the vials were then capped, gently vortexed and held for 24 h prior to the addition of scintillation cocktail. The sediment samples were then held for an additional 48 h to allow the subsidence of chemiluminescence prior to measurement of ^{3}H and ^{14}C activity.

FLU and TF desorption kinetics were determined at 22 ºC using a Tenax solid-phase extraction method (Cornelissen et al., 1997a). The experiment began at 11:24 AM on September 13, 2001. Spiked sediments (2.0 g), 38 ml of culture water, 1.9 mg $HgCl_2$, and 150 mg of Tenax beads were added to 40-ml amber, screw cap vials with Teflon®-lined closures. Triplicate vials for each of 4 concentrations (10, 40, 100 and 200 mg FLU and TF/kg dry wt) per sediment (i.e., Lakes Huron and Erie sediments) were prepared. The $HgCl_2$ (50 mg/L) was added to the vials to prevent any further microbial breakdown of the contaminants during the time course of the desorption experiment (ten Hulscher et al., 1999; Cornelissen et al., 2000). The vials were attached to the axles of a rolling mill and were continuously inverted (60 rpm) such that the sediments and Tenax beads were well mixed. The Tenax was refreshed at 12 sample times (2, 5, 9, 13, 24, 48, 96, 168, 288, 456, 672 and 816 h) for each of the test vials. At each sample time during the desorption experiment, vials were removed from the rolling mill and the Tenax separated from the sediment suspension rapidly as the sediments sank to the bottom of the vial and the Tenax beads floated to the

top of the aqueous phase and adhered to the walls of the amber vial. Removal of the Tenax beads from the vial was accomplished by using a solvent-washed spatula that was fashioned from a coiled piece of 0.794-mm OD copper wire. The Tenax beads were transferred to a 20-ml borosilicate glass scintillation vial, 12 ml of scintillation cocktail was added, the vial was capped, gently vortexed and held for 48 h prior to measurement of ^{3}H and ^{14}C activity by LSC.

After termination of desorption (at 816 h) samples of the remaining sediment (ca. 100 mg) and overlying water (5 mL) were taken from each vial and analyzed by LSC to check the mass balance. Sediment samples were processed as described above. The water samples were placed into 20-mL borosilicate glass scintillation vials and 12 mL of scintillation cocktail was added to each vial. Then the contents were vortexed for 10 sec and the samples were stored for >48 h in the dark at room temperature. After subsidence of chemiluminescence (=48 h), radioactivity was quantified by LSC. Mass balances were calculated by dividing the total mass of contaminant at the end of the experiment (mass in the end-of-experiment water and sediment samples plus the cumulative mass desorbed) by the total mass in the sediments measured at the beginning of the experiment.

Analytical methods

The measurement of FLU and TF equivalents in the sediment, Tenax and water samples by dual-label LSC followed the procedures described in Chapter 2, Analytical Methods. Counting time for each sample vial was 20 min. The total amounts of FLU and TF equivalents (parent compound and breakdown products

on a molar basis) in each sample were calculated using the nominal specific activities of the spiking solutions.

Desorption Modeling

Desorption of FLU and TF from the sediments was described by the following first-order three-compartment (triphasic) model (Kraaij et al., 2001):

$$S_t / S_0 = F_{rap} e^{-k_{rap} t} + F_{slow} e^{-k_{slow} t} + F_{vs} e^{-k_{vs} t}, \quad (4.1)$$

where S_t and S_0 are the sediment-sorbed amounts of contaminant at time t (h) and at the start of the experiment, respectively (µmol); F_{rap}, F_{slow} and F_{vs} are the fractions of the contaminant present in the rapidly, slowly and very slowly desorbing sediment compartments at time zero, respectively; and k_{rap}, k_{slow} and k_{vs} are the rate constants of rapid, slow and very slow desorption, respectively (1/h).

Three assumptions were made in order to apply this model. First, the amounts of FLU and TF in the aqueous phase were assumed to be negligible compared with the amounts in the rapidly, slowly and very slowly desorbing sediment compartments. The assumption was operationally met by the addition of Tenax to the system which was expected to strip the water of any desorbed chemicals (Pignatello, 1990; Cornelissen et al., 1997a). Second, it was assumed that the chemicals spiked onto the sediments were in either the rapidly, slowly or very slowly desorbing sediment compartments such that $F_{rap} + F_{slow} + F_{vs} = 1$. Finally, it was assumed that $k_{rap} >> k_{slow}$ and k_{vs}. The values of F_{rap}, F_{slow}, F_{vs}, k_{rap}, k_{slow}, and k_{vs} were determined by least squares nonlinear regression of the the desorption time course data (i.e., S_t/S_0 vs. t) using *SYSTAT for Windows,*

Version 9 (SYSTAT, Evanston, IL, USA). The use of the Gauss-Newton algorithm and exact sample times for the replicates with their corresponding ratio (S_t/S_0) values were as previously described in Chapter 2, Modeling.

The time at which 99.9% of a given fraction of FLU or TF takes to desorb from the sediments was calculated by the following set of equations:

$$F_x e^{-k_x t_1} = (1 - 0.999) F_x e^{-k_x t_2}, \quad (4.2)$$

where the subscript *x* denotes the compartment of interest (e.g., rapidly, slowly, very slowly desorbing); t_1 represents the time at which 99.9% of this initial fractional amount of contaminant has desorbed (h); and t_2 represents time zero (0 h). With t_2 =0 h, Equation 4.2 reduces to:

$$e^{-k_x t_1} = 0.001, \quad (4.3)$$

for which the linear form is:

$$t_1 = \frac{\ln(0.001)}{-k_x}. \quad (4.4)$$

Statistical analysis

The modeling of desorption using the three-phase model described by Equation 4.1 results in the simultaneous estimation of 6 parameters from the desorption-time profile. Therefore, entire curves of desorption data were compared with an F-test by the method of Ratkowsky (1983). This analysis operates on the hypothesis that common estimates of model parameters obtained by fitting the pooled data set (*i.e.,* all doses within a sediment type, both sediments within a dose level) are sufficient to describe individual data sets, and

are therefore invariant. This hypothesis is tested statistically by a one-tailed F-test with an alpha of 0.05 (Ratkowsky, 1983).

RESULTS

Sediment and test vial samples

The measured concentrations of FLU were between 81 to 97% of their target nominal concentrations for the Lake Erie sediments and for Lake Huron sediments this range was 79 to 95% (Table 4.1). TF concentrations in the sediments were 70 to 85% and 71 to 84% of their target nominal concentrations in the Lakes Erie and Huron sediments, respectively (Table 4.1). In general, the percent of the target concentration that was achieved decreased with treatment concentration. From the concentrations measured on the sediments, the amounts of FLU and TF that were added to each vial (2 g wet sediment per vial) were calculated. These masses (µg) of the test chemicals are shown in Table 4.2.

After the termination of the desorption experiment, samples of the water and sediments remaining in the vials were taken and analyzed by LSC for the determination of mass balances. The sums of the total masses of solutes desorbed to Tenax and solutes remaining in the vials after desorption were 74.8 ± 3.5% for FLU and 94.4 ± 2.5% for TF in the Lake Erie sediments. For the Lake Huron desorption vials, these mass balances were 77.3 ± 2.3 and 94.5 ± 1.7% for FLU and TF, respectively.

Fractions and rates of desorption

All of the desorption curves (plotted as S_t/S_0 versus time) were qualitatively similar in that they were characterized by a rapid decrease in the amount of contaminants sorbed to the sediments during the early part of the experiment, generally from 0-50 hours (Figures 4.1 and 4.2). This was followed by a transition period between 50 to 200 hours after which desorption appeared to be very slow. For FLU in the Lake Erie sediments, the least amount of desorption was observed to occur for the lowest and highest spiked concentrations (10 and 200 mg/kg). Slightly greater desorption was measured for FLU at 40 and 100 mg/kg which behaved similarly up to about 25 h after which time the 100 mg/kg treatment became more similar to and then converged with the 10 mg/kg treatment. Overall for TF in the Lake Erie sediments, the 10 mg/kg treatment desorbed the least, to 33.2 ± 0.01% of the initial concentration on the sediments by 816 h while the 40 and 100 mg/kg treatments apparently desorbed about equally (26.0 ± 0.002 and 25.0 ± 0.02%, respectively), and their standard deviations overlapped throughout much of the time course. The desorption pattern of TF at 200 mg/kg in the Lake Erie sediments was similar to the 10 mg/kg treatment until about 50 h, after which time the amount desorbed was between the 10 mg/kg and the 40 and 100 mg/kg time courses with a final amount of 28.5 ± 0.01% of the initial concentration remaining on the sediments. After about 400 h, the slopes of the desorption curves were nearly parallel to one another.

Desorption of FLU and TF in Lake Huron sediments generally followed a pattern of less total desorption of the initial concentrations at the lower doses (Figure 4.2). FLU at 10 mg/kg desorbed markedly less (42.7 ± 0.01% remaining on the sediments) than the amounts at 40-200 mg/kg (35 to 38% remaining on the sediments) by the end of the experiment. Desorption of FLU in the 200 mg/kg treatment was also intermediate to the 40 and 100 mg/kg desorption time courses until 288 h, after which time the slope decreased and led to convergence with the 40 mg/kg treatment. The slopes of the 10, 40 and 100 mg/kg sediments appeared to be parallel, indicating similar rates of desorption of FLU during the latter time points for these concentrations. TF desorption from the Lake Huron sediments increased with dose through the time course until the 288 h sample point. After this time it appeared that the 10 and 40 mg/kg treatments converged to final fractions of about 30% remaining on the sediments and the 100 and 200 mg/kg sediments converged to 27% of the initial amounts.

The values of the fractions of total sediment-associated FLU and TF in the rapidly, slowly and very slowly desorbing compartments and their rate constants are shown in Tables 4.3 and 4.4, respectively. These tables include the parameter estimates fit to the individual data sets for each concentration level as well as the entire (pooled) data set for FLU and TF spiked onto each sediment. The pooled fits are reported because these values were needed to carry out the statistical comparisons (*i.e.,* F-test results; see below). The fits of the data were in very good agreement with the individual treatment data sets (RSS range, 0.001 to 0.006; R^2 range, 0.994 to 0.999) (Table 4.5). Desorption rates, as

expected, followed the progression of $k_{rap} > k_{slow} > k_{vs}$ and were generally on the order of 10^{-1}, 10^{-2} and 10^{-4} per hour, respectively. In general, the slowly desorbing fraction (F_{slow}) of contaminants was the smallest compartment as indicated by the curve fits. The values of F_{slow} were =16.9% of FLU and =21.6% of TF in the sediments whereas the values of F_{rap} ranged across the sediments from 31.3 to 47.4% and 39.7 to 54.9% for FLU and TF, respectively. Very slowly desorbing amounts of the chemicals (F_{vs}) were similar to rapidly desorbing compartment and ranged from 40.6 to 52.9% for FLU and 30.5 to 42.0% for TF in the sediments.

Statistical comparisons between the curves for each treatment concentration of FLU and TF in Lake Erie sediments resulted in rejection of the null hypothesis (*i.e.,* that the values of F_{rap}, F_{slow}, F_{vs}, k_{rap}, k_{slow} and k_{vs} would be the same across the concentrations) ($F_{18,142}$ =54.5, p <0.00001 for FLU; $F_{18,142}$ =207, p <0.00001 for TF) (Tables 4.6 and 4.7). Further comparisons indicated that all three initial fractional amounts (F_{rap}, F_{slow} and F_{vs}) of desorbing FLU, and all parameters describing the desorption of TF except for k_{vs}, were not equivalent across the four treatment concentrations (all $F_{3,127} > 2.68$, p =0.02; Tables 4.6 and 4.7). The comparisons of the curves of the desorption of FLU and TF from the Lake Huron sediments are shown in Tables 4.8 and 4.9. Again, the null hypothesis of common parameter values across the treatment concentrations was rejected ($F_{18,150}$ =211, p <0.00001 for FLU; $F_{18,150}$ =51.7, p <0.00001 for TF). Supplementary statistical testing showed that all fitted parameters, with the exception of k_{slow}, were not equivalent across the treatment

concentrations for FLU in Lake Huron sediments (all $F_{3,135} > 2.68$, $p = 0.03$). The F-test on the TF data for these sediments indicated that the values of F_{rap}, F_{vs} and k_{vs} were significantly different with respect to the treatment concentrations.

Pairwise comparisons of the curves were performed between sediments with respect to dose (*i.e.,* 10 mg/kg Lake Huron versus 10 mg/kg Lake Erie). The detailed results of these analyses are provided in Appendix A. Each of these 8 comparisons (4 for each FLU and TF) resulted in a detection of significant differences ($F_{obs} > F_{crit}$; $p < 0.05$) between the curves. The major conclusion from these pairwise comparisons was that desorption rates of FLU and TF were all higher in the Lake Huron sediments (Tables 4.3 and 4.4), and were in many instances significantly faster than the rates of desorption from the Lake Erie sediments. Lake Huron sediment desorption rates for FLU, were significantly higher for k_{rap} at all 4 treatment concentrations and k_{slow} at 40, 100 and 200 mg/kg. There were no significant differences for the k_{vs} of FLU between sediments, but the values were higher for the Lake Huron sediments. The desorption of TF from Lake Huron sediments was faster than Lake Erie for k_{rap} at 10, 40 and 200 mg/kg, k_{slow} at 10 and 200 mg/kg and k_{vs} at 10 and 200 mg/kg. The fractions of the initial amounts of FLU and TF desorbing from the rapid, slow and very slow desorption compartments did not exhibit any general trends between sediments. However, the clearly faster rates of desorption from the Lake Huron sediments compared to the rates measured for the Lake Erie sediments is an important finding that will be addressed in the Discussion.

Desorption time

The time at which 99.9% ($t_{99.9}$) of the amounts of FLU and TF would have desorbed from each the rapidly, slowly and very slowly desorbing fractions were calculated from the corresponding desorption rates and the results are shown in Table 4.10. The times were on the order of hours, days, and years for the contaminant desorption from the rapid, slow, and very slow compartments, respectively. As would be expected from the finding that the desorption rates of FLU and TF were fastest in the Lake Huron compared to the Lake Erie sediments, $t_{99.9}$ values were also shorter for desorption from the Lake Huron sediments. The $t_{99.9}$ values of FLU desorption from both sediments ranged from 11.7 to 20.3 hours from the rapidly desorbing compartment, 8.42 to 15.1 days from the slowly desorbing compartment and 2.92 to 5.63 years from the very slowly desorbing compartment. The $t_{99.9}$ values determined for the desorption of TF from the sediments ranged from 9.85 to 17.9 h, 9.06 to 18.9 days and 1.88 to 3.29 years for the rapidly, slowly and very slowly desorbing fractions. Thus, assuming that the desorption rates are constant through time, one would expect that most all of the FLU and TF had desorbed from the rapid and slow desorption compartments of the sediments over the course (34 d) of the this study.

DISCUSSION

Triphasic desorption

The results of the 34-d desorption experiment indicated that this time frame was long enough to provide reasonable estimates of the rapid, slow and

very slow desorption of FLU and TF that were spiked onto Great Lakes sediments. The predicted $t_{99.9}$ values for rapid (=20.3 h) and slow (=18.9 d) desorption were less than the duration of the experiment, so it appeared that these fractions and their corresponding desorption rates were well characterized. In addition, since six of the twelve samples were taken at early points (*i.e.,* within the first 48 h) of the time course, as recommended by Opdyke and Loehr (1999), it was concluded that reliable estimates for F_{rap}, k_{rap}, F_{slow}, and k_{slow}, were obtained. However, there is considerable uncertainty in the estimates of k_{vs} values. This uncertainty arises due to the relatively short duration of the experiment in relation to the time scale of very slow desorption, which is on the order of years (Ball and Roberts, 1991; Ferrell and Reinhard, 1994; Opdyke and Loehr, 1999). Even with this uncertainty, three-phase models that include very slow desorption are applicable to laboratory studies and are useful for describing sediment desorption *in situ*, and an increasing number of triphasic models have been described in the recent literature (ten Hulscher et al., 1999; Cornelissen et al., 1997b, 2000, 2001).

Another source of uncertainty arises due to the degradation of TF in the sediments during the 4 months prior to the initiation of the desorption study (only 68-78% parent TF at T=0 h). Therefore, the estimates of the fractions and rates of TF desorption from the sediments should be viewed with caution. Since many of the TF breakdown products are more polar and more easily extracted from soils than the parent compound (Golab and Occolowitz, 1979; Malterre et al., 1997), it is possible that the parameter estimates were misrepresented and that

the values of F_r and k_r were overestimated. However, as discussed below, the desorption parameters estimated in the present study compare favorably with previously reported desorption rate constants and fractions and thus should be useful estimates of maximal desorption.

Desorption fractions and rates

The rate constants and fractions of FLU and TF that were estimated to be desorbing rapidly, slowly and very slowly in the present study were reasonably in accordance with values reported in other studies for PAHs that were spiked onto sediments. For example, the fraction of FLU desorbing rapidly was shown to vary from 27 to 87% in four different sediments (Cornelissen et al., 2001) and this range contains the range of F_{rap} values observed in the present study (31.3 to 47.4% for FLU; 39.7 to 54.9% for TF). In a study of sediments collected from Lake Oostvaardersplassen, The Netherlands, that were spiked with PAHs and allowed to equilibrate for 34 d, Cornelissen et al. (1997a) reported an F_{rap} of 40.1%, a k_{rap} of 0.202/h and a k_{slow} of 3.12×10^{-3}/h for FLU. The rapidly desorbing fraction in that study was similar to values obtained in the present study and k_{rap} was within a factor of 2-3 of the values reported in the present study. However, the value of k_{slow} reported by Cornelissen et al. (1997a) was an order of magnitude slower than the values estimated for the Great Lakes sediments. In another study with the same sediments, Cornelissen et al., (1997b) reported, for FLU, rapidly desorbing fractions of 60% at 20 °C and 73% at 65 °C, F_{slow} and F_{vs} values of 37% and 3.4%, respectively, at 65 °C, k_{slow} values of 3.12×10^{-3}/h and 128×10^{-3}/h, at 20 and 65 °C, respectively, and a k_{vs}

of 4.1 x 10^{-3}/h at 65 °C. The F_{rap} at 20 °C in that investigation was higher than their previous study (Cornelissen et al., 1997a) and higher than values obtained in the present study using sediments from Lakes Erie and Huron. Temperature elevation to 65 °C (Cornelissen et al., 1997b) increased the rates of slow and very slow desorption to levels that were faster than those observed for FLU in the present study at 22 °C by factors of about 4 to 7 for k_{slow} and by an order of magnitude for k_{vs}. Finally, in a study of the effect of contact time with sediments on the sequestration and bioavailability of contaminants to oligochaetes, it was shown for FLU that F_{rap} decreased from 76% after 5 d of contact to 56% by 959 d with a concomitant decrease in bioavailability, while F_{vs} increased from 13% to 26% over the same period (Kraaij et al., 2002). The contact time in the present study (up to approximately 120 d) was intermediate to the times used by Kraaij et al. (2002), but for FLU and TF desorption from Great Lakes sediments in the present study, the F_{rap} values were slightly lower and the F_{vs} values were up to a factor of 2 higher. These differences were likely due to different characteristics of the sediments, which will be addressed below.

TF desorption from sediments and soils has not been investigated with the same continuous desorption methods as were used in the present study and those cited above for FLU. However, Smith et al. (1988) reported that 35 to 47% of the initial amount of TF had desorbed by a first-order process over 84-d and there was no apparent effect of contact time since this range was similar for freshly spiked soils and those aged for 10 months following application of TF. The average half-life for the desorption of TF from the soils was 103 d (Smith et

al., 1988). In the present study, total TF desorption was between 26 and 33% of the initial amounts in the sediments by 34 d which is close to the amount that would be predicted to have desorbed by day 34 (21%) in the Smith et al., (1988) study based on their reported half-life.

Differences in F_i and k_i values between treatment concentrations

Inspection of the curves (Figure 4.1) and the values of the fractions and rate constants of the desorption compartments (Tables 4.3 and 4.4) for the Lake Erie sediment desorption data indicated that the behavior of the 200 mg/kg treatment was likely responsible for most of the statistical differences between the desorbing fractions for FLU and both the fractional amounts in the desorbing compartments and the desorption rates for TF. The explanation for these differences is that manipulation of the 200 mg/kg sediments at about 60 d of equilibration, when they were thoroughly mixed prior to their use for bioaccumulation testing with *H. azteca* (see Chapter 2), affected contaminant distribution and hence desorption. Since the concentrations of FLU and TF at 200 mg/kg in the Lake Erie sediments at the beginning of the desorption study (FLU, 0.801 ± 0.111 µmol/g dry wt; TF, 0.415 ± 0.045 µmol/g dry wt) were not less than the mean concentrations of these contaminants in the *H. azteca* bioaccumulation kinetics test (see Chapter 2, Table 2.5), then an explanation of a loss of FLU and TF from the sediments due to the manipulation was ruled out. A more plausible explanation of the effect of this manipulation would be that it led to a reduction of the rapidly desorbing fraction of contaminants from the Lake Erie sediments. This would come about by the redistribution of the contaminants

if: 1) dissaggregation of the sediments during the mixing process exposed new, high-energy (*i.e.,* slowly and very slowly desorbing) binding sites to the freely dissolved FLU and TF in the pore water, and then 2) any chemicals that were released by the kinetic energy of the mixing of particles from low-energy sites in the rapid fraction (activation enthalpies 0-50 kJ/mol) also were able to bind to these higher-energy sites in the slow and very slow fractions (activation enthalpies of 60-100 kJ/mol) (Weber and Miller, 1989; ten Hulscher and Cornelissen, 1996; Cornelissen et al., 1997b; Luthy et al., 1997; Schlebaum et al., 1999).

The explanation of a redistribution of FLU and TF in the Lake Erie sediments at 200 mg/kg toward binding sites from which desorption was slow or very slow appears to be supported by the data. For FLU, F_{rap}, F_{slow} and F_{vs} were determined to be significantly different across doses and the values of each fraction at 200 mg/kg were similar to the estimates at 10 mg/kg (Tables 4.4 and 4.6). However, these desorbing fractions at 40 and 100 mg/kg generally increased for F_{rap} and decreased for F_{slow} and F_{vs} in comparison to their values at 10 mg/kg. For the desorbing fractions of TF, similar trends between the 10 and 200 mg/kg treatments and with concentration for the 10, 40 and 100 mg/kg treatments were observed as for FLU, but there were also significant differences between the rapid and slow desorption rates (Tables 4.5 and 4.7). The value of k_{rap} tended to increase from 10 to 100 mg/kg while the value at 200 mg/kg was less than at 10 mg/kg but within its standard error, so overall they were similar between the lowest and highest treatments. The k_{slow} value of the 200 mg/kg

treatment was much lower than the similar rate estimates for slow desorption of TF from 10, 40 and 100 mg/kg sediments. The similarities of the desorbing fractional amounts of FLU and TF between the 10 and 200 mg/kg concentrations led to their similar desorption curves which nearly overlapped throughout the time course for FLU due to similarity among FLU desorption rates. These findings lend support to the argument that contaminants were redistributed to more slowly desorbing sites upon manipulation of the 200 mg/kg sediments.

For FLU and TF desorption from the Lake Huron sediments the effect of manipulation on the 200 mg/kg treatment was not as pronounced as in the sediments from Lake Erie. Only FLU seemed to be affected as the desorption curve for 200 mg/kg was between the 40 and 100 mg/kg treatments. This appeared to be driven by the lowest overall k_{rap} for FLU at 200 mg/kg compared to the other treatments that exhibited a concentration-dependent increase in the rate of rapid desorption (Table 4.3). In addition, F_{rap} at 200 mg/kg was between the estimates at 40 and 100 mg/kg. There was no good explanation for the lack of an effect of the manipulation on the Lake Huron sediments, but it may be related to the total organic carbon (TOC) contents of the sediments. The TOC of the Lake Huron sediments (3.64 ± 0.08%) was higher than Lake Erie sediments (2.08 ± 0.20%) and, assuming that organic carbon was the dominant sorbent in the sediment matrix for the hydrophobic chemicals (Karickhoff et al., 1979; Di Toro et al., 1991), this difference may have masked an effect of redistribution in the Lake Huron sediments since there was a larger pool of organic carbon and, presumably, total binding sites relative to the Lake Erie sediments. For the

Lake Huron sediments, the differences among the desorbing fractions and rate constants seemed to be due to concentration. Overall, for the treatment concentrations that were not manipulated (10, 40 and 100 mg/kg) there was less desorption of FLU and TF at lower doses as indicated by the lower percentages of the compounds remaining on the sediments as the concentration increased (Figures 4.1 and 4.2). The reasons for this are discussed in the next section.

Concentration dependence of the amount of contaminant desorbed

The percent of applied FLU and TF that desorbed from the sediments was directly related to concentration. A similar observation for trichlorobenzene at concentrations ranging from 0.016 to 27.6 ppm spiked onto sediments of about 13% organic carbon contents was reported by Cornelissen et al. (2000). In the present study where organic carbon contents ranged from approximately 2.1 to 3.6%, the total mass of organic carbon exceeded the total mass of the contaminants in the sediment by factors of at least 53 for Lake Erie and 93 for Lake Huron (*i.e.,* these factors calculated for a nominal concentration of 400 mg total contaminants/kg dry sediments). Therefore, one can assume that sorption to organic carbon was not limited, and that nearly all (99%) of the compounds in each treatment were sorbed to the organic carbon at the start of the experiment. This second assumption was supported by the predicted pore water concentrations of the compounds at a nominal sediment concentration of 200 mg/kg dry wt for each FLU and TF (*i.e.,* the maximum spiked concentration). The equations given by Di Toro et al. (1991) were used for the calculations with log K_{ow} values of 5.2 for FLU (MacKay et al., 1992) and 5.3 for TF (MacKay et al.,

1997). The predicted pore water concentrations for FLU were 76 and 42 µg/L for Lakes Erie and Huron sediments, respectively, and for TF these concentrations were 60 µg/L in Lake Erie sediments and 34 µg/L in Lake Huron sediments. These concentrations equate to dissolved (*i.e.,* non-sorbed) percentages of =0.12% of the mass of FLU or TF spiked onto either sediment at 200 mg/kg. For the sake of the following argument, the dissolved organic carbon- or colloid-bound fractions of the contaminants are assumed to be negligible.

Given the assumptions outlined above, a likely explanation for the observed lower desorption at lower treatment concentrations over the duration of the experiment (34 d) is related to the types of binding sites available for the contaminants in the sediment organic matrix. The triphasic model applied to the desorption data in the present study is not a mechanistic description of desorption, however it conceptually describes binding sites from which contaminants desorb rapidly, slowly, or very slowly. This is analogous to sites for which the activation enthalpies required for desorption from these sites range from nearly zero (rapid) to high (60-80 kJ/mol; slow and very slow) (ten Hulscher and Cornelissen, 1996; Cornelissen et al., 1997b). In a recent review, Pignatello and Xing (1996) reported that the slow fraction(s) of desorption were dependent on the inverse of the initial applied concentrations. More simply, this means that as the concentration of contaminants in the sediments decline, the slow desorption of HOCs is dominant. This effect at low contaminant concentrations is most likely because there are a limited number of high affinity or high energy binding sites (Shlebaum et al., 1999; Pignatello, 1990). Higher sorption

efficiencies are often observed at lower sorbate concentrations because of progressive saturation of the high-energy binding sites as the concentration increases (Weber and Miller, 1989; Cox et al., 1997; Celis et al., 1999). This, combined with kinetic hysteresis (*i.e.,* slower rates of "emptying" than "filling") following the binding of slowly desorbing sites, leads to slow desorption (Pignatello and Xing, 1996). In the present study, F_{rap} tended to increase with increasing concentration from 10 to 100 mg/kg, which suggests that the more slowly desorbing, higher energy binding sites approached saturation. Thus, the sorbed compounds at the lower concentrations were more resistant to desorption because proportionately more FLU and TF occupied slowly or very slowly desorbing compartments.

Differences between sediments

Overall, desorption of FLU and TF from Lake Huron sediments was faster than from the sediments from Lake Erie. Sediment characteristics including the nature of the organic carbon contents and particle size distributions may have had a role in this difference and they are discussed here. It is well established that organic carbon is the primary sorbent in sediments for HOCs such as FLU and TF (Di Toro et al., 1991; Burgess and Scott, 1992). Numerous studies have reported an inverse relationship between the rate/amount of desorption and the organic carbon contents of sediments when the same concentrations of compounds such as PAHs, PCBs, chlorinated benzenes and pesticides were applied (Karickhoff et al., 1979; Wu and Gschwend, 1986; Kan et al., 1998; Celis et al., 1999; Chen et al., 2000). Therefore, it was hypothesized that FLU and TF

desorption rates in the present study would be inversely related to the amount of organic matter in the test sediments. However, this was refuted as the opposite trend was observed between the Lake Huron sediments (3.6% TOC) and the sediments from Lake Erie (2.1% TOC).

With the failure of the organic carbon hypothesis, particle size was looked upon to explain the faster rates of FLU and TF desorption measured in Lake Huron sediments. Kukkonen and Landrum (1996) reported differential distribution of benzo[a]pyrene and hexachlorobiphenyl among sediment particles, with the largest fractions of the compounds being associated with relatively small particles <63 µm. A few studies have shown that desorption of sorbed contaminants increased inversely with particles size (Wu and Gshwend, 1986; Ball and Roberts, 1991), however pulverization of the soils and sediments in all these studies was required to obtain this result leaving to question whether the physical manipulation of the sediments led to the enhancement of desorption. In the present study, the percentage of small particles (<63 µm) was slightly higher in Lake Erie sediments (93.5%) than in sediments from Lake Huron (87.6%) (see Chapter 1, Table 1.1). A hypothesis that desorption rates of FLU and TF would be inversely related to particle size and thus would be higher for the Lake Erie sediments was not supported by the data. This was not a surprise as many recent studies have shown no correlations between desorption kinetics and particles size, down to 1 µm in some cases, for PAHs in Dutch sediments (Cornelissen et al., 1999a), PCBs from river and harbor sediments (Carroll et al.,

1994; Björklund et al., 1999) and field-aged pesticides in soils (Steinberg et al., 1987; Pignatello et al., 1993).

Finally, the polarity of the sediments, as indicated by their carbon-to-nitrogen (C/N) ratios was implicated to explain the higher desorption rates in the Lake Huron sediments. Sediment polarity, determined as the elemental ratios of C/N, H/O or O+N/C serves as a relative index of the amounts of hydrophilic, oxygen-containing functional groups and the aromatic content of sediments (Grathwohl, 1990). Many investigators have reported decreases in chemical sorption and organic carbon sorption coefficients for HOCs with increasing polarity of the sediments (Rutherford et al., 1992) which could have important implications on contaminant bioavailability in sediments depending on the dominant route of uptake for a given species (e.g., ingestion of contaminated particles or uptake from water) (Kukkonen and Landrum, 1996; Landrum et al., 1997). This indirect relationship between sorption and polarity has been observed for several chemical classes including chloroaliphatic chemicals (Grathwohl, 1990), benzene, toluene, xylene, carbon tetrachloride, α-naphthol (Xing et al., 1994), PCBs (Burgess et al., 1996), and PAHs (Landrum et al., 1997). In the present study, the organic carbon and nitrogen contents were measured for the sediments (see Chapter 2, Table 2.3), and the C/N ratios were very similar between Lake Erie (5.87-6.31) and Lake Huron (5.67-6.61). Overall, the quantity of organic carbon, the particle size distribution and the polarity of the sediment organic matter did not explain the observed difference in desorption rates between sediments.

Because a reason for the higher desorption rates observed for the Lake Huron sediments was not provided through other measurements taken during the study (*e.g.,* TOC, particle size distribution, N/C ratio), then some other characteristics of the sediments and/or sediment organic contents was responsible for this difference and it is only possible to speculate on those possibilities. Karapanogioti et al. (2000) reported on the heterogeneity of organic matter from a single sample of river sediments and that subsamples containing coal-derived organic matter showed markedly higher sorption capacities (K_{oc}) for the PAH phenanthrene than subsamples containing organic coated quartz particles. The samples of sediments used in the present study were from two different sources on the Great Lakes, and thus the differences in the type, age and quality of the organic matter may have been responsible for the observed differences in desorption rates. These differences may have been due to distributions or amounts of structurally distinct "soft carbon" which is analogous to a more flexible or rubbery polymer and "hard carbon" which is more like a glassy polymer (Huang et al., 1997). Differences such as these are thought to control the amounts of rapidly (soft carbon) and slowly (hard carbon) desorbing sites within the sediment organic matrix (Leboef and Weber, 1997, 1999; Cornelissen et al., 2000). Research on these specific aspects of organic carbon and their roles in desorption is ongoing by these investigators.

Utility of desorption data

The present study provided estimates of the rates of desorption and fractional distributions of FLU and TF among the rapid, slow and very slow

compartments after nearly 4 months of sediment aging. Since these desorption rate estimates were determined during constant mixing of spiked sediments at a stable temperature (22 ºC) in the presence of a strong sink (Tenax-TA) they are considered to represent maximum rates of desorption. In addition, the method used here also assumes that the rates are constant, whereas rates of desorption in the field can change with time (Pignatello and Xing, 1996). These artifacts increase the uncertainty in our current ability to predict desorption and hence bioavailability and acceptable remediation levels in the field from laboratory data, especially since very little of the rapidly desorbing fractions often remain in aged and weathered contaminated field sediments (ten Hulscher et al., 1999). However, the estimated values of the rapid, slow and very slow rates desorption of FLU and TF were within the ranges reported for HOCs in the literature (*i.e.,* k_{rap}, 10^{-1}/h; k_{slow}, 10^{-2-3}/h; k_{vs}, 10^{-4}/h) from both laboratory- and field-contaminated sediments and soils (McCall and Agin, 1985; ten Hulscher et al., 1999; Cornelissen et al., 2001). Therefore, it is reasonable to assume that the $t_{99.9}$ values (Table 4.10) that were calculated for FLU and TF give a realistic indication of the persistence of these contaminants in field sediments both after an input event (*e.g.,* spill, runoff, atmospheric deposition, etc.) and after aging of the sediments as desorption of most of the fast fraction occurs within hours and can take years for the very slowly desorbing fraction (Chung and Alexander, 1998). However, predicting the bioavailability of sediment-associated contaminants from desorption data is still problematic.

The fraction of sediment-associated contaminants in the rapidly desorbing compartment is increasingly considered to be bioavailable for accumulation or biodegradation (Cornelissen et al., 1998; Lamoureux and Brownawell, 1999; Kraaij et al., 2001, 2002). Recently, a proposed method for roughly determining the bioavailable amount of HOCs was based on the rapidly desorbing fraction whereby F_{rap}*Cs gives a better estimate of bioavailable concentration than equilibrium partitioning equations that rely on K_{oc} values (van Noort et al., 1999; Cornelissen, 1999b). An assumption of this approach was that the rapidly desorbing fraction represents HOCs that are adsorbed to external surfaces of particles and thus are not entrapped within pores (Cornelissen et al., 1997a; 2000). This assumption has been supported by particle sectioning studies using X-ray spectroscopy (Ghosh et al., 2000) and in modeling studies of chlorinated benzenes, PAHs and PCBs (Cornelissen et al., 1997a). Furthermore, it has been shown that the bioavailability of sediment-associated HOCs to benthic species including *L. variegatus* and *Diporeia* sp. decreases with aging (Landrum et al., 1992b; Loonen et al., 1997; Alexander, 2000) which can be due to the increasing resistance to desorption (Lamoureux and Brownawell, 1999) or, more simply, to loss of the rapid fraction by desorption or erosion in more dynamic (*i.e.,* riverine) environments (van Metre et al., 1998). Thus, field-collected sediments that have undergone significant aging (months to years) would be expected to have very small F_{rap} values and high fractions for the slow and very slow desorption compartments. Some authors suggest that there is little or no uptake in biota from the slowly and very slowly desorbing fractions (van Noort et al.,

1999; Kan et al., 2000). This generalization should be viewed with caution however, as pore water is assumed to be the dominant route of uptake and thus uptake by ingestion, which has been shown to be important to deposit-feeding benthic species (Leppänen and Kukkonen, 1998b), is ignored. Therefore, based on the values of F_{rap} for FLU and TF in the present study for which the aging time was relatively short, the bioavailable concentration in the sediments would be roughly predicted to range from approximately 31 to 47% of the measured concentrations of FLU and from 40 to 55% of the bulk sediment levels of TF.

Conclusions

The triphasic model of desorption led to estimates of F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs} and k_{vs} for FLU and TF that are similar to previously reported values of these parameters for hydrophobic organic chemicals. However, due to the relatively short duration of the experiment (~1 month) relative to the temporal scale of very slow desorption (years), the values of k_{vs} have a high degree of uncertainty. The rapidly desorbing fraction for FLU and TF in sediments that were aged for 4 months ranged from 31.3 to 54.9% of the initial concentrations and k_{rap}, k_{slow} and k_{vs} values were on the order of 10^{-1}/h, $10^{-2–3}$/h and 10^{-4}/h, respectively. In general, the total fraction of the initial contaminant amount that desorbed over the time course was directly related to concentration, even though the mass of organic carbon in the sediments far exceeded (by a factor of 50-90) the applied masses of the test chemicals. It was postulated that this trend was due to the combined effects of saturation of high energy (slow and very slow) binding sites in the organic carbon matrix and hysteresis. However, the highest concentration

treatment (200 mg/kg) in the Lake Erie sediments did not follow this general trend which was likely due to its manipulation (*i.e.,* mixing that disturbed the equilibrium) at 60 d post-spiking. Higher rates of desorption were observed for FLU and TF from the Lake Huron sediments and this was not apparently related to the TOC, particle size distribution or polarity (C/N ratio) of the sediments. A reasonable explanation for this difference between the sediment types would be that the relative amounts of “soft” and “hard” carbon were dissimilar for Lakes Erie and Huron sediments. Laboratory-to-field extrapolations are difficult, but overall, FLU and TF in these sediments were predicted to persist for years due to the very slow desorption of an estimated 30.5-52.9% of the bulk sediment concentrations. Finally, based on the rapidly desorbing fractions, bioavailable amounts of the contaminants were predicted to be between 31-47% of sediment concentrations.

Table 4.1. Concentrations of fluoranthene and trifluralin in sediment samples taken at the beginning of the desorption experiment. Samples from each treatment were taken in triplicate.

Sediment ID	Nominal sediment concentration (mg/kg dry wt)	Measured fluoranthene in sediments (mg/kg dry wt)			Measured trifluralin in sediments (mg/kg dry wt)		
		Mean	±	SD	Mean	±	SD
Lake Erie	10	9.67	±	0.32	8.54	±	0.39
	40	35.7	±	0.21	32.1	±	0.6
	100	80.5	±	2.07	70.6	±	2.4
	200	162	±	22.4	139	±	15.0
Lake Huron	10	9.09	±	0.15	8.13	±	0.06
	40	37.8	±	0.52	33.4	±	0.19
	100	86.0	±	1.57	76.2	±	1.03
	200	158	±	8.3	141	±	2.1

Table 4.2. Amounts of fluoranthene and trifluralin in the desorption vials (n =3 per treatment) at the beginning of the desorption experiment.

Sediment ID	Nominal sediment concentration (mg/kg dry wt)	Amount of fluoranthene in desorption vials at T=0 h (µg)		Amount of trifluralin in desorption vials at T=0 h (µg)	
		Mean	± SD	Mean	± SD
Lake Erie	10	4.63	0.05	4.08	0.04
	40	17.4	0.04	15.7	0.04
	100	39.3	0.28	34.5	0.25
	200	77.8	1.41	66.5	1.21
Lake Huron	10	3.47	0.02	3.11	0.02
	40	13.9	0.09	12.3	0.08
	100	31.8	0.60	28.2	0.53
	200	58.2	0.39	51.7	0.34

Table 4.3. Fluoranthene desorption parameters. The desorbing fractions (F_{rap}, F_{slow}, F_{vs}; %) and their corresponding desorption rate constants (k_{rap}, k_{slow}, k_{vs}; 10^{-3}/h) are shown as the estimated value ± asymptotic standard error (± A.S.E.).

		10 mg/kg	40 mg/kg	100 mg/kg	200 mg/kg	Pooled data
Sediment	**Parameter**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Lake Erie	F_{rap}	37.2 ± 1.78	45.1 ± 1.03	43.8 ± 0.79	37.9 ± 0.75	41.5 ± 1.47
	F_{slow}	16.5 ± 1.52	12.4 ± 0.89	12.3 ± 0.66	16.9 ± 0.64	14.0 ± 1.25
	F_{vs}	46.2 ± 0.90	42.4 ± 0.60	43.9 ± 0.37	45.2 ± 0.54	44.5 ± 0.95
	k_{rap}	341 ± 32.1	387 ± 19.0	411 ± 17.7	377 ± 19.0	372 ± 30.3
	k_{slow}	25.0 ± 5.42	25.6 ± 4.81	27.5 ± 3.46	19.1 ± 2.00	21.5 ± 5.13
	k_{vs}	0.22 ± 0.04	0.19 ± 0.03	0.15 ± 0.02	0.14 ± 0.02	0.17 ± 0.04
Lake Huron	F_{rap}	31.3 ± 0.92	41.4 ± 0.68	47.4 ± 1.46	45.0 ± 0.87	42.4 ± 2.49
	F_{slow}	15.6 ± 0.78	13.1 ± 0.57	11.9 ± 1.22	12.0 ± 0.74	12.4 ± 2.10
	F_{vs}	52.9 ± 0.47	45.4 ± 0.32	40.6 ± 0.73	43.0 ± 0.41	45.3 ± 1.38
	k_{rap}	520 ± 31.9	586 ± 22.3	591 ± 47.4	500 ± 23.1	518 ± 70.8
	k_{slow}	31.3 ± 3.82	34.2 ± 3.52	31.5 ± 7.88	31.2 ± 4.49	28.2 ± 12.1
	k_{vs}	0.27 ± 0.02	0.23 ± 0.01	0.19 ± 0.04	0.17 ± 0.02	0.21 ± 0.06

Table 4.4. Trifluralin desorption parameters. The desorbing fractions (F_{rap}, F_{slow}, F_{vs}; %) and their corresponding desorption rate constants (k_{rap}, k_{slow}, k_{vs}; 10^{-3}/h) are shown as the estimated value ± asymptotic standard error (± A.S.E.).

		10 mg/kg	40 mg/kg	100 mg/kg	200 mg/kg	Pooled data
Sediment	**Parameter**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Lake Erie	F_{rap}	41.7 ± 1.01	53.1 ± 0.91	54.9 ± 1.27	44.0 ± 0.78	49.1 ± 2.41
	F_{slow}	17.0 ± 0.85	14.3 ± 0.79	14.6 ± 1.06	21.6 ± 0.77	16.2 ± 2.15
	F_{vs}	41.2 ± 0.57	32.6 ± 0.58	30.5 ± 0.74	34.4 ± 0.79	34.7 ± 1.95
	k_{rap}	430 ± 24.4	449 ± 18.9	500 ± 35.7	387 ± 18.8	429 ± 56.8
	k_{slow}	25.6 ± 3.21	26.1 ± 3.89	25.5 ± 4.79	15.2 ± 1.48	19.4 ± 7.23
	k_{vs}	0.28 ± 0.03	0.29 ± 0.03	0.25 ± 0.05	0.24 ± 0.04	0.26 ± 0.10
Lake Huron	F_{rap}	39.7 ± 0.99	45.0 ± 1.05	48.5 ± 1.62	50.8 ± 0.69	46.6 ± 1.47
	F_{slow}	18.2 ± 0.83	16.3 ± 0.88	16.6 ± 1.36	15.5 ± 0.58	16.2 ± 1.25
	F_{vs}	42.0 ± 0.58	38.7 ± 0.60	34.8 ± 1.00	33.7 ± 0.43	37.2 ± 0.94
	k_{rap}	701 ± 43.0	661 ± 40.9	571 ± 50.0	576 ± 22.6	598 ± 49.3
	k_{slow}	31.8 ± 3.75	30.4 ± 4.22	26.8 ± 5.79	26.3 ± 2.63	27.0 ± 5.53
	k_{vs}	0.42 ± 0.03	0.35 ± 0.03	0.34 ± 0.06	0.30 ± 0.02	0.35 ± 0.05

Table 4.5. Goodness of fit indicators for the desorption parameters of fluoranthene and trifluralin[a].

Sediment	Concentration (mg/kg)	Fluoranthene		Trifluralin	
		RSS	R^2	RSS	R^2
Lake Erie	10	0.006	0.994	0.002	0.998
	40	0.001	0.998	0.001	0.999
	100	0.001	0.999	0.004	0.997
	200	0.002	0.998	0.002	0.998
	Pooled data	0.092	0.977	0.314	0.943
Lake Huron	10	0.002	0.998	0.003	0.998
	40	0.001	0.999	0.003	0.997
	100	0.005	0.995	0.008	0.994
	200	0.002	0.998	0.001	0.999
	Pooled data	0.308	0.928	0.125	0.977

[a]See Tables 4.3 and 4.4 for the values of the fitted parameters.

Table 4.6. Comparison of fits for the fluoranthene desorption data measured in Lake Erie sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe all four concentrations (10, 40, 100 and 200 mg FLU/kg dry sediments). All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are: No. param. = number of parameters fitted; No. obs. = number of observations; *df* = degrees of freedom; RSS = residual sum-of-squares; RMS = residual mean square; ΔRSS = change in RSS; MS = mean square; Var. Ratio = variance ratio; *p* = *p*-value from the F-distribution; Signif. = significance (* = significant, *p* <0.05; ns = no significant difference).

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	21	148	127	0.01066	
(G)	Common F_{vs}	21	148	127	0.01153	
(F)	Common k_{slow}	21	148	127	0.01049	
(E)	Common F_{slow}	21	148	127	0.01193	
(D)	Common k_{rap}	21	148	127	0.01060	
(C)	Common F_{rap}	21	148	127	0.01288	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	148	142	0.09156	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	24	148	124	0.01028	0.00008

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	18	0.08128	0.00452	54.46779	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	3	0.00260	0.00087	10.45396	<0.00001	*
(D) - (A)	test of invariant k_{rap}	3	0.00032	0.00011	1.28664	0.28188	ns
(E) - (A)	test of invariant F_{slow}	3	0.00165	0.00055	6.63424	0.00034	*
(F) - (A)	test of invariant k_{slow}	3	0.00021	0.00007	0.84436	0.47205	ns
(G) - (A)	test of invariant F_{vs}	3	0.00125	0.00042	5.02594	0.00252	*
(H) - (A)	test of invariant k_{vs}	3	0.00038	0.00013	1.52789	0.21047	ns

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),18,142} = 1.658$
[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),3,127} = 2.679$
Reject H_0 if $F > F_{crit}$

Table 4.7. Comparison of fits for the trifluralin desorption data measured in Lake Erie sediments. An *F*-test was used to test the hypothesis that common parameter estimates described all four concentrations (10, 40, 100 and 200 mg FLU/kg dry sediments). All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	21	148	127	0.01022	
(G)	Common F_{vs}	21	148	127	0.01928	
(F)	Common k_{slow}	21	148	127	0.01099	
(E)	Common F_{slow}	21	148	127	0.01379	
(D)	Common k_{rap}	21	148	127	0.01105	
(C)	Common F_{rap}	21	148	127	0.01927	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	148	142	0.31447	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	24	148	124	0.01014	0.00008

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	18	0.30433	0.01691	206.75499	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	3	0.00913	0.00304	37.21631	<0.00001	*
(D) - (A)	test of invariant k_{rap}	3	0.00091	0.00030	3.70940	0.01341	*
(E) - (A)	test of invariant F_{slow}	3	0.00365	0.00122	14.87837	<0.00001	*
(F) - (A)	test of invariant k_{slow}	3	0.00085	0.00028	3.46483	0.01831	*
(G) - (A)	test of invariant F_{vs}	3	0.00914	0.00305	37.25707	<0.00001	*
(H) - (A)	test of invariant k_{vs}	3	0.00008	0.00003	0.32610	0.80648	ns

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),18,142} = 1.658$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),3,127} = 2.679$

Reject H_0 if $F > F_{crit}$

Table 4.8. Comparison of fits for the fluoranthene desorption data measured in Lake Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates described all four concentrations (10, 40, 100 and 200 mg FLU/kg dry sediments). Comparisons were performed at α=0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	21	156	135	0.01122	
(G)	Common F_{vs}	21	156	135	0.02464	
(F)	Common k_{slow}	21	156	135	0.01046	
(E)	Common F_{slow}	21	156	135	0.01139	
(D)	Common k_{rap}	21	156	135	0.01105	
(C)	Common F_{rap}	21	156	135	0.02341	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	156	150	0.30801	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	24	156	132	0.01032	0.00008

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	18	0.29769	0.01654	211.53682	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	3	0.01309	0.00436	55.81008	<0.00001	*
(D) - (A)	test of invariant k_{rap}	3	0.00073	0.00024	3.11240	0.02848	*
(E) - (A)	test of invariant F_{slow}	3	0.00107	0.00036	4.56202	0.00446	*
(F) - (A)	test of invariant k_{slow}	3	0.00014	0.00005	0.59690	0.61810	ns
(G) - (A)	test of invariant F_{vs}	3	0.01432	0.00477	61.05426	<0.00001	*
(H) - (A)	test of invariant k_{vs}	3	0.00090	0.00030	3.83721	0.01126	*

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),18,150}$ = 1.657

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),3,135}$ = 2.679

Reject H_0 if $F > F_{crit}$

Table 4.9. Comparison of fits for the trifluralin desorption data measured in Lake Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates described all four concentrations (10, 40, 100 and 200 mg FLU/kg dry sediments). Comparisons were performed at α=0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	21	156	135	0.02594	
(G)	Common F_{vs}	21	156	135	0.02294	
(F)	Common k_{slow}	21	156	135	0.01566	
(E)	Common F_{slow}	21	156	135	0.01467	
(D)	Common k_{rap}	21	156	135	0.01632	
(C)	Common F_{rap}	21	156	135	0.02126	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	156	150	0.12504	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	24	156	132	0.01553	0.00012

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	18	0.10951	0.00608	51.71110	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	3	0.00573	0.00191	16.23439	<0.00001	*
(D) - (A)	test of invariant k_{rap}	3	0.00079	0.00026	2.23825	0.08667	ns
(E) - (A)	test of invariant F_{slow}	3	0.00086	0.00029	2.43657	0.06742	ns
(F) - (A)	test of invariant k_{slow}	3	0.00013	0.00004	0.36832	0.77598	ns
(G) - (A)	test of invariant F_{vs}	3	0.00741	0.00247	20.99420	<0.00001	*
(H) - (A)	test of invariant k_{vs}	3	0.01041	0.00347	29.49388	<0.00001	*

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),18,150} = 1.657$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),3,135} = 2.679$

Reject H_0 if $F > F_{crit}$

Table 4.10. Times at which 99.9% ($t_{99.9}$) of the sediment-associated fluoranthene and trifluralin will be desorbed from the rapid, slow and very slow desorption compartments[a].

Sediment	Compound	Desorption compartment	Time Scale[b]	Concentration (mg/kg) 10	40	100	200	Pooled
Lake Erie	Fluoranthene	rapid	h	20.3	17.9	16.8	18.3	18.6
			d	0.844	0.744	0.700	0.763	0.775
			y	0.002	0.002	0.002	0.002	0.002
		slow	h	276	270	251	361	322
			d	11.5	11.2	10.5	15.1	13.4
			y	0.032	0.031	0.029	0.041	0.037
		very slow	h	31399	36357	46052	49341	40634
			d	1308	1515	1919	2056	1693
			y	3.58	4.15	5.26	5.63	4.64
	Trifluralin	rapid	h	16.1	15.4	13.8	17.9	16.1
			d	0.670	0.641	0.576	0.744	0.670
			y	0.002	0.002	0.002	0.002	0.002
		slow	h	270	265	270	455	357
			d	11.2	11.0	11.3	18.9	14.9
			y	0.031	0.030	0.031	0.052	0.041
		very slow	h	24671	23820	27631	28782	26568

			d	1028	992	1151	1199	1107
			y	2.82	2.72	3.15	3.29	3.03
Lake Huron	Fluoranthene	rapid	h	13.3	11.8	11.7	13.8	13.3
			d	0.553	0.491	0.487	0.575	0.555
			y	0.002	0.001	0.001	0.002	0.002
		slow	h	221	202	219	221	245
			d	9.20	8.42	9.14	9.23	10.2
			y	0.025	0.023	0.025	0.025	0.028
		very slow	h	25584	30034	36357	40634	32894
			d	1066	1251	1515	1693	1371
			y	2.92	3.43	4.15	4.64	3.76
	Trifluralin	rapid	h	9.85	10.4	12.1	12.0	11.6
			d	0.411	0.435	0.504	0.500	0.481
			y	0.001	0.001	0.001	0.001	0.001
		slow	h	217	227	258	262	256
			d	9.06	9.48	10.8	10.9	10.7
			y	0.025	0.026	0.029	0.030	0.029
		very slow	h	16447	19736	20317	23026	19736
			d	685	822	847	959	822
			y	1.88	2.25	2.32	2.63	2.25

[a]Calculated from the values of k_{rap}, k_{slow} and k_{vs} using Equations 4.2-4.4.
[b]Time scales: h, hours; d, days; y, years.

Figure 4.1. Plots of the fractional mass (S_t/S_0) **(A)** fluoranthene and **(B)** trifluralin in spiked Lake Erie sediments versus desorption time. Measurements are indicated by symbols. Error bars represent standard deviation of three samples. Solid lines represent best-fit model results.

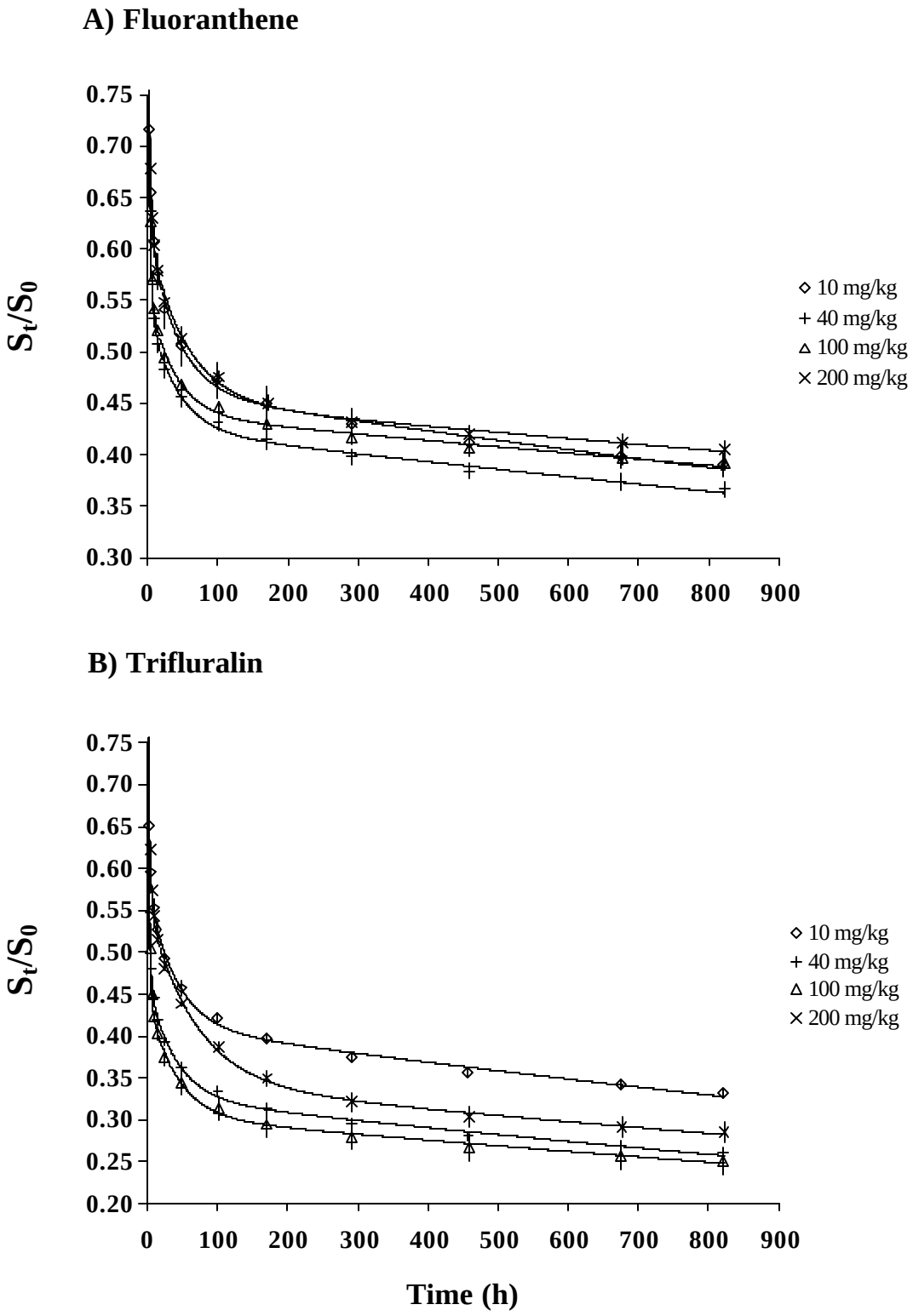
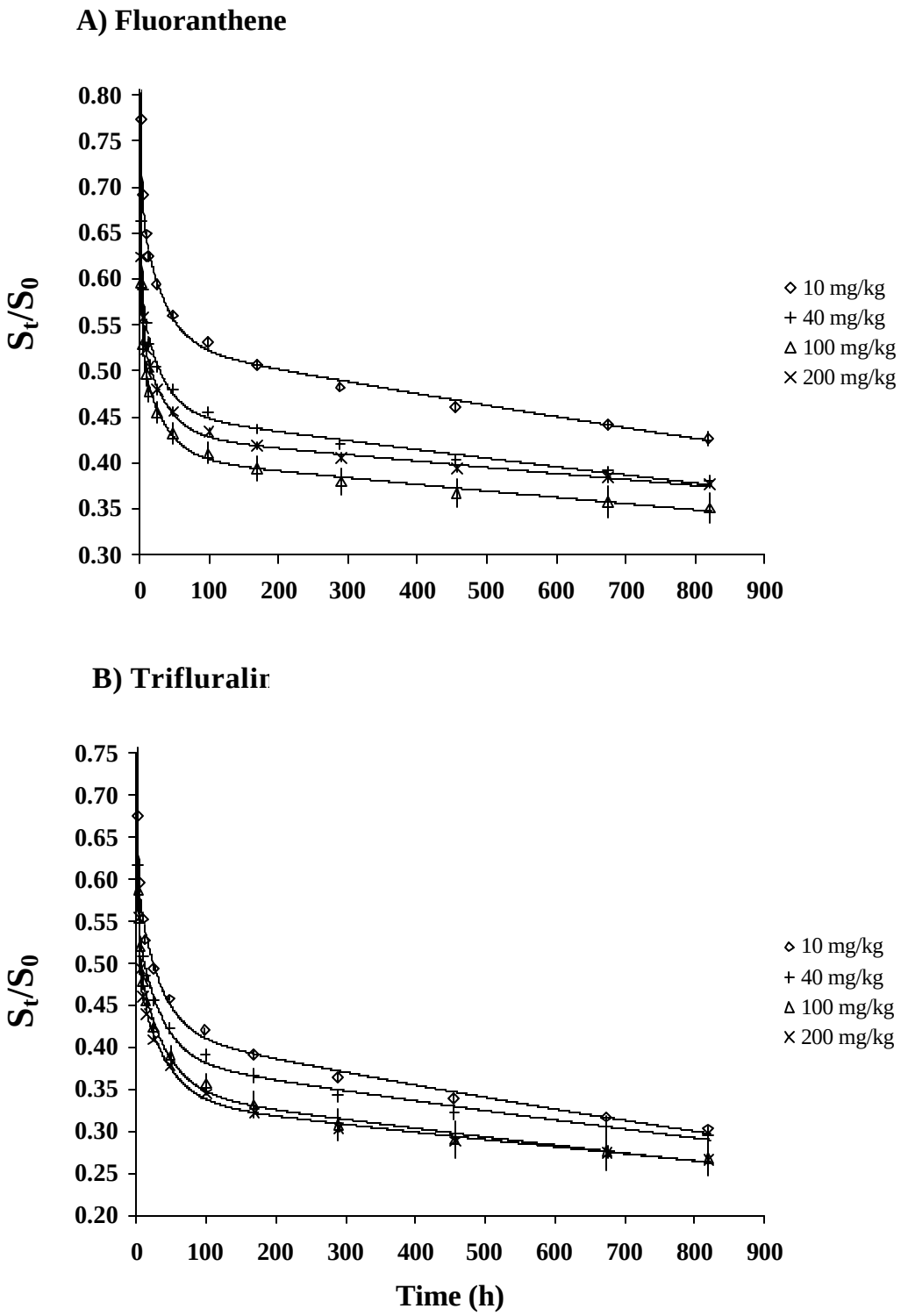


Figure 4.2. Plots of the fractional mass (S_t/S_0) of **(A)** fluoranthene and **(B)** trifluralin in spiked Lake Huron sediments versus desorption time. Measurements are indicated by symbols. Error bars represent standard deviation of three samples. Solid lines represent best-fit model results.



CHAPTER 5

Optimizing Interpretation of *In Situ* Effects of Riverine Pollutants: Impact of Upwelling and Downwelling

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Environmental Toxicology and Chemistry 21: 289-297, 2002

ABSTRACT

In situ toxicity and bioaccumulation tests with *Ceriodaphnia dubia* (48-h), *Chironomus tentans* (96-h), *Hyalella azteca* (96-h) and *Lumbriculus variegatus* (96-h) were conducted at three stations on a river that was contaminated primarily with chlorobenzenes (CBs) and results were compared to a nearby reference site. Exposures were characterized by: 1) using mini-piezometers for contaminant profiling and determination of hydraulic heads and vertical flow direction within the sediments, and 2) measuring contaminants in sediment, surface water and exposure chamber water samples. Localized zones of upwelling and downwelling existed in the exposure areas at contaminated sites 5 and 18, while site 23 was downwelling at all measurement positions. Pore water samples from mini-piezometers contained CBs at the three contaminated sites which were highest at site 23. However, sediment and water samples from

exposure chambers at site 23 contained the lowest levels of CBs among the contaminated sites. CBs were not detected at the reference site, but other organic contaminants and metals were detected at all sites with the highest concentrations occurring at sites 5 and 18. In water column exposures, there were no significant ($p > 0.05$) differences in species survival between the contaminated sites and the reference. Mean percent survival of *H. azteca*, *C. dubia* and *C. tentans* exposed to surficial sediments (SS) at sites 5 and 18 was significantly ($p < 0.05$) reduced compared to the reference, whereas only *C. tentans* survival was significantly reduced at site 23. Body residues of total CB congeners in *L. variegatus* exposed to SS were highest at site 18 (618 µmol/kg lipid) and lowest at site 23 (21 µmol/kg lipid). The data suggest that downwelling reduced the bioavailability, of CBs in surficial sediments most likely by mobilizing the freely-dissolved and colloid-bound fractions to deeper sediments. Overall, downwelling conditions reduced the *in situ* exposure of organisms in surficial sediments, and hence, the toxicity and bioaccumulation of CBs. Hydrologic and chemistry data from nested mini-piezometers improved the interpretation of exposure-effects relationships.

Keywords: Upwelling, Downwelling, Sediments, *In situ* testing, Chlorobenzenes

INTRODUCTION

Sediment contamination in rivers and streams receiving inputs from industrial, agricultural and municipal sources is a persistent problem that places the health of these aquatic ecosystems at risk (USEPA, 1998a). There are many

approaches currently used to assess and manage contaminated sediments and most are a component of the "sediment quality triad" (Chapman, 1990). Chemical benchmarks or sediment quality guidelines (SQGs) have been developed for the screening of contaminated sediments (MacDonald et al., 2000). In more comprehensive studies, surveys of benthic macroinvertebrate and fish community structure are used to further evaluate sediment quality (La Point and Fairchild, 1990). And, laboratory and *in situ* methods for a variety of freshwater invertebrates have been developed to assess the toxicity and bioaccumulation of sediment-associated contaminants and non-contaminant stressors (ASTM, 1995b; Ireland et al., 1996; Chappie and Burton, 1997; USEPA, 2000a). Although these chemistry-, community- and toxicity-based approaches to evaluating sediment contamination and its potential effects to biota are useful, there is a lack of information in the literature regarding hydraulic exchange, such as groundwater-surface water (GW-SW) interaction, and its relationship to sediment toxicity.

River and stream locations where GW-SW interactions occur can be identified by detecting areas of upwelling and downwelling. The sediments in these transitional zones (TZ), where either pore water or groundwater discharges to surface water (upwelling) or where surface water flows downward into the sediment bed (downwelling), play important roles in lotic ecosystem processes including nutrient cycling, retention and storage (Valett et al., 1997), organic matter processing (Storey et al., 1999), and serving as refugia or sources of organism recolonization following disturbance or stress events (Ward et al.,

1998). Because biological and physicochemical conditions within groundwater, surface water and the TZ are different, upwelling or downwelling conditions may affect the fate, dynamics and hence bioavailability of sediment-associated contaminants by either mobilizing aqueous phase contaminants or affecting partitioning. For example, changes in pH may affect the binding of metals (Benner et al., 1995) whereas the rate and extent of microbial processing of sediment organic matter may alter the partitioning of persistent organic contaminants (USEPA, 2000b).

There are a number of situations in which knowledge of GW-SW interactions would be useful in evaluations of the *in situ* exposure and toxicity of sediment-associated contaminants. Upwelling conditions can lead to exposure of benthos and surface water biota if either or both the groundwater and sediments are contaminated. Aqueous phase chemicals (*e.g.,* freely dissolved, colloid-bound) in the upward flowing groundwater and/or the mobilization of sediment-bound contaminants by upwelling groundwater are potential inputs to surficial environments. Downwelling surface water can affect benthic, hyporheic (TZ-associated) and phreatic (groundwater-associated) biota if surface waters and sediments are contaminated. Under such conditions, contaminants can be transported to deeper layers within the streambed and groundwaters. Concern over these scenarios has recently emerged as an issue in the scientific and regulatory community (USEPA, 2000b).

The objectives of the present study were to: 1) evaluate the role of upwelling and downwelling on sediment toxicity and bioaccumulation observed *in*

situ, 2) increase our ability to measure exposure accurately, and 3) improve interpretation of complex field data. This was accomplished through a study design that combined hydrological measurements using mini-piezometers for the detection of upwelling groundwater and downwelling surface water on a local scale (*i.e.,* cm to m), depth-integrated sampling of pore water for chemical profiling, and *in situ* exposures of *Ceriodaphnia dubia, Hyalella azteca, Chironomus tentans* and *Lumbriculus variegatus*.

METHODS

Study sites

Discharges from a former woolen mill located on the East Sebasticook River (ESR) in Corinna, ME, USA were the source of chlorinated benzenes in the aquifer and bed sediments between Corundel Lake and Lake Sebasticook. In the area of concern, the river is lined by a moderate riparian zone, the streambed consists of sediments ranging from coarse sand to cobble overlying bedrock, and the surrounding area is predominantly residential and agricultural. Three separate locations were chosen for *in situ* evaluations of toxicity and bioaccumulation, physicochemical characterization and hydrological measurements. Site 5 was located at the outfall of the former mill and sites 18 and 23 were 96 and 130 m downstream of the mill, respectively. The reference site (Pristine), with streambed characteristics similar to the ESR, was located approximately 56 km from the contaminated sites on a tributary of Kingsbury

Stream, Abbot Village, ME. The study took place during the week of August 30, 1999.

Test organisms

Culturing methods for *C. dubia, C. tentans, H. azteca* and *L. variegatus* followed protocols recommended by the U.S. EPA as reported in previous studies from our laboratory (Ireland et al., 1996; Chappie and Burton, 1997; USEPA, 1994, 2000a). The organisms were reared at 24 °C in diluted well-water that was adjusted to the appropriate hardness for each species with Milli-Q® water (Millipore, Bedford, MA, USA). The organisms were transported to the test site by overnight courier. The ages of the *C. dubia, C. tentans* and *H. azteca* used for *in situ* toxicity testing were 24 h post-hatch, 8 to 12 d post-hatch (second to third instar), and less than 14 d old, respectively.

In situ exposure chambers

In situ chambers were constructed of transparent core tubing (cellulose acetate butyrate, 6.67-cm ID, 6.98-cm OD, 0.16-cm wall thickness) cut to a length of 12.7 cm. Polyethylene closures capped each end. Two rectangular windows (4 x 8 cm) were cut on each core tube opposite each other and covered with polypropylene mesh (74-µm). *In situ* chambers exposed to the overlying water column (WC chambers) were tethered inside wire baskets. One polyethylene cap of each WC chamber was equipped with a 0.3-cm ID water sampling tube that was covered with mesh on the end extending into the chamber. Organisms were exposed to the surficial sediments (top 2-4 cm) using

chambers that were identical to the water column *in situ* chambers except for the addition of inlet (0.9-cm ID) and outlet (0.3-cm ID) ports constructed of plastic tubing equipped with pinch clamps. The outlet port functioned as a water sampling tube as described above. Surficial sediment (SS) chambers were buried to approximately half their depth into the streambed and left to equilibrate to surrounding conditions for 24-36 h prior to organism addition via the inlet tube. Chambers were installed such that one mesh window was embedded in the surficial sediments and the opposite widow was exposed to the overlying water column. These designs provided specific compartmentalized exposures.

***In situ* toxicity and bioaccumulation**

In situ evaluations of toxicity and bioaccumulation included three sites with chlorobenzene (CB) contamination (sites 5, 18 and 23), a field reference (Pristine), and a laboratory control for each test species. Quadruplicates of the two *in situ* treatments (WC and SS exposures) were deployed containing the test organisms. *H. azteca, C. tentans* and *L. variegatus* were exposed for 96 h, and *C. dubia* were exposed for 48 h. Ten *H. azteca, C. tentans* and *C. dubia*, and 2 g of *L. variegatus* were placed in each replicate. *C. tentans* and *H. azteca* were grouped together while *C. dubia* and *L. variegatus* were exposed individually in separate chambers. Test organism transport to the test sites, acclimation to site conditions, addition to exposure chambers and exposure termination procedures were as described elsewhere (Ireland et al., 1996; Chappie and Burton, 1997; Burton et al., *in press*).

On August 29, 1999 SS chambers were installed at all field sites and on August 30, 1999 (time 0), water column *in situ* chambers were deployed and organisms were added to all chambers at all field locations. During the exposure period, stream conditions were at base flow. Water quality parameters were measured at test initiation then again at test termination at each field site, including conductivity (µS/cm), pH, alkalinity (mg/L $CaCO_3$), hardness (mg/L $CaCO_3$) and ammonia (mg/L) (Table 5.1). Temperature (°C) and dissolved oxygen (mg/L) and were measured daily beginning at –24 h.

Laboratory controls were maintained at the field laboratory. Test water for laboratory controls was 20% diluted mineral water (hardness ~100-150 mg/L $CaCO_3$) prepared on site with bottled Perrier® water and distilled water. Percent survival in these controls was 90.0 ± 8.2, 87.5 ± 9.6 and 72.5 ± 5.0% for *H. azteca, C. dubia* and *C. tentans*, respectively. These laboratory controls verified that the organisms used for *in situ* toxicity testing were healthy and were within established test acceptability criteria for toxicity tests (USEPA, 2000a).

Hydrological measurements using mini-piezometers

To detect areas of upwelling and downwelling, nested mini-piezometers were installed at the test sites on 29 August 1999 following established methods (Lee and Cherry, 1978). Briefly, mini-piezometers were comprised of lengths of 0.3-cm ID plastic tubing that was perforated and screened with 300-µm mesh along the bottom 5 cm. A nest is a cluster of four mini-piezometers of specific lengths attached to a 1-m dowel rod that will sample at various levels (*e.g.,* 20, 40, 60, 80 cm) beneath the sediment surface. Installation was accomplished by

hammering a plugged 2-cm ID galvanized steel pipe into the sediment bed, inserting a mini-piezometer nest and then withdrawing the pipe slowly to allow the cavity to fill in around the nest. Three nests were installed at *in situ* testing locations as close to the exposure chambers as possible (within 10-15 cm) and all nests were within 1 m from one another.

Mini-piezometers at sites 5, 18, 23 and Pristine were measured on 1 September 1999. Hydraulic heads (Δh; in cm) were determined with a manometer by measuring the heights of water columns drawn simultaneously from the inserted mini-piezometer and overlying surface water (Bouton, 1993). Relative to surface water, a positive or negative Δh indicates an upwelling or downwelling zone, respectively. Vertical hydraulic gradient (VHG) was calculated by normalizing hydraulic head difference to the sampling depth of the mini-piezometer using the following relationship:

$$VHG = \Delta h/L, \quad (1)$$

where Δh = hydraulic head (cm) and L = depth to the top of the piezometer screen in the sediment bed (cm). The VHG is another indicator of the vertical direction of ground water or interstitial water flow within the sediment bed (*i.e.,* upwelling or downwelling) and it gives relative driving forces of such flows.

Chemical sampling

Samples of surface water were collected from each site for analysis of semivolatile and volatile organic compounds (S/VOCs), and total metals. Water from within *in situ* exposure chambers was sampled on days 0, 2, and 4 for detection of chlorobenzenes (CBs) and other VOCs, and total metals by

unclipping the end-cap sampling port and withdrawing approximately 40 mL with a sterile 60-mL syringe. Pore water samples for VOC analysis were withdrawn from installed mini-piezometers at each site prior to the determination of hydraulic head. This strategy prevented dilution of the potentially contaminated pore water surrounding each mini-piezometer screen since the measurement of hydraulic head using a manometer requires pumping large volumes of water through the apparatus. Samples were obtained from each mini-piezometer by first purging the tube and then slowly withdrawing 2 x 40 mL aliquots of hyporheic water with a 60-mL syringe.

Aqueous samples were placed into duplicate 40-mL vials with Teflon®-lined septa and preserved with HCl for VOC analysis, 1-L amber bottles for SVOCs, and 1-L polyethylene bottles with nitric acid preservative for total metals. Surficial sediments from each site were sampled by scraping the top 2-4 cm with a clean core tube and placed into 250-mL amber bottles with MeOH as a preservative for analysis of SVOCs and into 500-mL polyethylene jars for metals analysis. Water and sediment samples were extracted and analyzed to determine the concentrations of VOCs, SVOCs, pesticides and PCBs, and total metals by following EPA Methods 1668, 5030B, 5035, 8021B, 8260B (USEPA, 1997, 1998b), and by methods described in EPA's Contract Laboratory Program Statement of Work (http://www.epa.gov/superfund/programs/clp/methods.htm).

The concentrations of CBs accumulated by *L. variegatus* during *in situ* exposures were measured. The surviving worms in the four replicates for each treatment were pooled into a clean beaker of culture water for a short period

(≤ 6 h) to allow for gut purging, wet weighed and placed into amber, screw-cap vials with Teflon®-lined caps. CB concentrations in tissues were determined from 1-g samples using methanol extraction-sonication followed by VOC analysis using EPA Method 8260B (USEPA, 1998b). Lipid contents of tissues were quantified gravimetrically following extraction with hexane (Randall et al., 1998). Body residues of CBs measured in *L. variegatus* were calculated by converting the mass-based concentrations of each congener group to molar-based concentrations.

Data analysis

Test organisms were exposed in both WC and SS chambers. For *C. dubia, H. azteca* and *C. tentans*, mean percent survival and standard deviation were calculated. Survival from exposures at the contaminated sites was compared to the reference site (Pristine). Data met assumptions of normality and homogeneity of variance and were subjected to analysis of variance (ANOVA), followed by pairwise comparisons via Dunnett's test (*C. dubia*) or Bonferroni's t-test (*H. azteca, C. tentans*) using Toxstat® programs (WEST, 1994). CB concentrations measured in exposure chambers were evaluated with ANOVA followed by Tukey's Test to determine statistically significant differences ($p < 0.05$) using Statistica® programs (Statsoft, 1997).

RESULTS

In situ **toxicity**

In situ toxicity tests were conducted with three species and the results are shown in Figure 5.1. Mean percent survival of *C. dubia* (48-h), *H. azteca* (96-h) and *C. tentans* (96-h) in WC exposures was not significantly ($p > 0.05$) reduced at any of the contaminated sites compared to the field reference (Pristine). Mean percent survival of SS-exposed *C. dubia* was significantly ($p < 0.05$) reduced at contaminated sites 5 and 18 compared to the Pristine site; but was high (> 80%) and not significantly different ($p > 0.05$) than Pristine at site 23. Complete mortality (100%) was observed in *H. azteca* exposed to surficial sediments at sites 5 and 18. Mean percent survival of *C. tentans* in SS exposures at all three contaminated sites was significantly ($p < 0.05$) lower than at Pristine.

Dissolved oxygen (DO) levels measured within *in situ* chambers from sites 5, 18 and 23 at the end of the 96-h exposures were 2.3, 3.1 and 3.3 mg/L, respectively, for WC exposures and were 1.2, 1.8 and 1.7 mg/L, respectively, for SS treatments. DO levels at Pristine were 5.87 mg/L in WC chambers and 5.03 mg/L in SS exposures. These low DO levels could have been an additional stressor to the test species during the exposure period. However, survival for *H. azteca*, *C. tentans* and *C. dubia* was high for all WC exposures and for SS exposures at site 23 which suggests that chemical contamination rather than low DO was the primary stressor below the mill.

Hydrological measurements and pore water chemistry using mini-piezometers

Mini-piezometer measurements of interstitial water head pressures (Δh) are shown in Figure 5.2. Site 5 contained localized zones of upwelling and downwelling around the *in situ* chambers and head pressure differences ranged from absolute differences of 0.2-0.4 cm. Downwelling was detected below 50 cm depth in the sediment. The vertical hydraulic gradient (VHG) ranged from –0.008 to +0.040 cm/cm. Site 18 mini-piezometer measurements indicated the presence of both upwelling and downwelling zones. No exchange of groundwater with surface water was detected in nest A, whereas upwelling and downwelling were shown by nests B and C, respectively, with absolute differences of head pressures ranging from 0.4-0.6 cm. VHG ranged from –0.024 to +0.012 cm/cm. All mini-piezometer nests installed at site 23 indicated that the sample locations surrounding *in situ* chambers were downwelling. These mini-piezometers characterized the stream bed from 10-40 cm depths and indicated absolute head pressure differences of 0.2-0.6 cm and VHG ranging from –0.008 to –0.017 cm/cm. At Pristine, mini-piezometer nests A, B and C all showed upwelling conditions.

Chlorobenzene (CB) concentrations in pore water samples from mini-piezometer nests are summarized in Table 5.2. Mean ± SD concentrations of total CB in samples from sites 5, 18 and 23 were 2,048 ± 1,442, 4,662 ± 1,674 and 16,859 ± 22,923 μg/L, respectively. The highest concentration measured for a congener at site 5 was 2,000 μg/L for 1,2,4-triCB. 1,4-diCB was the highest single congener detected at sites 18 (2,300 μg/L) and 23 (17,000 μg/L). Levels

of all CB congeners were below the limits of detection in mini-piezometer samples from the Pristine reference site.

Other analytes detected in mini-piezometer samples from site 5 were benzene (range 7.3-42 μg/L; all samples), ethylbenzene (540 μg/L; 1 sample) and toluene (11 μg/L; 1 sample). Benzene was detected in all mini-piezometers from site 18 (range 2.5-14 μg/L). Other analytes in samples from site 23 included benzene (≤ 9.5 μg/L; 2 samples), ethylbenzene (20 μg/L; 1 sample) and tetrachloroethane (1.7 μg/L; 1 sample).

Contaminants in sediments and surface water

Concentrations of polycyclic aromatic hydrocarbons (PAHs), organic pesticides and total metals in sediment samples (*n* = 1 per site) are shown in Table 5.3. The only CB congener that was detected in sediment solids was 1,2,4-triCB at 56, 44 and 21 μg/kg dry weight (dry wt) at sites 5, 18 and 23, respectively. 4-Methylphenol (56 μg/kg dry wt) and dibenzofuran (18 μg/kg dry wt) were detected at site 5. Sediments at site 18 also contained 2-methylnaphthalene (15 μg/kg dry wt) and dibenzofuran (37 μg/kg dry wt). Polychlorinated biphenyls were not detected in sediment samples.

Surface water samples (*n* = 3) from site 18, the midpoint of the contaminated zone on the ESR, were taken on days 0, 2 and 4 of the study and analyzed for contaminants. The results were highly variable. The mean ± SD concentrations (μg/L) of CB congeners were: 37.5 ± 20.5 (monoCB), 23.9 ± 21.4 (1,2-diCB), 11.6 ± 14.7 (1,3-diCB), 22.8 ± 22.9 (1,4-diCB), 0.94 ± 0.65 (1,2,3-

triCB), and 48.8 ± 61.2 (1,2,4-triCB). Pesticides detected at site 18 included α-lindane, γ-lindane and endosulfan II at concentrations of 2.1, 1.0 and 1.2 ng/L, respectively. The mean ± SD concentrations of total metals detected at site 18 were 22.9 ± 14.5, 5.7 ± 5.23 and 267.3 ± 426.7 μg/L for Cu, Ni and Zn, respectively. At the reference site, Pristine, endosulfan II (1.4 ng/L) and total metals including Ni (5.3 μg/L) and Zn (5.5 μg/L) were detected in a surface water sample (n = 1). CBs were not detected at Pristine.

Contaminant exposure levels within *in situ* chambers

The aqueous concentrations of CBs within *in situ* chambers are shown in Figure 5.3. These contaminant levels represent the actual or real exposures to the test organisms. CBs were below the limits of detection in both WC and SS exposure chambers at Pristine. There were no significant (p > 0.05) differences between mean concentrations of total CBs in WC exposures at sites 5, 18 and 23. In comparisons between WC and SS exposures, mean total CBs were significantly (p < 0.05) higher in SS chambers at sites 5 and 18. Across the contaminated sites, total CBs in SS chambers were significantly (p < 0.05) lower at site 23 than at sites 5 and 18.

Hardness-adjusted water quality criteria (WQC) (USEPA, 1987) for the total metals concentrations of copper (Cu) and lead (Pb) were exceeded in both the WC and SS *in situ* chambers. Exposure concentrations of Cu exceeded the acute WQC in both treatments at all study sites and were 22.0 ± 3.2, 22.6 ± 7.4, 14.5 ± 1.4 and 11.7 ± 4.7 μg/L in WC chambers at sites 5, 18, 23 and Pristine, respectively; SS chamber concentrations of Cu were 30.0 ± 8.68, 23.5 ± 5.1,

16.8 ± 2.9 and 13.7 ± 0.1 μg/L, respectively. Pb exceeded the chronic WQC in WC exposures at site 18 only (6.6 ± 0.0 μg/L) and in SS exposures at sites 5, 18 and 23 (8.8 ± 8.1, 7.7 ± 3.4 and 4.8 ± 1.0 μg/L, respectively).

Tissue concentrations of chlorobenzenes

Accumulation of CB congeners by the oligochaete, *Lumbriculus variegatus*, after 4-d *in situ* exposures is shown in Figure 5.4. For worms exposed in WC exposure chambers, the highest total CB levels (75.7 μmol/kg lipid) were observed at site 5, followed by site 18 (33.2 μmol/kg lipid) and site 23 (19.8 μmol/kg lipid). For *L. variegatus* exposed to surficial sediments, the highest total CB levels (618.1 μmol/kg lipid) were observed at site 18, followed by site 5 (276.5 μmol/kg lipid) and site 23 (21.0 μmol/kg lipid). CBs were not detected in tissue samples from the Pristine reference site. 1,4-diCB represented the highest proportion of the total CB body burden in 5 of the 8 pooled samples.

1,4-diCB was the only congener detected in the tissues of *L. variegatus* exposed to WC at site 18 and to both treatments at site 23. MonoCB was a large portion of the total CB tissue burden for worms exposed to surficial sediments at site 5.

DISCUSSION

In situ toxicity

In situ testing was both effective and sensitive at sites containing contaminated sediments on the East Sebasticook River (ESR). *C. dubia* and

H. azteca did not survive the surficial sediment (SS) exposures at sites 5 and 18, and *C. tentans* mean percent survival increased in SS exposures at sites away from the mill suggesting that adverse effects were due to sediment-associated sources. However, pore water measurements indicated that chlorobenzene (CB) contamination was highest at site 23 where toxicity and bioaccumulation were lowest among the test species. It was only after evaluation of the chemical and survival data in combination with the hydrologic data (which indicated whether site conditions were upwelling or downwelling) that these non-concordances could be explained, and hence the exposure-effects relationships could be elucidated.

Mini-piezometers: Chemical profiling and hydrologic measurements

CB concentrations in samples withdrawn from mini-piezometers installed on the ESR (Table 5.2) show increasing pore water contamination by CBs downstream of the former mill. Mean levels of total CBs at site 23 are higher than sites 5 and 18 by factors of 8.2 and 3.6, respectively. The measured levels of CBs in many pore water samples from mini-piezometers either approached or were above published aqueous concentrations that cause toxicity in freshwater macroinvertebrate species including *D. magna*, *C. dubia*, and *Chironomus riparius*. These toxicity values were recently reviewed by Fuchsman et al. (1999). For example, 10th percentile 48-h LC_{50} (acute toxicity) values for diCB are 2,100 and 12,000 µg/L for *D. magna* and *C. riparius*, respectively, and the 16-d EC_{50} (chronic toxicity) value is 1,400 µg/L for *D. magna*. For triCB, the 10th percentile 48-h LC_{50}s are 1,820 and 1,700 µg/L for *D. magna* and *C. riparius*,

respectively. Concentrations in pore water from mini-piezometers exceeded these acute and/or chronic values for 1,4-diCB at sites 18 and 23, 1,2-diCB and 1,2,3-triCB at site 23, and 1,2,4-triCB at all three contaminated sites below the mill. Therefore, where upwelling conditions occurred, the potential for sediment- and pore water-associated organisms (*e.g.,* benthos, hyporheos) to encounter toxic levels of these CBs existed at the contaminated sites below the mill.

The placement of mini-piezometer nests at test locations within centimeters of the exposure chambers was an important consideration because it allowed us to measure dynamic hydrologic conditions and pore water chemistry at meso-scale resolution (*i.e.,* cm to m), and therefore provided improved characterization of real environmental exposures. The extent of upwelling and downwelling determined by potentiomanometric measurements of mini-piezometers at sites 5, 18 and 23 was subtle. However, vertical hydraulic gradients (VHGs) similar to the values reported in the present study have been reported at other stream sites in the U.S. and these gentle vertical forces were sufficient to significantly affect ecological processes such as the supply and metabolism of dissolved and particulate organic matter, and hyporheic respiration (Hendricks and White, 1995; Jones et al., 1995). Therefore, it is plausible that aqueous phase CBs were mobilized by upwelling or downwelling conditions at contaminated sites on the ESR and that these stream dynamics could have influenced exposures in the surficial sediment environment (*i.e.,* top 2-4 cm).

Upwelling conditions would likely increase the probability of exposure and effects in benthos whereas downwelling would reduce or effectively remove the

bioavailable fraction of contaminants from surficial sediments. For example, pore water CB concentrations were elevated at site 23 but the hydraulic information indicated that downwelling conditions dominated the site. Such a situation would favor the drawing of CBs away from the surficial sediments. Other evidence supporting this conclusion includes observations of high survival at this site (site 23), low bioaccumulation of CBs by *L. variegatus*, lower concentrations of sediment contaminants and low SS chamber CB levels relative to other contaminated sites.

Sediment contaminants and exposures

The only CB congener that was detected in sediment samples was 1,2,4-triCB at 56, 44 and 21 μg/kg at sites 5, 18 and 23, respectively. These levels were below the sediment quality guideline (SQG) for threshold effects in freshwater environments (92 μg/kg) (USEPA, 1996) and the New York State Department of Environmental Conservation (NYDEC) acute toxicity SQG (91 μg/kg) (NYSDEC, 1994) for triCB. However, the concentrations of 1,2,4-triCB in sediments from all three contaminated sites were above the NYDEC SQG for chronic toxicity (9.1 μg/kg) (NYSDEC, 1994). Therefore, the sediment chemistry samples suggest that toxic levels of CBs exist in the ESR sediments below the mill.

A number of sediment-associated contaminants (Table 5.3) exceeded consensus-based numerical SQGs for freshwater environments (MacDonald et al., 2000). The threshold effects concentration (TEC; below which adverse effects are not expected to occur) was exceeded at sites 5, 18 and Pristine for

PAHs including anthracene, phenanthrene, benz[*a*]anthracene, benzo[*a*]pyrene, chrysene, dibenz[*a,h*]anthracene, fluoranthene, pyrene and total PAHs. Probable effects concentrations (PEC; above which adverse affects are expected to occur more often than not) of PAHs were exceeded at site 18 only for anthracene, benz[*a*]anthracene, chrysene, fluoranthene and pyrene. For the metals and pesticides detected, only a few of the measured concentrations were above consensus-based SQGs (MacDonald et al., 2000). Specifically, the PEC for Pb at site 5 and the TECs for As, Cu, Pb, Ni and Zn at site 18 were exceeded. Interestingly, no SQGs for PAHs or metals were exceeded at site 23 (*i.e.,* where surface water was downwelling).

CB and metal concentrations were measured from aqueous samples withdrawn from *in situ* chambers and therefore these values represent expected exposure levels (Figure 5.3). The concentration of total aqueous CBs in SS exposure chambers at site 23 was the lowest among the contaminated sites and this level was similar to total CBs measured in water column (WC) chambers across all sites below the former mill. This observation, taken alone, would have been unexpected given that pore water contamination by CBs was highest at this site (Table 5.2). However, the hydrologic data indicated that pore water and hence CB contamination was moving in a downward direction, not upward toward the SS chambers in the surficial sediments or the WC exposure chambers in the overlying surface water.

Because some sediment-associated PAHs and metals were above SQGs and since Cu and Pb detected within *in situ* exposure chambers exceeded some

WQC values for acute or chronic effects at sites including the reference (Pristine), one may argue that these contaminants may have contributed to the observed toxicity (Figure 5.1). However, survival at Pristine was high in the presence of PAHs and metals, and CBs were not detected at this reference. Thus, we concluded that CBs were the primary chemical stressor causing toxicity at contaminated sites on the ESR. However, because effects on survival were observed *in situ* at aqueous levels of CBs that were nearly an order of magnitude below the laboratory-based effect concentrations reported in Fuchsman et al. (1999), the issue of multiple contaminant stressors is addressed below.

Body residues of chlorobenzenes in *L. variegatus*

The tissue levels of total CBs in *L. variegatus* exposed at site 23 were similar between treatments, which would be expected based on the similarity of the *in situ* exposure chamber levels of total CBs. Bioaccumulation was higher at sites 5 and 18, where upwelling was detected. We conclude that downwelling at site 23 reduced the bioavailability of CBs in the surficial sediments most likely by mobilizing the freely-dissolved and colloid-bound fractions to deeper sediments. This hypothesis is reflected by the lower levels of contaminants in surficial sediments, exposure chambers and tissues at site 23, but the higher pore water concentrations in mini-piezometer samples relative to sites 5 and 18 where upwelling and downwelling were measured.

On a lipid-normalized basis, body residues of total CBs ranged from 19.8 to 618.1 μmol/kg lipid across the contaminated sites of the ESR (Figure 5.4). As mentioned above in *Methods,* the *L. variegatus* samples were not all completely

purged of their gut contents (*i.e.,* ≤ 6 hrs of depuration) prior to tissue CB analysis. Mount et al. (1999) reported that potentially contaminated gut contents can lead to overestimation of bioaccumulation by *L. variegatus*. However, worms exposed at contaminated sites on the ESR were allowed to purge their guts for 6 h, the recommended time for *L. variegatus* bioaccumulation studies (Mount et al., 1999). *L. variegatus* exposed at Prisine (for which no tissue CBs were detected) were collected from the field last and were subjected to the shortest depuration times (ca. 2-4 h). Therefore, incomplete gut purging was not an issue.

The critical body residue (CBR) for neutral, lipophilic chemicals that act by narcosis is the tissue concentration at which mortality will occur in 50% of an exposed population (McCarty et al., 1992a). In aquatic organisms, acute lethality for non-polar narcotic chemicals, including CBs, has been reported for body residues ranging from 0.1 to 8.5 mmol/kg wet weight or 40 to 160 mmol/kg lipid in *D. magna* (Pawlisz and Peters, 1993), amphipods (Landrum et al., 1991), sand crab, *Portunus pelagicus* (L) (Mortimer and Connell, 1994) and fish (van Wezel et al., 1995). The body residues of CBs measured in *L. variegatus* exposed during the present field study were 2-3 orders of magnitude below the published acute CBRs from laboratory studies. We have also observed this in an *in situ* study in a river system containing sediments contaminated by PCBs (unpublished manuscript).

It is possible that *H. azteca* and *C. tentans* had accumulated body residue levels of total CBs that were similar to *L. variegatus*. This accumulation would be

possible because the lipid levels of daphnids (zooplankton, 1.3% by wet weight [Kucklick et al., 1996]; *D. magna*, 7.2% by dry weight [Cauchie et al., 1999]), chironomids (*Chironomus tentans* 3rd and 4th instar larvae, 0.82-1.08% by wet weight [West et al., 1997]) and amphipods (*Acanthogammarus* sp., 1.6% by wet weight [Kucklick et al., 1996]; *H. azteca*, 1.8% by wet weight, [Lotufo et al., 2000]) are within the same range as lipids in the *L. variegatus* used in the present study (0.8-1.6%).

Assuming that *C. dubia*, *H. azteca* and *C. tentans* accumulated CBs to levels similar to those measured in *L. variegatus* as described above, then for these species mortality was observed at body residues in the μmol/kg lipid range. Since *L. variegatus* are tolerant to organic contaminants when compared to other aquatic species (Schuytema et al., 1990; Phipps et al., 1993), it was not surprising that acute effects were not observed in *L. variegatus* whereas mortality occurred in the other test species. Thus, given that tissue residues were 2-3 orders of magnitude below CBR values, it appears that within the mixture of sediment-associated chemicals detected in samples from the ESR, CBs were not acting by narcosis to cause the mortality endpoint. Landrum et al. (1989) observed similar results with amphipods in a study of mixtures of narcotic chlorinated hydrocarbons, and non-narcotic chemicals. It is unknown whether other suspect contaminants in the present study sediments (*e.g.,* PAHs, metals, pesticides) acted additively, antagonistically, or synergistically with the CBs. When xenobiotics exist in contaminated environments, they are often in mixtures with several other chemical classes, so the effective concentrations of individual

compounds are difficult to determine (Burton, 1991). The body residues of CBs accumulated *in situ* were below laboratory-derived values for acute lethality of non-polar narcotics and since the CBs existed in a complex mixture of other chemicals in the study area, it was not unexpected that deleterious effects in aquatic organisms occurred despite lower than acute CBR levels.

Conclusions

In this study, mini-piezometers provided useful information regarding site characteristics and chemical dynamics. Their application to pore water sampling and hydrologic measurements (which indicated whether site conditions were upwelling or downwelling) has improved our ability to interpret often complex exposure-effects relationships that result from *in situ* toxicity tests. We have shown that contaminant concentrations in samples of sediments and pore water were not always predictive of *in situ* chamber exposure levels and observed effects in multiple test species. Furthermore, this was the first field study to demonstrate that downwelling surface water at sites containing contaminated sediments can reduce the bioavailability and exposure to organisms in surficial sediments (top 2-4 cm) and pore water. Hence, bioaccumulation and toxicity were reduced.

Changes in hydrologic conditions such as stream depth, groundwater recharge and the water table level can vary seasonally and with dry periods or storm events. These changes can alter, or even reverse, the upwelling or downwelling vertical flow regime on the local scale. Equilibrium conditions may not exist at these dynamic locations and thus changes in vertical flow and

chemical conditions (e.g., Eh, pH gradients) within the sediments can potentially influence contaminant bioavailability at contaminated sites, leading to toxic impacts on benthic community structure. The relationships between temporal and seasonal changes in vertical hydrology and chemistry, and sediment toxicity need to be evaluated.

Acknowledgement—The assistance of numerous personnel from Harding ESE is greatly appreciated. We thank M. McElroy and J. Heitmeyer for technical assistance in the field, C. Rosiu, P.B. Duncan and P. Landrum for helpful discussions and two anonymous referees for comments that helped improve the paper.

Table 5.1. Characteristics of water quality samples taken from the East Sebasticook River study, Corrina, ME, USA, from August 29 to September 3, 1999.

Location	Temperature (^{o}C)	pH	Conductivity (µS/cm)	DO (mg/L)	Alkalinity (mg/L, $CaCO_3$)	Hardness (mg/L, $CaCO_3$)	Ammonia (mg/L)
Site 5	20.3 ± 1.3	7.2 ± 0.4	131.2 ± 47.6	5.5 ± 0.3	58.0 ± 8.5	87.3 ± 4.6	0.23 ± 0.06
Site 18	20.3 ± 1.7	7.1 ± 0.0	130.0 ± 51.8	5.4 ± 0.5	51.0 ± 1.4	102.6 ± 28.9	0.13 ± 0.06
Site 23	20.5 ± 2.0	7.1 ± 0.2	129.8 ± 50.6	5.3 ± 0.8	50.0 ± 2.8	79.3 ± 9.2	0.23 ± 0.15
Pristine	19.4 ± 2.3	7.0 ± 0.5	26.2 ± 1.2	8.2 ± 0.5	18.0 ± 2.8	22.3 ± 4.6	0.09 ± 0.01
Lab Control	20.7 ± 0.7	—	—	5.9 ± 1.2	—	—	—

Table 5.2. Chlorobenzene concentrations (µg/L) in mini-piezometers at contaminated sites on the East Sebasticook River, Corinna, Maine, USA.

	Site 5 mini-piezometers										
Compound	**A20[a]**	**A40**	**B10**	**B30**	**B50**	**C16**	**C36**	**C56**	**C76**	**Mean**	**SD[b]**
Chlorobenzene	720	1,100	1,100	1,800	1,300	110	500	1,100	320	894	529
1,2-Dichlorobenzene	50	16	8	20	23	33	150	450	170	102	143
1,3-Dichlorobenzene	ND[c]	23	23	210	ND	53	130	410	110	137	138
1,4-Dichlorobenzene	310	250	58	550	260	93	560	1,100	450	403	317
1,2,3-Trichlorobenzene	49	17	11	9	8	180	ND	350	ND	89	131
1,2,4-Trichlorobenzene	ND	ND	ND	ND	ND	ND	1,400	2,000	800	1,400	600
1,3,5-Trichlorobenzene	8	ND	ND	ND	ND	43	ND	ND	ND	25	25
Total Chlorobenzenes	1,137	1,406	1,200	2,589	1,591	512	2,740	5,410	1,850	2,048	1,442

	Site 18 mini-piezometers								
	A28	**A48**	**B28**	**B48**	**B68**	**C30**	**C50**	**Mean**	**SD**
Chlorobenzene	1,600	710	1,100	540	470	750	350	789	432
1,2-Dichlorobenzene	1,603	1,800	550	690	870	760	950	1,032	478
1,3-Dichlorobenzene	220	210	160	360	ND	210	170	222	72
1,4-Dichlorobenzene	1,900	2,300	730	1,300	1,400	1,200	1,400	1,461	505
1,2,3-Trichlorobenzene	ND	520	ND	210	ND	280	ND	337	163
1,2,4-Trichlorobenzene	730	2,200	230	980	980	1,100	1,100	1,046	593
1,3,5-Trichlorobenzene	ND	ND	ND	ND	ND	ND	ND	-	-
Total Chlorobenzenes	6,053	7,740	2,770	4,080	3,720	4,300	3,970	4,662	1,675

	Site 23 mini-piezometers						
	A20	**B20**	**B40**	**C10**	**C30**	**Mean**	**SD**
Chlorobenzene	35	2,900	6,500	170	460	2,013	2,768
1,2-Dichlorobenzene	ND	7,400	14,000	ND	ND	10,700	4,667
1,3-Dichlorobenzene	ND	ND	ND	18	ND	-	-
1,4-Dichlorobenzene	13	9,000	17,000	590	790	5,479	7,431
1,2,3-Trichlorobenzene	ND	2,900	4,000	ND	ND	3,450	778
1,2,4-Trichlorobenzene	ND	8,600	9,200	390	330	4,630	4,937
1,3,5-Trichlorobenzene	ND	ND	ND	ND	ND	-	-
Total Chlorobenzenes	48	30,800	50,700	1,168	1,580	16,859	22,923

[a] A, B or C indicates piezometer location in the sediments; numbers following letters indicates the depth of the mini-piezometer screen (cm).
[b] SD = standard deviation.
[c] ND = not detected.

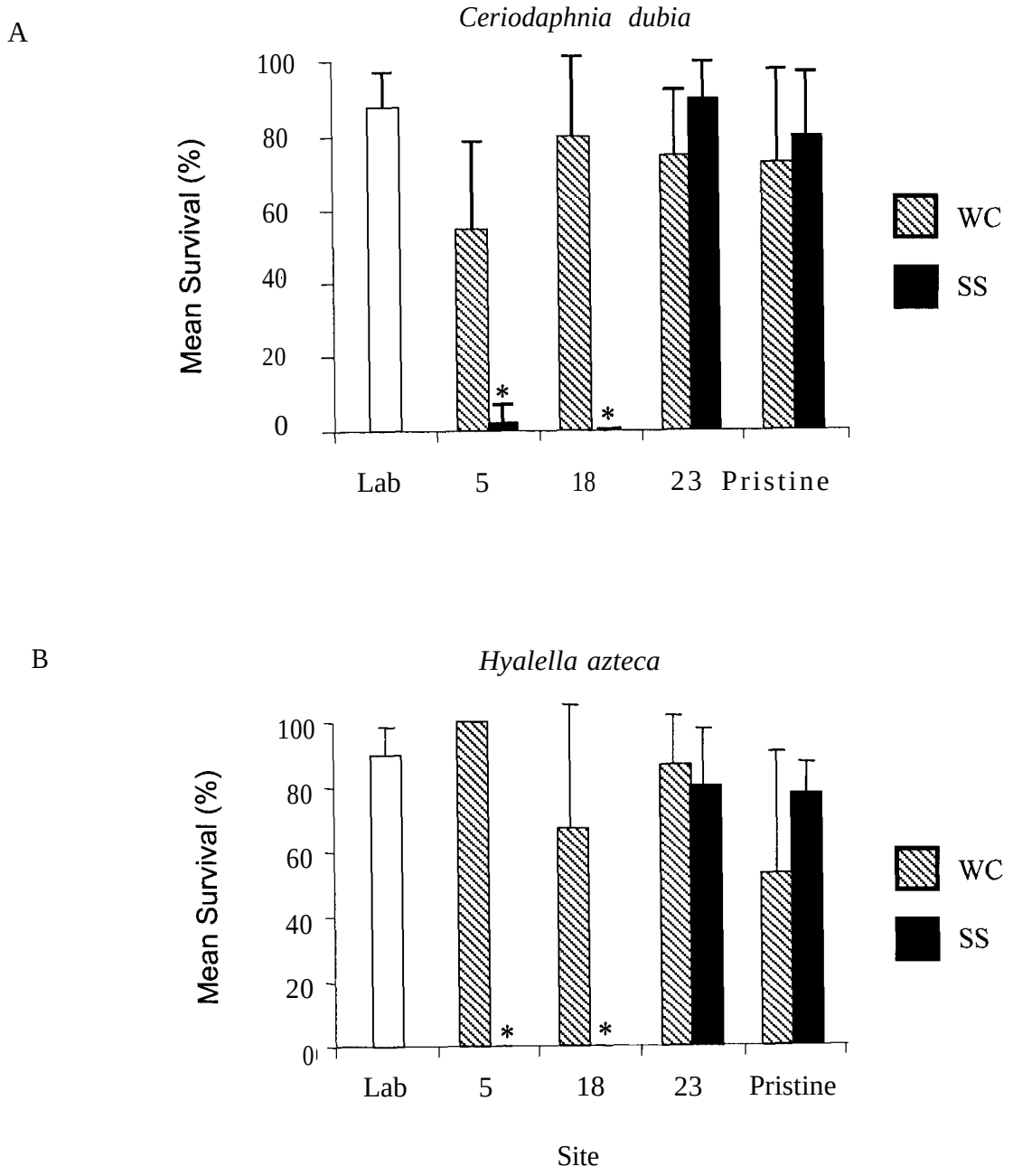
Table 5.3. Polycyclic aromatic hydrocarbon (PAH), pesticide organics, and total metals concentrations in sediments.

	Site			
PAH (mg/kg, dry wt)	**5**	**18**	**23**	**Pristine**
Naphthalene	12	18	ND[a]	ND
Acenaphthylene	160	220	20	160
Acenaphthene	31	53	ND	ND
Fluorene	28	95	ND	30
Phenanthrene	460	1,000	70	390
Anthracene	140	2,400	20	120
Fluoranthene	930	2,400	120	920
Pyrene	950	2,200	120	910
Benzo(a)anthracene	500	1,300	54	540
Chrysene	570	1,500	73	630
Benzo(b)fluoranthene	430	890	53	440
Benzo(k)fluoranthene	470	1,200	49	550
Benzo(a)pyrene	520	1,100	52	540
Indeno(1,2,3-*cd*)pyrene	380	780	37	290
Dibenz(a,h)anthracene	140	300	18	100
Benzo(g,h,i)perylene	460	900	43	310
Total PAHs	6,181	16,356	729	5,930

Pesticide organics (mg/kg, dry wt)				
Aldrin	0.15	0.39	0.34	ND
Heptachlor Epoxide	ND	1.4	1.1	1
Dieldrin	0.39	2.3	0.7	ND
Endrin	0.6	ND	0.99	ND
Endosulfan II	0.84	2.7	0.65	ND
4,4'-DDD[b]	0.74	2.4	0.8	ND
Endosulfan Sulfate	1.2	5.5	8.7	2.7
4,4'-DDT	0.44	1.5	ND	ND
alpha-Chlordane	ND	0.51	0.46	ND
gamma-Chlordane	ND	0.13	ND	0.067
Metals (mg/kg, dry wt)				
As	6.0	13.1	6.0	9.3
Cr	23.3	37.1	19.9	16.6
Cu	15.7	77.1	21.3	5.8
Ni	18.9	73.5	19.6	17.7
Pb	328	56.7	19.7	25.2
Zn	119	391	68.2	121

[a] ND = not detected.
[b] DDD = dichlorodiphenyldichloroethane.

Figure 5.1. Mean percent survival (± 1 standard deviation) of **(A)** *Ceriodaphnia dubia,* **(B)** *Hyalella azteca,* and **(C)** *Chironomus tentans* exposed *in situ* to contaminated sediments at the East Sebasticook River and a nearby pristine reference site (Kingsbury Stream, Corrina, ME, USA). Open bars represents laboratory controls, stippled bars represent water column (WC) exposures and black bars represent surficial sediment (SS) exposures. Asterisks denote significant differences *(p* < 0.05) between treatment responses compared to the pristine reference site.



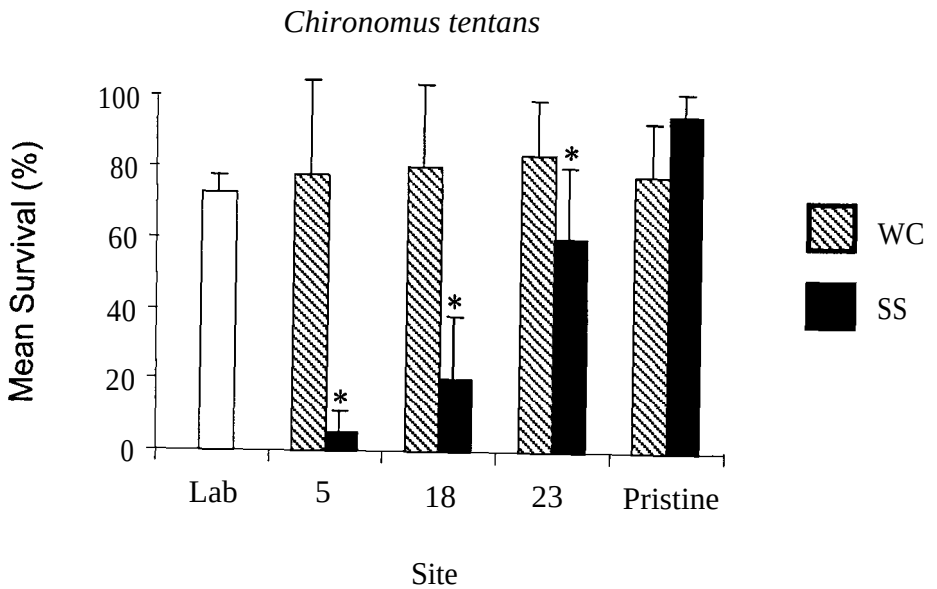

Chironomus tentans
Mean Survival (%)
100
80
60
40
20
0
Lab
5
18
23
Pristine
Site
WC
SS
*
*
*

Figure 5.2. Hydrologic data from mini-piezometers installed at **(A)** Site 5, **(B)** Site 18 and (C) Site 23 on the East Sebasticook River (Corrina, ME, USA), and **(D)** Pristine, a nearby reference site. Mini-piezometer nests are indicated on the x-axis, and the axis can be considered to represent the sediment-water interface. Head pressure difference *(Δh,* cm) between the pore water at the mini-piezometer screen and surface water is indicated on the y-axis. Bar patterns represent the depths of individual mini-piezometer tubes on a given nest. Upward or downward bars indicate upwelling or downwelling flows, respectively.

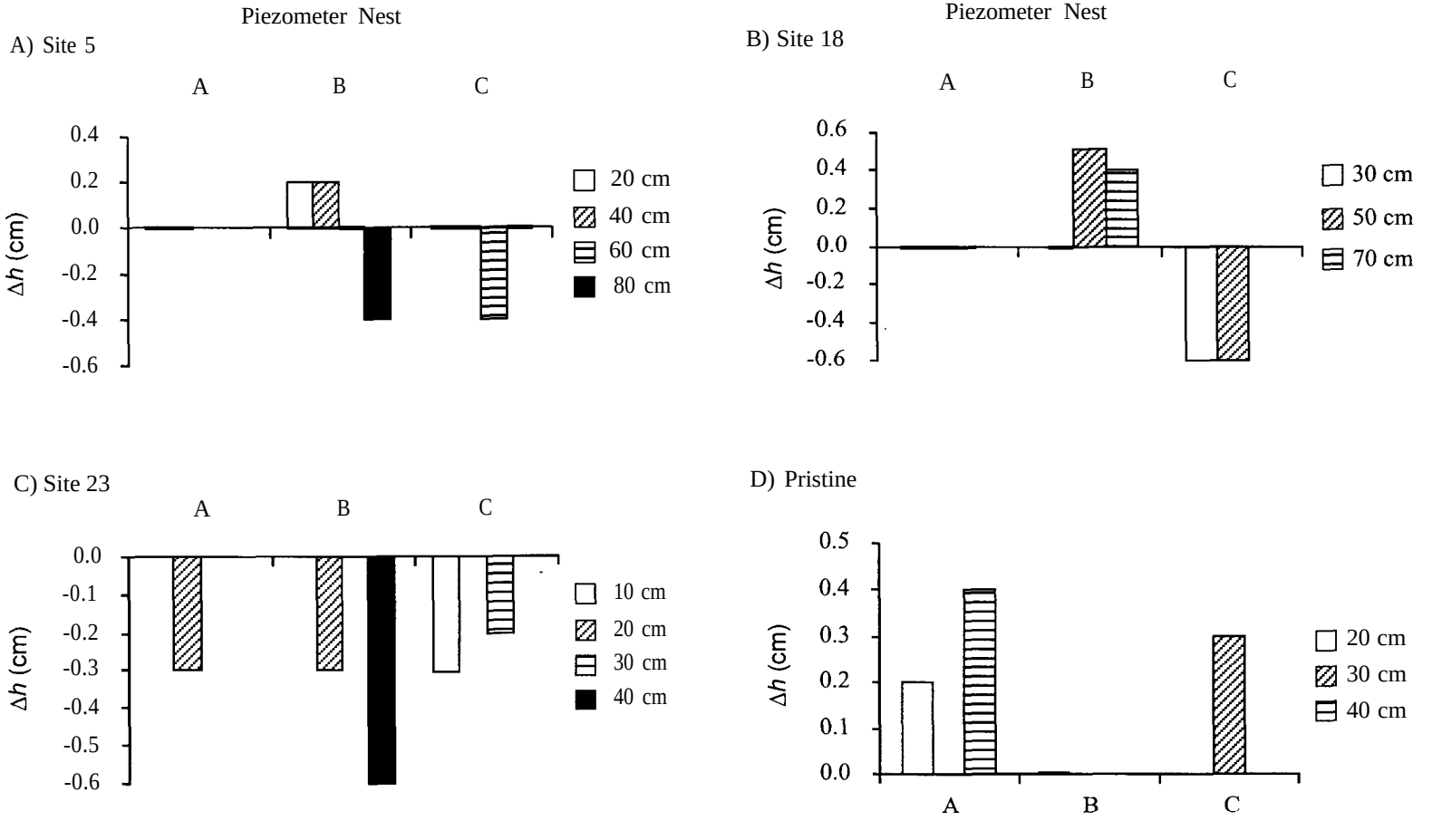


Figure 5.3. Mean (± 1 standard deviation) total chlorobenzene (CB) concentrations in aqueous samples taken from *in situ* exposure chambers installed at contaminated sites on the East Sebasticook River (Corrina, ME, USA). Stippled bars indicate water column (WC) exposures and black bars represent surficial sediment (SS) exposures. Letters that are different denote significant differences ($p < 0.05$) between the total CB concentrations of a single exposure treatment at sites 5, 18 and 23, and between the total CB concentrations in exposure treatments with respect to site.

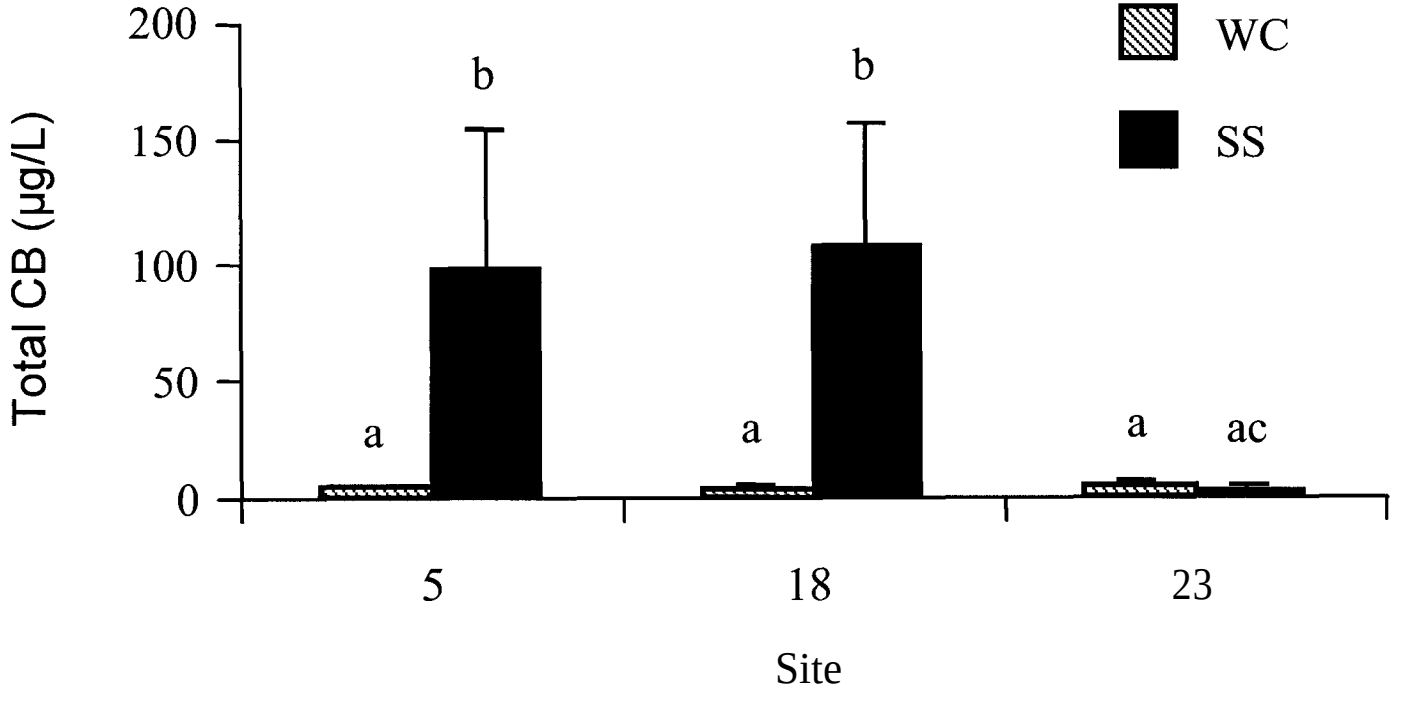
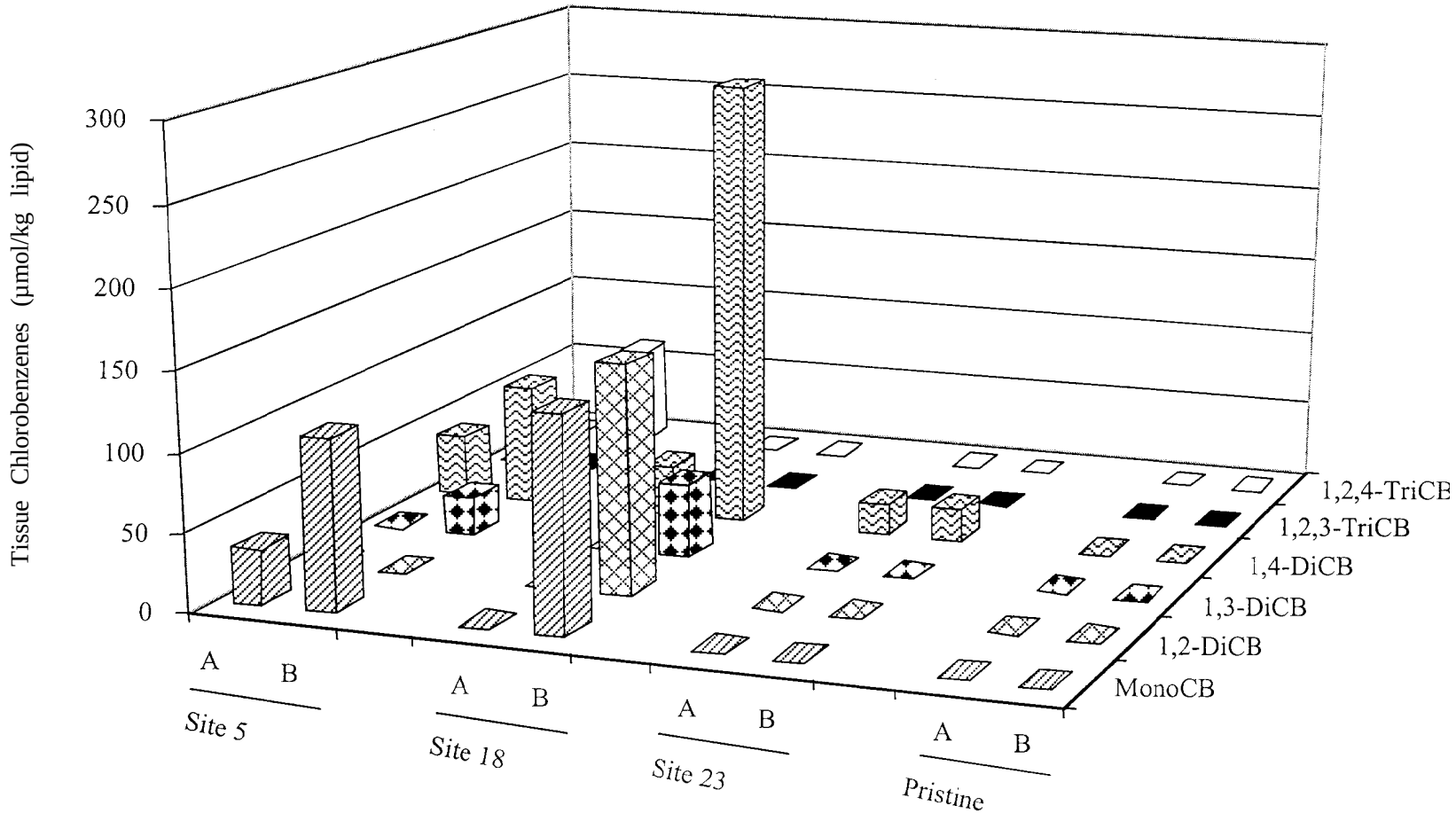


Figure 5.4. Body residues of chlorobenzene congeners in *Lumbriculus variegatus*. Bars represent the concentration in a pooled sample from four replicate exposure chambers. A = water column (WC) exposures, B = surficial sediment (SS) exposures.



A = Water Column
B = Surficial Sediments

CHAPTER 6

A Model of Bioaccumulation in Stream Systems Where Groundwater-Surface Water Interactions Affect the Bioavailability of Sediment-Associated Contaminants

INTRODUCTION

Stream environments are dynamic ecosystems that often contain localized areas of upwelling and downwelling (Ward et al., 1998). These groundwater-surface water interactions (GSI) have been shown to affect the transport and bioavailability of nutrients (Dahm et al., 1998) and contaminants (Greenberg et al., 2002) in rivers and streams. For benthic species encountering contaminated sediments in aquatic systems containing GSI, current exposure and accumulation models that do not take such dynamic conditions into account will be insufficient to accurately predict bioaccumulation. Therefore, a model that builds upon widely used and accepted approaches–including equilibrium partitioning (Gobas et al., 1989; Di Toro et al., 1991), toxicokinetics (Landrum, 1989; Landrum and Robbins, 1990) and contaminant desorption from solid phases (Pignatello and Xing, 1996; Cornelissen et al., 1997a)–with parameters to describe GSI in streams, will improve the predictions of the bioavailability, uptake, and accumulation of sediment-associated organic contaminants.

The main objective of this work was to develop a bioaccumulation model for sediment-associated organic contaminants that accounted for GSI and was

capable of predicting body burdens in exposed benthic invertebrates. Data from the laboratory investigations on the toxicokinetics and desorption rates of fluoranthene (FLU) and trifluralin (TF) and the field study of the impact of upwelling and downwelling on *in situ* toxicity and bioaccumulation were used to parameterize the model. These studies are described in previous chapters of this thesis. In addition, an attempt was made to validate the model using the *in situ* bioaccumulation of chlorobenzenes measured during the field study with parameters obtained from the literature. Finally, the hypothesis that upwelling and/or downwelling conditions can affect the bioavailability of sediment-associated contaminants, and hence their accumulation by organisms, was evaluated through simulations of the model.

MATERIALS AND METHODS

Model development, structure and conceptualization

An exposure and accumulation model of sediment-associated organic contaminants for benthic invertebrates was developed using data from the experiments described earlier in this thesis. Experimental measurements from investigations of: 1) the toxicokinetics of sediment-bound (Chapter 2) and waterborne (Chapter 3) FLU and TF in *Lumbriculus variegatus*, 2) the rates of desorption of FLU and TF from sediments (Chapter 4), and 3) the impact of upwelling and downwelling on contaminant bioavailability and accumulation (Greenberg et al., 2002; Chapter 5) were used to parameterize the model. The model was based upon an earlier model describing the bioavailability of

sediment-associated contaminants to benthic invertebrates (Landrum and Robbins, 1990). However, the present model has included the process of pore water flow due to upwelling or downwelling which has improved our ability to predict *in situ* bioaccumulation.

The model structure is diagrammed in Figure 6.1 and describes the accumulation of an organic contaminant (*e.g.,* FLU, TF) by the infaunal oligochaete, *L. variegatus,* using first-order processes. Conceptually, two submodels, or sectors, were linked to fully characterize the behavior of organic contaminants in sediments and organisms. The organism sector describes the uptake of organic chemicals from pore water and ingestion, and elimination from the body. The sediment and pore water sector describes: 1) the partitioning of the contaminant between sediment particles and pore water, 2) the removal of the contaminant from the sediment environment by organisms, 3) the loss of the contaminant from the system by the flow of uncontaminated pore water, and 4) the re-addition of contaminant to the sediment environment by the elimination of parent compound by the organisms. For the purposes of the model, contaminant concentrations were assumed to represent parent compound. The model terms including compartments, inflows, outflows, parameters and symbols are shown in Table 6.1, and the equations from which the model code (Appendix B) was derived are given below.

Although the amphipod, *Hyalella azteca*, was also studied in the research described earlier in this thesis (Chapters 2 and 3) they were not considered in this development of the bioaccumulation model. The reasons for this decision

were twofold. First, *H. azteca* are epibenthic (de March, 1981; USEPA, 1989; Pickard and Benke, 1996) and thus do not represent an infaunal, sediment-dwelling amphipod (*e.g., Diporeia* sp.). Second, *H. azteca* avoided sediments that were contaminated with FLU and TF (Chapter 2) and this observation raises important issues regarding the suitability of these organisms in sediment bioaccumulation and toxic effects testing.

Model equations and simulations

Organism sector. The organism was treated as a single, homogenous compartment and the body burden was described as a result of the uptake and elimination processes. Two uptake routes, pore water and ingestion of contaminated sediments, and a single elimination term were included to model bioaccumulation.

$$\frac{dC_a}{dt} = C_{pw} \bullet k_u + C_s \bullet k_f - C_a \bullet k_e , \qquad (6.1)$$

where C_{pw} is the concentration of the contaminant in the pore water (µmol/mL), k_u is the conditional uptake clearance coefficient (mL/g wet animal/h), C_s is the concentration in the sediments (µmol/g dry wt), k_f is the uptake clearance from ingestion of contaminated sediments (g dry sediment/g wet animal/h), C_a is the concentration in the organism (µmol/g wet animal), k_e is the conditional elimination rate constant (1/h), and *t* is time (h). The uptake clearance from ingestion (k_f) was calculated by:

$$k_f = FR \bullet AE , \qquad (6.2)$$

where FR is the feeding rate of the organism on the sediments (g dry sediment/g wet animal/h) and AE is the chemical assimilation efficiency from ingested sediments (fractional value).

Sediments and pore water sector. The sediments and pore water were each considered as individual compartments in the model description with the desorption/adsorption processes controlling the pore water concentrations. Inflows and outflows from these compartments due to organism uptake and elimination were also described. The flow of pore water was included to account for the effect of upwelling pore water (ground water) or downwelling surface water on pore water concentrations. It was assumed that this pore water flow resulted in the replacement of contaminated water with "fresh" water.

The concentration of a contaminant in the sediments was described by:

$$\frac{dC_s}{dt} = \left\lceil \frac{K_p \bullet k_{des} \bullet C_{pw} \bullet r_s \bullet (1-f)}{f} \right\rceil + \left\lceil \frac{C_a \bullet k_e \bullet r \bullet f}{r_s \bullet (1-f)} \right\rceil - \left\lceil \frac{k_{des} \bullet C_s \bullet r_s \bullet (1-f)}{f} \right\rceil - \left\lceil C_s \bullet \left(\frac{(k_f \bullet r \bullet f)}{r_s \bullet (1-f)} \right) \right\rceil . \quad (6.3)$$

where k_{des} is the desorption rate (1/h), r_s is the mean density of particles (2.5 ± 0.2 g/mL) in the sediments (Robbins, 1980), ø is the fraction of pore water in the sediments (*i.e.,* volume of pore water per volume of sediments), and r is the density of organisms per volume of pore water (g/mL). The partition coefficient of the chemical between the sediments and the pore water (K_p; mL/g) was calculated from the organic carbon partition coefficient (K_{oc}) (Di Toro et al., 1991):

$$K_p = K_{oc} \bullet f_{oc}, \quad (6.4)$$

where f_{oc} represents to fraction of organic carbon in the sediments. The log K_{oc} values were determined from the log K_{ow} values of FLU (5.2; MacKay et al., 1992) and TF (5.3, Mackay et al., 1997) with the following equation (Di Toro et al., 1991):

$$\log(K_{oc}) = 0.00028 + 0.983 \bullet \log(K_{ow}), \quad (6.5)$$

The concentration of a contaminant in the pore water was described by:

$$\frac{dC_{pw}}{dt} = \left[\frac{k_{des} \bullet C_s \bullet r_s \bullet (1-f)}{f}\right] - \left[\frac{K_p \bullet k_{des} \bullet C_{pw} \bullet r_s \bullet (1-f)}{f}\right] -$$

$$[k_u \bullet C_{pw} \bullet r] - [C_{pw} \bullet q], \quad (6.6)$$

where q is the fractional rate of pore water flow through the sediments (1/h).

The model simulations and visual parameter optimizations were carried out using Structural Thinking Experiential Learning Laboratory with Animation for Research (STELLA for Research) software (High Performance Systems, Hanover, NH, USA) on a Macintosh personal computer. The time-step (DT) for simulations was set to 0.005 h = DT =0.01 h and was determined by the software depending on the length of the simulation. The integration method was the fourth-order Runga-Kutta algorithm. Visual optimization was carried out by repeated simulation of the experimental conditions (*e.g.,* sediment concentrations, animal loading, length of exposures) used in studies of the bioaccumulation of sediment-associated FLU and TF by *L. variegatus* followed by comparisons to the data sets.

Model Parameter Estimation

The model was initially parameterized with values from the literature and from experimental measurements (Table 6.2). The default assimilation efficiency (AE) for *L. variegatus* (0.26) was chosen from studies with the PAH benzo[a]pyrene (BaP) in which AE ranged from 0 to 35% (Kukkonen and Landrum, 1995; Schuler and Lydy, 2001). A general AE of 72 ± 28% for oligochaetes has been reported by others for models of contaminant biomagnification in food-webs (Morrison et al., 1996). The default feeding rate of *L. variegatus* (0.08 g dry sediment/g wet animal/h) was taken from the measured egestion rates (ca. 0.01-0.08 g dry sediment/g wet animal/h) reported in Leppänen and Kukkonen (1998a,b,c). The choice of this default feeding rate was supported because it was within the range (0.03-0.17 g dry sediment/g wet animal/h) of other reported rates of ingestion by *L. variegatus* exposed to various chemicals (Kukkonen and Landrum, 1995; Schuler and Lydy, 2001).

Numerous parameters for the model (Table 6.2) were obtained from the experiments described in the previous chapters (Chapters 2-5) of this thesis. General parameters including ø and ρ were obtained from the toxicokinetics of FLU and TF in *L. variegatus* exposed to spiked Great Lakes sediments (Chapter 2). The value of ø (mL pore water/mL sediment) was determined from the mass fraction of pore water per wet mass (f_{pw}; assuming 1 g pore water = 1 mL pore water) of the Lakes Erie (0.774) and Huron (0.828) sediments divided by the respective measured volume per mass of wet sediment (Lake Erie, 0.870 mL sed/g wet sed; Lake Huron, 0.909 mL sed/g wet sed).

The value of ρ (g wet organism/mL pore water) represents the amount of pore water encountered by an animal, and in theory this value should be system-independent. In order to provide theoretical minimum and maximum values of ρ, literature values of oligochaete worm densities and burrowing depths were used in simple calculations to estimate the bounds of this parameter. The observed densities of tubificids in Lake Erie ranged from 6,600 to 55,300 individuals/m^2 (Robbins et al., 1989) and the reported vertical distributions of *L. variegatus* in lake sediments ranged between 0 and 5 cm depth (Krezoski and Robbins, 1985). It was assumed that: 1) the organisms experienced 100% of the pore water available, which is reasonable for *L. variegatus* based on studies of their reworking rates of sediments (Keilty et al., 1988a,b; Landrum et al., 2002); 2) that conversions of the density (abundance per unit area) measurements given above to abundance per volume sediment using burrowing depths of 1 cm and 5 cm would provide realistic bounded estimates of ρ, and 3) that the values of f in Lakes Erie and Huron sediments (Table 6.2) used to convert wet sediment volume to pore water volume would provide realistic estimates of ρ. Following these assumptions, the bounded estimate of ρ ranged from 0.00061 to 0.026 g animal/mL pore water.

Since the true value of ρ is unknown, it was reasoned that a value based on the experimental conditions (laboratory or *in situ*) would be usable in simulations if it fell within the natural-density-based bounded estimate given above. These experimental values of ρ were calculated by:

$$r = \frac{(n \bullet miw)}{(g_{sed} \bullet f_{pw})}, \qquad (6.7)$$

where *n* is the number of *L. variegatus* placed in each test beaker (10 individuals), *miw* is the mean individual wet wt of the test organisms (4.12 and 4.20 mg for Lakes Erie and Huron tests, respectively) and g_{sed} is the grams of wet sediments in each beaker (50 g).

The fractional flow of pore water through the sediments (q; 1/h), was set at a default value of 0 and could be increased to 1 (*i.e.,* 100% replacement of the pore water volume). This parameter was included in the model to provide a tool capable of mimicking the reduced exposure to contaminants observed in the presence of groundwater-surface water interactions (GSI) during the *in situ* toxicity and bioaccumulation testing on the East Sebasticook River, Corinna, ME, USA (Greenberg et al., 2002; Chapter 5). Therefore, a q value of 0/h indicates that no exchange of groundwater and surface water occurs (*i.e.,* no pore water flow; stagnant system) and a value of 1.0 indicates continuous flow of pore water (*e.g.,* all piezometer nests indicated downwelling at exposure site 23 in Greenberg et al., 2002). The value of q could be changed by the operator to investigate the potential impact of upwelling and downwelling on predictions of field tissue data.

Starting values for chemical-specific model parameters (Table 6.2) were taken from the desorption experiments (Chapter 4) and from investigations of the toxicokinetics of waterborne FLU and TF in *L. variegatus* (Chapter 3). The default k_u values for FLU and TF used in the model were the means of the estimates listed in Table 3.8 (*L. variegatus*, 154 and 121 mL/g/h, respectively).

Assuming that the mean value represented a good initial estimate was justified because there were no statistical differences determined between the multiple comparisons of these estimates and their associated errors (t-test, $p>0.05$; Table 3.9). The k_e value of FLU for *L. variegatus* (0.116/h) was the mean of the estimates for the 5 and 50 µg/L treatments given in Table 3.12. The default value of k_e for TF was the mean estimate of this parameter for *L. variegatus* (0.103/h; Table 3.12).

The rates of FLU and TF desorption from Lakes Erie and Huron sediments were measured in the experiments described in Chapter 4. These estimates represent maximal rates of desorption due to the method (*i.e.,* Tenax® extraction under continuously mixed conditions), and therefore, there is uncertainty regarding the true value of k_{des} achieved during the sediment bioaccumulation experiments (Chapter 2). Since the time courses of FLU and TF desorption from Lakes Erie and Huron sediments were fitted with a three-phase kinetic model that described rapid, slow and very slow desorbing fractions and their associated desorption rates, values within these ranges were used in model verification simulations (Table 6.2). It was assumed that the true value of k_{des} for FLU and TF during the sediment bioaccumulation tests fell within the extremes of the rapid and very slow desorption rates estimated during the desorption experiments. The rationale for this assumption was that in the sediment bioaccumulation tests, the sediments were bedded within a beaker and thus the processes of desorption from the sediments, readsorption to the sediments, and animal movement through the sediments would determine the true value of k_{des}.

Sensitivity analysis

To evaluate the sensitivity of the parameters in the bioaccumulation model each parameter or initial value was individually increased or decreased from its original value given in Tables 6.2 or 6.3. The following parameters were changed by a factor of 2 (*i.e.,* original value multiplied or divided by 2): k_e, k_u, and ρ_s. In other cases, (*e.g.,* AE and FR) the minimum and maximum values reported in the literature were used. Exceptions to these general approaches included the following parameters: q, K_p, ø, ρ, and k_{des}. Since the default value of the fractional rate of pore water flowing out of the system (q) was set to zero, its sensitivity was evaluated by setting q to 0.01 and 1.0 (*i.e.,* 1 to 100% pore water flow). The sensitivity of K_p was tested by setting its values for FLU to 1436 and 5942, which corresponded to log K_{ow} values of 4.9 and 5.3, respectively. For TF, the sensitivity analysis for K_p used values of 1254 (at log K_{ow} = 4.9) and 6506 (at log K_{ow} = 5.34). A factor of 2 increase and decrease was not performed for ø. Since values of ø in the sediments from Lakes Erie and Huron were 0.89 and 0.91, respectively, the upper value used in sensitivity analyses was 0.99 because a fraction of 1.0 would imply an absence of sediment particles. The sensitivity of ρ was evaluated by increasing and decreasing the value to the literature-based upper and lower bounded estimates, respectively. For the sensitivity analysis of the rates of FLU and TF desorption (k_{des}) from the sediments, a default value (0.0003/h) was selected from the range of measured values based on previous visualization of the model predictions. The upper and lower values from the

measured range (0.00017/h to 0.37/h; see Chapter 4) were used for comparisons.

Following these adjustments of individual parameter values, the percentage change in the concentrations of FLU and TF in the body of *L. variegatus*, the sediments and the pore water were recorded and compared with unadjusted model simulation outputs.

Model verification with laboratory experimental data

The model structure and parameters were verified by simulating the accumulation time course data from the exposures of *L. variegatus* sediment-associated FLU and TF (see Chapter 2). Parameter values listed in Table 6.2 and sediment concentrations (Table 6.3) were entered respective of the sediment exposure (*i.e.,* Lake Erie or Huron), and chemical (*i.e.,* FLU or TF) data that were to be simulated. Since these simulations were meant to model the experiments conducted under static conditions within the beaker, q (the fractional flow of pore water; 1/h) was left at its default value of zero. Then, the initial (time = 0 h) non-zero values for the mean sediment concentration (C_s) of FLU or TF from each specific experimental treatment (Table 6.3) were entered. The initial concentration in the pore water compartment was calculated as C_s/K_p (Di Toro et al., 1991). The compartment representing the body burden (C_a) began with an initial value of zero. The lengths of the simulations (96 h) were the same duration as the bioaccumulation experiments conducted with the *L. variegatus* (Chapter 2). The results of the simulations were compared to the experimentally measured tissue concentration-time profiles.

The relative magnitudes of uptake from feeding and uptake from contaminated pore water is an important issue in our understanding of the bioaccumulation of sediment-associated contaminants by benthic organisms. Therefore, the model was used with the laboratory bioaccumulation data set in order to evaluate whether its structure could not only describe the experimentally measured bioaccumulation of FLU and TF, but also whether it could describe the data under different assumptions of the relative roles of the two uptake routes. This was done by conducting three separate verification simulations with adjustments made to FR and k_{des}, while keeping all other parameter values at their defaults. The assumptions, values of FR, and general values of k_{des} in these simulations were as follows:

1. Assumption: Uptake from feeding was of greater relative importance compared to uptake from pore water. Simulation: FR maximum, 0.08 g dry sediment/g wet animal/h; k_{des} minimum, 10^{-4} to 10^{-3}/h.
2. Assumption: The importance of uptake from feeding and from pore water was relatively equal. Simulation: FR medium, 0.04 g dry sediment/g wet animal/h; k_{des} medium, 10^{-4} to 10^{-2}/h.
3. Assumption: Uptake from feeding was of less relative importance compared to uptake from pore water. Simulation: FR low, 0.01 g dry sediment/g wet animal/h; k_{des} medium, 10^{-3} to 10^{-1}/h.

For the infaunal oligochaete worms exposed to both FLU and TF, it was believed that both uptake routes were important in determining the accumulated tissue concentrations. However, this exercise was conducted because the feeding rate was not measured in the bioaccumulation experiments. Thus, different hypothesis regarding FR and k_{des} (for which the true value is unknown) could be tested for their ability to accurately predict the observed tissue concentration-time profiles. The values of FR and k_{des} in these simulations are shown in Table 6.4)

Model validation with field data

An attempt was made to validate the model with an independent set of field data. Chlorobenzene (CB) concentrations in the sediments, pore water, and tissues of *in situ* exposed *L. variegatus* from the study on the East Sebasticook River (Greenberg et al., 2002; Chapter 5) were simulated. However, prior to running the simulations a number of parameters specific to the field study conditions and the CB congeners required estimation. The value of ø (0.42 ± 0.16, n=14) was the mean (±SD) percentage water in the sediments. The value of ρ (0.024 g wet organism/mL; near the upper value of the natural-density-based bounded estimate) was calculated with Equation 6.7 using 2 g as the numerator (*i.e.,* wet mass of *L. variegatus* placed in each *in situ* exposure chamber), a g_{sed} of 200 g (*i.e.,* approximate wet mass of sediments placed in each chamber), and a f_{pw} of 0.42.

The values of K_p were calculated for the five CBs that were detected in the tissues of *in situ*-exposed *L. variegatus*. The congeners and their mean (±SD, n) log K_{ow} values (Mackay et al., 1992) included: monoCB (log K_{ow}, 2.77 ± 0.22,

n=57), 1,2-diCB (log K_{ow}, 3.46 ± 0.15, n=47), 1,3-diCB (log K_{ow}, 3.52 ± 0.14, n=49), 1,4-diCB (log K_{ow}, 3.45 ± 0.14, n=56) and 1,2,4-triCB (log K_{ow}, 4.08 ± 0.15, n=55). The mean (±SD) TOC of the sediments across the three contaminated sites was 2.26 (±1.44)% (n=13). The mean log K_{ow} and f_{oc} values were used in Equations 6.4 and 6.5 to calculate K_p for each CB congener (Table 6.5).

Values for other parameters including the uptake and elimination rate constants, and desorption rates were obtained from the literature. The toxicokinetic parameters (k_u and k_e) for oligochaete worms exposed to di- and triCBs were obtained from numerous studies of the toxicokinetics in both invertebrates and fish (Oliver, 1987; Legierse et al., 1998; Van Hoogen and Opperhuizen, 1988; Gabric et al., 1990; Belfroid et al., 1993; Sijm et al., 1993; Sijm and van der Linde, 1995). The mean (±SD) value for k_u was 18.2 (± 18.0) mL/g wet organism/h among the studies cited above. The value of k_u used for the simulations mono-, di- and triCBs in *L. variegatus* was 36.25 mL/g wet organism/h (*i.e.,* the value of the mean + SD). This value was chosen because it was thought to represent a realistic value for an uptake rate compared to other chemicals with log K_{ow} values between 2.8 and 4.1 (Mackay et al., 1992) and because it was similar to a recently measured value for pentachlorobenzene in amphipods (35-57 mL/g wet organism/h; P. F. Landrum, personal communication, Great Lakes Environmental Research Laboratory, NOAA, Ann Arbor, MI).

The mean (±SD) values for k_e were 0.0990 ± 0.105/h among all organisms and 0.132 ± 0.125 in oligochaetes (Oliver, 1987; Legierse et al., 1998; Van Hoogen and Opperhuizen, 1988; Gabric et al., 1990; Belfroid et al., 1993; Sijm et al., 1993; Sijm and van der Linde, 1995). The values of k_e used in simulations were 0.099/h (*i.e.,* the overall mean) and 0.265/h (i.e., the mean + SD for elimination by oligochaetes only). The choice of using these values was logical as the higher k_e represented a literature-based maximum elimination rate for oligochaetes, whereas, the lower generic species value (0.099/h) fell within the standard deviation of the oligochaete-specific elimination rates. It should be noted that this approach could not be taken with the k_u values obtained from the same sources (see previous paragraph) because the oligochaete-specific mean (±SD) (0.070 ± 0.106 mL/g/h) was unrealistically low.

The values of k_{des} were taken from numerous studies of field-contaminated sediments and the mean (±SD) desorption rates from slowly and very slowly desorbing fractions ranged from 4.20 (± 2.00) x 10^{-5} to 5.55 (± 1.90) x 10^{-3} (Cornelissen et al., 1997c,2000; ten Hulscher et al., 1999; Kan et al., 2000). Ten Hulscher et al. (1999) measured the triphasic desorption of CBs in field-contaminated sediments with concentrations of di- and triCBs in the range of 13-459 µg/kg dry wt (Ten Hulscher et al., 1999) and these levels encompassed the range measured for 1,2,4-triCB in sediment samples taken during the *in situ* study conducted on the East Sebasticook River (21-56 µg/kg dry wt) (Greenberg et al., 2002). Furthermore, a rapidly desorbing pool was not observed in the field-contaminated sediments used in the ten Hulscher et al.

(1999) study. They concluded that under field conditions, the rates associated with the slowly and very slowly desorbing fractions are expected to more accurately reflect actual desorption rates for sediments historically contaminated by volatile organic contaminants like chlorobenzenes (ten Hulscher et al., 1999). Therefore, it was assumed that the desorption rates reported for the slowly and very slowly desorbing fractions of the chlorobenzenes (Cornelissen et al., 1997c,2000; ten Hulscher et al., 1999; Kan et al., 2000) were the best values to use in the present model of chlorobenzene bioaccumulation by oligochaetes exposed at the historically contaminated Eastland Woolen Mill. The means of the low (4.20×10^{-5}/h) and high (5.55×10^{-3}/h) values from the reported range were used in simulations of the model.

Sediment concentrations of the CBs that were accumulated by *L. variegatus* over the 96-h *in situ* exposures were estimated from pore water samples taken from minipiezometers. It was necessary to calculate the expected sediment concentrations because only one congener, 1,2,4-triCB, was detected in sediment samples collected during the study. The model structure was such that either the pore water or sediment concentration must be known as the initial concentrations of these compartments are related by the partition coefficient, K_p. In the case of the CB study, pore water data for numerous CBs were available and thus sediment concentrations were estimated by (Di Toro et al., 1991):

$$C_s = K_p * C_{pw}. \qquad (6.8)$$

Pore water samples taken from shallow minipiezometers (*i.e.,* 10-30 cm depth in the sediment) were assumed to be most representative of surficial sediment

conditions (top 0-10 cm) where *L. variegatus* were exposed and therefore these levels were used to estimate sediment concentrations. The pore water concentrations of CBs from sites 5, 18 and 23 on the East Sebasticook River are summarized in Table 5.2 of Greenberg et al., 2002 (Chapter 5). The minipiezometer samples that were used for the estimation of C_s by Equation 6.8 were as follows: 1) A20, B10 and C16 at site 5, 2) A28, B28 and C30 at site 18 and 3) A20, C10 and C30 at site 23. At site 23, the minpiezometers at position B were inserted into a pocket of non-aqueous phase liquid (NAPL) which contained very high levels of CBs. Since this sample did not represent pore water, the values from tube B20 were not used in the estimation. The estimates of the sediment concentrations are shown in Table 6.6.

The validity of the model was then assessed for *L. variegatus* by performing numerous simulations in which parameters describing the feeding rate (FR), the elimination (*i.e.,* k_e) of CBs by organisms, the desorption rate (k_{des}), and the fractional flow of pore water (q) were varied. The feeding rate was evaluated to simulate bioaccumulation under physiological conditions of *no* feeding, low (0.01 g dry sediment/g wet organism/h), medium (0.04 g/g/h) and high (0.08 g/g/h) rates of feeding. The elimination rate was evaluated because two reasonable estimates of k_e (low, 0.099/h; high, 0.265/h) were obtained from the literature as described above. Likewise, two literature values representing low and high rates of desorption (4.20×10^{-5}/h and 5.55×10^{-3}/h, respectively) were evaluated to demonstrate the impact of a two order-of-magnitude change in the desorption rate (a parameter whose true value has a high degree of

uncertainty). Finally, because mixed upwelling and downwelling conditions were detected with mini-piezometers at study sites 5 and 18 on the East Sebasticook River, and downwelling conditions were measured at site 23, five values of the pore water flow rate (q; 0, 0.1, 0.25, 0.5, 1.0/h) were used in the simulations to evaluate the role of GSI on bioavailability and bioaccumulation. The value of k_u was not adjusted from its chosen value (36.25 mL/g wet organism/h; see above) and all other parameters were left at their default values (Table 6.2).

The parameters whose values were varied were combined in a factorial design such that their combination (*i.e.,* 4 levels FR x 2 levels k_e x 2 levels k_{des} x 5 levels of q) led to 80 iterations of the bioaccumulation model for a given chlorobenzene congener-site datum. Each iteration simulated the 96-h body burdens for the CB congeners that were measured in the *L. variegatus* exposed *in situ* to surficial sediments at each contaminated study site (see Figure 5.4, Greenberg et al., 2002; Chapter 5). Performing this large number of simulations within the parameter variations described above was important because it provided an uncertainty analysis of the model by resulting in a range of tissue concentration predictions. The results of the simulations using the bioaccumulation model were compared to the experimental tissue concentrations from the *in situ* bioaccumulation study.

RESULTS AND DISCUSSION

General behavior of the model

The simulations shown in Figures 6.2 and 6.3 demonstrate the general patterns of the model predictions (*e.g.,* sediment and pore water concentrations of contaminants, and bioaccumulation by organisms) and were not meant to be predictive of the data. Comparisons of model predictions to both laboratory and field measurements are addressed in following sections. In these examples of typical model predictions (Figures 6.2 and 6.3), 96-h exposures of *L. variegatus* to 200 mg FLU/kg dry Lake Erie sediments were simulated using the initial parameter values given in Table 6.2 with a sediment concentration of 0.659 µmol/g dry wt (Table 6.3). To demonstrate the effect of pore water flow through the sediments, the value of q (1/h) was set at its default (zero/h) in the first simulation (Figure 6.2) and at its maximum value (1.0/h) in the second simulation (Figure 6.3). With no pore water flow, the predicted body burden of FLU in the worms at 96 h was 0.314 µmol/g wet wt. However, when 100% pore water flow was included the estimated 96-h body burden was 0.173 µmol/g wet wt, which represented a reduction of approximately 45%. Pore water concentrations of FLU over the 96-h simulations began at 2.45×10^{-4} µmol/mL and decreased to 1.49×10^{-4} (factor of 1.6) with no pore water flow and to 4.29×10^{-5} (factor of 5.7) with q =1.0/h. Thus, the maximum rate of pore water flow reduced the end-of-simulation predicted pore water concentration by 71% compared to the case of no pore water flow (q =0/h). FLU in the sediments decreased from its initial concentration by less than 0.5% in the first simulation (0.656 at 96 h), and when

pore water flow was included the predicted sediment concentration at 96-h (0.651 µmol/g dry wt) was lowered by only 1.2%.

The model behaved reasonably for a short-term sediment exposure of benthic invertebrates, as steady state was reached by the end of the simulations. For FLU and TF, this was expected based on laboratory studies (Chapter 2). The sediment concentration remained relatively constant throughout the 96-h simulations (=1.2% decrease), which is important because this result supports a common assumption of *basic* bioaccumulation models (*i.e.,* constant pool of contaminant) (Lee, 1992). Relatively constant concentrations of hydrophobic sediment-asssociated contaminants have been demonstrated in laboratory experiments of up to 30 d under both static and flow-through conditions (Landrum, 1989). In the presence of prolonged pore water flow over time frames of months to years, sediment concentrations may be expected to decline. Simulations of FLU sediment concentrations after one year using the same parameters as in the simulations shown in Figures 6.2 and 6.3 resulted in levels that were reduced by 41 and 69%, of the initial concentration (0.659 µmol/g dry wt), respectively. Comparing the results of the 96-h and one year simulations suggests that in short-term exposures (*i.e.,* hours to days) the flow of pore water does not greatly enhance the decline in bulk sediment concentration. This was likely because the desorption rate (10^{-4}/h) was relatively slow (Cornelissen et al., 1998) and within this short exposure duration, the mass of contaminant desorbed to pore water was small. When a year-long exposure scenario was considered, the impacts on predictions of bulk sediment concentration by desorption alone

and by desorption and pore water flow were significant. However, even at the slow rate of desorption simulated in these examples, the flow of pore water did show marked effects on the pore water concentration of FLU in only 96-h (reduced approximately 6-fold), and on the steady state tissue concentration (reduced by a factor of 1.8).

Parameter sensitivity

Analytical sensitivity coefficients were calculated as a percentage change in model output (body burden, sediment and pore water concentrations) associated with a change (*e.g.,* factor of 2, literature or measured range) in the value of each input parameter. The results of these procedures for 96-h simulations of *L. variegatus* exposed to 100 mg FLU/kg dry Lake Erie sediments are shown in Table 6.7. All model parameters were sensitive for describing the body burdens of FLU as most changes in parameter values, except in the case of increasing ø, led to over or underpredictions of the body burden by >12%. A given change in any parameter, led to a change in tissue concentration predictions that ranged in magnitude from –62% (ρ) to nearly 100% (k_e).

The model-predicted concentrations of FLU in the pore water of Lakes Erie and Huron sediments were also sensitive to numerous parameters. For simulations of bioaccumulation by *L. variegatus*, a factor of 2 change in k_u and ρ_s led to a =24% change in pore water concentrations (Table 6.7). When the input parameters were changed by the upper and lower values obtained from the literature (K_p, ρ) or measurements (ø, k_{des}) the concentrations of FLU in the pore water of the simulated worm exposures varied from –92% to 64%. Sediment

concentrations were not very sensitive to changes in model input parameter values. The greatest percentage change was a decrease in sediment FLU levels by 14% when ρ was increased to its literature-based theoretical maximum value (0.026 g wet organism/mL pore water) 10% in the simulation. The results of sensitivity analyses for TF bioaccumulation by *L. variegatus* were nearly identical to those obtained for FLU as described above (data not shown).

The sensitivity analyses conducted for the bioaccumulation model indicated that accurate estimates were necessary for all parameters (Tables 6.7-6.10). The parameters describing accumulation in the organisms (k_u, k_e, FR and AE) were all shown to be very sensitive. Sediment-specific parameters such as K_p and ø were important, particularly in predicting body burdens and pore water concentrations. Thus the TOC, % water and the volume to weight ratio of the sediments must be accurately measured for reliable representation of the system. The estimated sediment concentrations were, overall, not very sensitive to changes in the values of the model parameters. This insensitivity of the sediment compartment assured that sediment concentrations will remain relatively stable throughout the short-term (96-h) simulations, as discussed above.

The value of ρ (density of organisms per volume interstitial water) was the most sensitive single parameter as it exhibited the widest ranges of changes in the predictions resulting from the analysis procedures (body burden, 12 to –62%; pore water, 20 to –92%; sediment, 0.31 to 14%) (Table 6.7). For benthic organisms occurring naturally, the true value of ρ should be independent of the

specific system and the mass of sediment in the exposure. This implies that when the system becomes sufficiently large (*e.g.,* sediment bed in a lake or river), there is a maximum value beyond which ρ remains constant. However, the true value of ρ for *L. variegatus* remains unknown. The best estimates of minimum and maximum values of ρ for oligochaetes were calculated from reported densities of worms in the field (Krezoski and Robbins, 1985; Robbins et al., 1989). These estimates ranged over two orders of magnitude (0.00061 to 0.026 g wet animal/mL pore water). Although the values of ρ used in the present model as defaults (e.g., 0.00104 g/mL for FLU and TF in the laboratory; 0.024 g/mL for CBs in the field) were based on the density loading of organisms used in experiments, these values were within the literature-based range for this parameter. And, the value of ρ calculated for the *in situ* study was near the maximum theoretical value. Furthermore, the experiment-specific calculations of ρ assumed that the worms were capable of processing all of the sediments presented during the exposures (50 g wet, laboratory; 200 g wet, field), and thus experienced 100% of the pore water. This was a reasonable assumption considering the reworking of greater masses of sediments by *L. variegatus* (Landrum et al., 2002). Even with these best estimates of ρ calculated from natural densities, this parameter was likely a major source of uncertainty in the model.

Simulations of laboratory bioaccumulation

The tissue concentration-time profiles from laboratory exposures of *L. variegatus* to FLU- and TF-spiked sediments from Lakes Erie and Huron were

simulated (Figures 6.4-6.5). In general, the model was in good agreement with observations of the apparent steady state concentrations of FLU and TF in the tissues of the oligochaetes except in the cases of Simulation 3 (*i.e.,* assumption of a low rate of feeding) for the Lake Huron experiment where FLU in the 100 mg/kg treatment and TF in both exposure concentrations were underpredicted. The observed and model-predicted curves for the bioaccumulation of FLU and TF by *L. variegatus* that were exposed to spiked Lake Erie sediments are shown in Figure 6.4. For the 100 mg/kg treatment, the model predicted FLU body burdens of 0.160, 0.163, and 0.163 µmol/g wet wt at 96 h in simulations 1, 2 and 3, respectively (see Table 6.4 for FR and k_{des} settings). The observed mean (± SD) apparent C_{ss} was 0.161 ± 0.0243 µmol/g wet wt. At 200 mg/kg, the observed C_{ss} for FLU was 0.312 ± 0.0367 µmol/g wet wt and the predicted values at 96 h were, respectively, 0.314, 0.320, and 0.320 µmol/g wet wt in simulations 1, 2 and 3. The respective model predictions of TF concentrations at 96 h in the *L. variegatus* following simulations 1, 2 and 3 were 0.0732, 0.0674, and 0.0644 µmol/g wet wt in the 100 mg/kg exposure group and were 0.149, 0.138, and 0.131 µmol/g wet wt in the worms exposed to 200 mg/kg. These values were nearly identical to the observed apparent C_{ss} values of 0.0668 ± 0.0138 µmol/g wet wt at 100 mg/kg and 0.137 ± 0.0183 µmol/g wet wt at 200 mg/kg.

The comparisons between the model predictions and observed tissue concentrations for the bioaccumulation of FLU and TF by *L. variegatus* exposed to spiked Lake Huron sediments are shown in Figure 6.5. The model adequately

predicted FLU body burdens (simulations 1,2, and 3, respectively) of 0.200, 0.168, and 0.149 µmol/g wet wt for worms exposed at 100 mg/kg and 0.314, 0.302, and 0.274 µmol/g wet wt at 200 mg/kg. The observed C_{ss} values for FLU were 0.194 ± 0.0268 and 0.326 ± 0.0616 µmol/g wet wt in the 100 and 200 mg/kg treatments, respectively. The model estimates for TF at 96 h were 0.0896, 0.0745, and 0.0582 µmol TF/g wet wt at 100 mg/kg in simulations 1,2, and 3, respectively. The predictions from simulations 1 and 2 were in close agreement with measured C_{ss} values of 0.0918 ± 0.0147 µmol/g wet wt. In the 200 mg/kg exposure group, experimental body burdens at C_{ss} were and 0.145 ± 0.0182 µmol/g wet wt and the model accurately predicted this observation in simulations 1 (0.145 µmol/g wet wt) and 2 (0.137 µmol/g wet wt).

Conducting the three simulations of each data set (see Table 6.4; Figures 6.4 and 6.5) that focused on the effect of changing the input values of FR and k_{des} was important. This allowed for an evaluation—through comparisons of the predicted tissue concentration-time curves—of the conditions under which the relative importance of uptake by feeding vs. uptake via pore water in determining the body burden at steady state could be hypothesized. It should be stated that each simulation (*i.e.,* simulations 1, 2 and 3) was conducted with all other parameters at their default values and that for both FLU and TF predictions, the organism-specific FR was the same within a given simulation. Thus, for a given simulation, the predictions for each chemical were the result of the same assumed physiological state of the organism. It was shown that, in general, at a high rates of feeding in the model (FR, 0.08 g dry sediment/g wet organism/h)

lower rates of desorption of FLU (10^{-4} to 10^{-3}/h) and TF (10^{-4} to 10^{-2}/h) were required to adequately describe the data. When the conditions of simulation 1 for FLU at 100 and 200 mg/kg exposures of *L. variegatus* to spiked Lake Erie sediments were repeated with no ingestion (*i.e.,* FR =0 g/g/h; k_{des} = 0.0003/h), the body burdens were reduced by 37 and 39%, respectively. In the environment, these condition would imply that when desorption rates of chemicals approach values that are kinetically described as "very slow" (on the order of 10^{-4} to 10^{-5}/h, Cornelissen, 1999b) uptake from feeding is nearly equal in importance to pore water accumulation in determining the body burden. This would be a reasonable hypothesis and is supported by a recent study in which up to 61% of the benzo[a]pyrene body burden in *L. variegatus* was observed to have accumulated via ingested sediments (Leppänen and Kukkonen, 1998b).

At low rates of feeding (FR, 0.01 g dry sediment/g wet organism/h), higher rates of desorption (10^{-3} to 10^{-1}/h) were needed as input values to describe the observed C_{ss} of the FLU and TF. When simulation 3 was repeated for the Lake Erie exposures to FLU with an assumption of no feeding, the predicted body burdens were reduced by only about 5%. This suggested that pore water uptake may dominate when rates of field desorption are in the range of kinetically slow (10^{-3} to 10^{-2}/h) to rapid (10^{-1}/h) desorption rates (*i.e.,* greater than 10^{-4}/h; Cornelissen, 1999b). Therefore, in evaluating the potential hypotheses originally outlined by the three simulations (see Methods), the modeling results do not support a condition in which ingestion is the *dominant* source of uptake of FLU and TF. However, the conditions during which uptake from feeding is nearly

equal to uptake from pore water were demonstrated when desorption rates were similar to the kinetic rates associated with very slow desorption (10^{-4}/h) (Chapter 4; ten Hulscher et al., 1999; Cornelissen, 1999b). This important conclusion underscores the need to obtain accurate estimates of desorption rates for organic contaminants when predicting their bioavailability and bioaccumulation.

In all simulations of the bioaccumulation of FLU and TF from Lake Erie sediments and in most simulations of the Lake Huron data, the kinetics of uptake for time points prior to the plateau of the accumulation curve were overestimated. Although the latter time points were adequately described by the model, there is considerable uncertainty associated with the predicted body burdens of FLU and TF at earlier time points (*e.g.,* 0-12 h). This problem of the present model limits its applicability to exposure scenarios in which the organisms have reached steady state. One source of the uncertainty may have been that the model uptake and elimination parameters were derived from toxicokinetic rates that were determined in water-only exposures. In the sediment exposures, it was possible that organism uptake at the earlier time points was slower than the model predicted due to a delay in the onset of activity and feeding while the worms adjusted to the test conditions during the first few hours of exposure to the spiked sediments. If the organisms at first did not move much, or rework the sediments (Keilty et al., 1988a,b; Landrum et al., 2002), then it was possible that lower concentrations of contaminants were available for uptake to the organisms than were predicted at the earlier times. For *L. variegatus* exposed to the spiked sediments, fecal pellets were not observed until about 12 h into the exposure

(see Chapter 2). This implied that organism activity was low at the earlier time points and thus the worms may not have experienced as much contaminated pore water or ingested as much sediment as the model simulations assumed a constant rate of ingestion throughout the exposure. Although the earlier data points were often overestimated, the model was developed to accurately predict steady state body burdens of the contaminants, which it did successfully. Future versions of the model should attempt to include the effects of changes in organism behavior during exposure. For example, ramping the feeding rate during first few hours of the simulations may alleviate this problem of poor prediction of the tissue concentrations at the earlier time points.

Field validation

The ability of the model to predict the measured body burdens following 4-d *in situ* exposures of *L. variegatus* to chlorobenzene (CB) contaminated sediments was evaluated by simulating exposures for each congener detected in the tissues of the worms with the factorial parameter set-ups (*i.e.,* 80 iterations; 4 levels FR x 2 levels k_e x 2 levels k_{des} x 5 levels of q). This approach provided a consistent protocol for simulating the bioaccumulation of specific congeners at each contaminated site with varying input values for critical parameters including FR, k_e, k_{des} and q. The uptake rate coefficient (k_u; 36.25 mL/g/h) was not adjusted in the protocol because only a single reasonable estimated value was obtained from the literature, whereas for k_e there were two reasonable literature-based values requiring evaluation. The approach also provided a means to quantify uncertainty in the model predictions by resulting in ranges of predicted

body burdens that are reported as closest and furthest estimates from the measured tissue concentrations (Table 6.8). Overall, the predicted body burdens ranged from values that were within a factor of 1.0 of the observations to maximum factors of 1565 and 260 for overestimates and underestimates of the measured tissue residues, respectively. Meaningful or reasonable body burden estimates resulting from the simulations were considered to be predictions that were within an order of magnitude (factor of 10) of the observed *in situ* bioaccumulation. The simulation conditions (*i.e.,* initial values for sensitive parameters) that generally led to predictions within this limit are discussed below.

MonoCB was predicted to bioaccumulate in worms to levels within a factor of approximately 1.0 of the observed values at sites 5 and 18 when the lower desorption rate (k_{des} = 0.4.2 x 10^{-5}/h), higher (*i.e.,* mean + SD; oligochaete values) elimination rate (k_e = 0.265/h), no feeding (FR = 0.0 g/g/h) and 50% pore water flow (q) values were used in the simulations. Overestimates of the monoCB body burdens of nearly 160-fold higher than observed tissue concentrations were obtained when k_e (0.099/h) and FR (0.08 g/g/h) were changed to their respective low and maximal parameter values, q was set to zero, and k_{des} was set to the higher literature-based value of 5.6 x 10^{-3}/h. Between these extremes, the body burdens were predicted within an order of magnitude of the measured tissue concentrations when k_{des} = 4.2 x 10^{-5}/h (slowest rate) in various combinations of feeding, elimination, fractional pore water flow and desorption. For example, at site 5 under the highest feeding and elimination rates (FR = 0.08 g/g/h; k_e = 0.265/h) and with no pore water flow

(q = 0/h), the body burden (9.89 x 10^{-3} µmol/g wet wt) was overpredicted by a factor of 6.95.

The predicted body burdens of diCB congeners (1,2-, 1,3-, and 1,4-diCB) that most closely agreed with *in situ* bioaccumulation measurements across the contaminated sites were within a factor of 8 of the observations (Table 6.8). In general, very good agreement (factor =2.07) between model-predicted and observed values for 1,2-diCB (site 18), 1,3-diCB (sites 5 and 18) and 1,4-diCB (sites 5 and 18) were obtained with assumptions of: 1) no feeding by *L. variegatus* over the 4-d exposure; 2) full (1.0/h) pore water flow; 3) slower desorption (k_{des}, 4.2 x 10^{-5}/h); and higher elimination (0.265/h). However, as discussed for monCB above, simulated body burdens for these congeners to within a factor of 10 were obtained using other initial values of parameters and included simulations where FR = 0.1 g/g/d (i.e., non-zero rates of feeding) and q = 0.25/h (*i.e.,* low fractional flow of pore water) (data not shown).

The 1,4-diCB was the only congener that was bioaccumulated by *L. variegatus* across the three study sites. At site 23,the site on the Sebasticook River at which downwelling pore water was detected in all piezometer samples, the best predicted body burden (1.84 x 10^{-3} µmol/g wet wt) exceeded the *in situ* observation by a factor of nearly 8. This simulation assumed no feeding, full pore water flow, the fastest elimination rate, and the slowest rate of desorption. When minimal, medium, and maximal levels of feeding (FR = 0.01, 0,04 and 0.08 g/g/h, respectively) were considered along with full fractional pore water flow, and the maximum elimination and slowest desorption rates, the predictions exceeded the

measured body burdens by factors of 15, 37 and 66, respectively. Thus at site 23, maximum pore water flow was required to provide a reasonable estimate of the body burden. This is an important finding because for monoCB, 1,2-, 1,3-, and 1,4-diCB, closer predictions (*i.e.,* better than a factor of 8) resulted from similar simulations in which higher rates of feeding (*i.e.,* 0.04 and 0.08 g/g/h) at lower values of q (*i.e.,* 0.1, 0.25/h) were modeled. This qualitatively supports both the observation on the Sebasticook River that there was a greater occurrence of downwelling at site 23 and that this flow of pore water reduced the bioavailable fraction of the contaminant to the exposed oligochaetes (Greenberg et al., 2002).

The 1,2,4-triCB was the only congener for which a sediment concentration (3.09×10^{-4} µmol/g dry wt) was measured from bulk sediment samples taken from site 5 and therefore, bioaccumulation for 1,2,4-triCB was simulated using this measured value. Relative to *in situ* bioaccumulation (8.27×10^{-4} µmol/g wet wt), all predictions from the 80 iterations (*i.e.,* the factorial simulation protocol) resulted in underprediction of the observed body burden (Table 6.8). The closest reasonably simulated body burdens obtained from the measured bulk sediment concentration were underpredicted by factors ranging from 2 to 10. These respective predicted body burdens were 3.61×10^{-4} µmol/g wet wt (see Table 6.8 for input parameter values) and 8.11×10^{-5} with settings of k_e = 0.099/h (lowest evaluated), FR =0.08 (maximum), $k_{des} = 4.2 \times 10^{-5}$/h (slowest), and q = zero or 0.10/h. The major difference between the predictions for this congener and all others discussed previously, is that the best predictions of the measured tissue

concentrations were obtained when the fastest desorption rate (k_{des} = 5.6 x 10^{-3}/h) was used. However, predictions of about an order of magnitude (factor of 10.3) below the observed tissue concentration were obtained at the slower rate of desorption.

Some general trends were observed from the simulations of bioaccumulation of CB congeners at each site. In all simulations that led to predicted body burdens within an order of magnitude of the measured values, the desorption rate (k_{des}) input to the model was the slower value obtained for CBs from the literature (4.2 x 10^{-5}/h). The impact of the fractional flow of pore water (q) on the predictions of the body burden data was important as full flow was required to reasonably predict 1,4-diCB bioaccumulation by *L. variegatus* at site 23, where conditions were completely downwelling. Without consideration of full pore water flow at site 23, the body burden was greatly overpredicted. At sites 5 and 18 where piezometer measurements indicated conditions of no pore water flow, or mixed upwelling or downwelling flows, the value of q was not required to obtain predictions within an order of magnitude of the observations, but a q =1.0/h was necessary to predict to a factor of near 1.0. Another important point is that predictability of the model to within an order of magnitude of the observed body burdens was possible for all accumulated congeners at a given site when initial parameters of FR, k_e, k_{des} and q were the same value for each modeled CB.

The parameters for the uptake and elimination of CBs by oligochaetes, the partitioning of CB congeners between sediments and pore water, and the

desorption of CBs from field sediments were obtained from the literature. All other parameters were defined by experimental procedures of measurements taken during the *in situ* exposures (*i.e.,* sediment TOC, pore water concentrations, organism densities used, hydrologic flow). In general, the model was capable of predicting the observed tissue levels of the CB congeners to within an order of magnitude and when feeding rate (*i.e.,* uptake from ingestion) was set to low values or turned off, the predictions were often within a factor of 4. However, the model-predicted tissue concentrations that resulted from simulations in which feeding was absent should be viewed with caution because gut contents were observed in the *L. variegatus* collected after the 4-d *in situ* exposure, and thus an assumption of no ingestion was invalid. Therefore, the simulations for which FR was 0.01 or 0.04 g dry sediment/g wet wt/h were more realistic.

The present model developed for *L. variegatus* compared well with other model predictions of the bioaccumulation of organic contaminants by benthic invertebrates. Model predictions for PCB concentrations in benthic invertebrates indigenous to Lake Erie (*e.g.,* amphipods, mayflies, caddisflies, crayfish, zebra mussels) were within a factor of two of observed concentrations (Morrison et al., 1996, 1997). Their food-web bioaccumulation model was parameterized with concentrations of the contaminants in the sediments and water, ingestion rates and gill ventilation rates but did not use kinetic rate constants for uptake and elimination or desorption parameters (Morrison et al., 1996, 1997). Considering the number of parameters that required estimation in the present model, the

ability to predict within a factor of 10 difference from observations was encouraging.

There were a number of uncertainties in the parameters used in the model validation. First, the kinetic rate constants for the uptake from pore water and elimination of chlorobenzenes used to simulate bioaccumulation in *L. variegatus* were measured in fish or other species of invertebrates including the oligochaetes *Tubifex tubifex* and *Limnodrillus hoffmeisteri* (Oliver, 1987; Legierse et al., 1998; Van Hoogen and Opperhuizen, 1988; Gabric et al., 1990; Belfroid et al., 1993; Sijm et al., 1993; Sijm and van der Linde, 1995; P. F. Landrum, personal communication, Great Lakes Environmental Research Laboratory, Ann Arbor, MI). Therefore species-specific characteristics including lipid contents and behavior in the sediments, which are incorporated into the conditional rate estimates for these parameters, may be different between *L. variegatus* and the other species. Also, these estimates of k_u and k_e were values for di- and triCB congeners and these rate estimates were used across all simulated congeners. Therefore there was uncertainty surrounding these toxicokinetic rate estimates.

The parameters for desorption of CBs were taken from studies of field-contaminated sediments (Cornelissen et al., 1997c,2000; ten Hulscher et al., 1999; Kan et al., 2000), however the characteristics of those sediments were likely different than the sediments underlying the East Sebasticook River. These differences (*e.g.,* grain size distribution, TOC) would add uncertainty to the model predictions. In addition, the desorption rates in both the laboratory data for FLU and TF and the field-contaminated sediments are based on biphasic or triphasic

kinetic descriptions of desorption as measured by Tenax® extraction in completely mixed conditions. Therefore, as has been suggested for desorption rates determined by gas stripping, the desorption rates in the natural environment and experienced by the organisms are likely smaller than would be measured for rapid and perhaps slow desorption under Tenax® extraction (Landrum and Robbins, 1990). These methods of determining desorption rate only consider the one-way transfer of contaminant off the sediment particle and do not consider the net flux of compound off of the sediments as a result of the processes of both desorption and readsorption.

The value of K_p was a sensitive model parameter that required the log K_{ow} as an input parameter to its calculation (Di Toro et al., 1991). A recent report identified many issues regarding the accuracy and quality of available K_{ow} and water solubility data (Renner, 2002). For example, log K_{ow} values for compounds such as DDT and DDE were found to range over 4 orders of magnitude (Renner, 2002). If the log K_{ow} estimates used for FLU, TF and the CBs in the present model have a degree of uncertainty about their estimates, then this uncertainty would be inherent in the model and could be exacerbated due to the importance of K_p to the calculation of sediment and pore water concentrations during the simulations.

Lastly, a weakness in the validation data set was that initial sediment concentrations for most congeners were estimated from CBs measured in pore water samples. It is preferred that these values come from actual measurements rather than estimates from pore water. However, the latter was necessary

because many of the congeners were consistently detected in samples of pore water and in the tissues of *in situ*-exposed *L. variegatus*, yet they were not detected in the analysis of sediment samples. In the case of the CBs, these compounds are volatile (Henry's Law constants ca. 150-500 Pa m^3/mol, Mackay et al., 1992) and reliable sediment concentrations from bulk samples are often difficult to measure. In the sediments collected from the East Sebasticook River study, only 1,2,4-triCB was detected and the mono-, and diCB levels measured in pore water samples were used to estimate sediment concentrations. Even with the uncertainty added from such estimates, the simulations carried out within the boundaries of the model parameters led to generally good agreement (within a factor of 10) between the observed and model-predicted bioaccumulation. Therefore, the use of the model as a screening tool at sites containing contaminated sediments seems to be an appropriate application.

Model simulations of field bioaccumulation were very sensitive to the input value for the feeding rate. Lower feeding rates (=0.04 g/g/h) often led to more accurate predictions. This suggests the feeding rates of organisms used for *in situ* bioaccumulation tests should be measured in future studies. A concern of investigators using short-term *in situ* toxicity and bioaccumulation test methods is whether the deployed organisms are behaving normally. Due to the stresses of handling and placement in an unknown environment, physiological conditions including the feeding rates can be depressed and thus future studies with *L. variegatus* should quantify any changes in feeding rate compared to appropriate reference site and/or laboratory controls. To date, the only feeding

rates reported from measurements taken during *in situ* toxicity testing were for the amphipod, *Gammarus pulex* (Maltby, 1999; Maltby et al., 2000). Other investigators have measured feeding rates of *D. magna* in the laboratory following an *in situ* exposure (McWilliam and Baird, 2002).

An important life-history aspect of *L. variegatus* that may impact its bioaccumulation of contaminants is its mode of asexual reproduction by architomy or splitting (Cook, 1969). After splitting, the worms do not feed for about 7 days as they regenerate their anterior or posterior parts (Leppänen and Kukkonen, 1998b). It is common practice that the worms used for *in situ* tests are taken from laboratory cultures and placed directly into chambers (Burton et al., 2001; Greenberg et al., 2002). In these studies, there was no attempt to minimize the impacts of reproduction during *in situ* bioaccumulation testing by selecting smaller (<9 mg wet wt), feeding individuals who have completed regeneration of their heads and tails (Leppänen and Kukkonen, 1998c; Van Hoof et al., 2001). The feeding rates used in the model were from measurements of selected worms that were known to be feeding (Leppänen and Kukkonen, 1998a,b,c). Therefore, *in situ* measured feeding rates on these mixed groups of feeders and non-feeders could improve the parameter estimate for FR and the model-predictions of the observations and may be useful to simulations of long-term accumulation.

The model supported the hypothesis that GSI could influence bioavailability and hence exposure and bioaccumulation of sediment-associated chemicals in benthic organisms. The parameter for the fractional flow of pore

water (q) was not necessary to obtain good fits to the bioaccumulation data for sites 5 and 18, where mixed upwelling and downwelling occurred (Greenberg et al., 2002; Chapter 5). Although at higher rates of feeding (0.01 and 0.04 g/g/h) a q of 10-25% also resulted in good predictions of the body burden data for these sites. These q values below full pore water flow (*i.e.,* $q < 1.0$) indicated that a smaller proportion of the exposure site was characterized by GSI (Greenberg et al., 2002). The value of q was most important at site 23, where downwelling conditions were detected in all sampled minipiezometers. *In situ* observations of toxicity in three species (*H. azteca*, *Ceriodaphnia dubia* and *Chironomus tentans*) and bioaccumulaton by *L. variegatus* suggested that the downwelling conditions at site 23 mobilized the bioavailable fraction of contaminants to deeper zones in the sediment bed, thus reducing exposures, effects and bioaccumulation in organisms in the surficial sediments (Greenberg et al., 2002; Chapter 5). Model simulations in which FR and was turned off and q was set to 1.0/h led to predictions that were near the observed body burden of 1,4-diCB at site 23 (Table 6.8). However, since it was unrealistic to assume that the worms did not feed during the test, using a minimal feeding rate of 0.01 g/g/h led to predicted body burdens that were within an order of magnitude of the measured value. With no pore water flow at this rate of feeding, the model predictions exceeded a factor of 10 of the measured tissue concentration and they were seen to be as high as 1565 (Table 6.10). These simulations suggested that pore water flow due to downwelling was a major determinant in the bioaccumulation of 1,4-diCB

at site 23 since adequate agreement between predictions and observations was only obtained when this flow was considered in the model.

Conclusions

The bioaccumulation model that was developed for infaunal *L. variegatus* described system dynamics including adsorption/desorption processes and pore water flow through the sediments. The model adequately predicted steady state tissue concentrations and the approach may be useful for exposure characterization in screening level ecological risk assessments. Because simulated pore water flow affected (decreased) predictions of both pore water and tissue concentrations in the exposures, the model gave support to the hypothesis that GSI (*i.e.,* upwelling, downwelling) can influence contaminant bioavailability and hence the exposure and bioaccumulation of sediment-associated chemicals in benthic species.

The model was successfully developed and verified using both laboratory and field bioaccumulation data. The bioaccumulation of sediment-associated FLU and TF by *L. variegatus* in laboratory exposures to spiked sediments was simulated using the model and there was good general agreement between the observed tissue concentrations and model-predicted body burdens of the test compounds. The model was sufficiently validated by simulation of CB body burden data from an *in situ* bioaccumulation study using *L. variegatus.* Upwelling and downwelling, or GSI, was qualitatively described with the fractional flow of pore water through the sediments (q, 1/h). The fractional pore water flow was shown to be an important determinant in the bioaccumulation of CBs when

conditions were downwelling in an exposure area, but was not as important as ingestion when mixed hydrologic conditions (*i.e.,* both upwelling and downwelling) were detected. In general, the model was capable of predicting the *in situ*-measured tissue levels of the CB congeners to within an order of magnitude, and many simulations that used rates of feeding =0.04 g dry sed/g wet animal/h and desorption rates on the order of 10^{-5}/h often resulted in predictions that were within a factor of 4 of the observations. Many input values including k_u, k_e, FR and AE, K_p and ρ were shown to be sensitive parameters and considerable uncertainty surrounded estimates of the kinetic rate constants and desorption terms for CBs due to their derivation in different test species and sediments.

The model can be improved by consideration of a number of additional factors. More research is needed to understand the relationship between laboratory measures of maximal desorption and field (*in situ*) desorption rates. Since the simulations of the field bioaccumulation study were very sensitive to the feeding rate, future *in situ* bioaccumulation tests should attempt to directly measure the feeding rates of field-deployed *L. variegatus*. In addition, future studies should also quantify additional stream bed characteristics that are important in GSI including the hydraulic conductivity of the sediments, pore water seepage rates, and physical changes to the stream bed over the exposure time. This will allow for improved correspondence between the value of q and the vectorial pore water flow rate through the sediments. Alternatively, with hydraulic conductivity data, the mathematical expression of the pore water flow from a

fractional value to a volumetric rate should be possible. These factors can be used to further describe the role of GSI in bioavailability, exposure and bioaccumulation beyond the simplistic approach of a pore water flow term that is currently in the model.

Table 6.1. Model terms including compartments, inflows and outflows (*i.e.,* equation types) and parameters. Each term is defined and the units are given. These terms are shown in the diagram of the uptake and accumulation model (Figure 6.1). Symbols used in the descriptions of the model equations (see text) are also included.

Name (symbol)	Type	Definition	Units
		Organism Sector	
Body Burden (C_a)	Compartment	Concentrations of chemical in body	µmol/g wet animal
uptake from water	Inflow	Uptake flux from water	µmol/g wet animal/h
uptake from feeding	Inflow	Uptake flux from feeding on sediments	µmol/g wet animal/h
elimination	Outflow	Elimination flux from organism	µmol/g wet animal/h
AE (AE)	Parameter	Assimilation efficiency of contaminant from ingestion	fraction
Cpw (C_{pw})	Parameter	Pore Water Concentration (see below)	µmol/mL
feeding rate (FR)	Parameter	Organism feeding rate on sediments	g dry sed/g animal/h
ke (k_e)	Parameter	Conditional elimination rate constant of chemical	1/h
kf (k_f)	Parameter	Uptake rate of chemical from feeding	g dry sed/g animal/h
ku (k_u)	Parameter	Conditional uptake clearance constant of chemical from pore water	mL/g wet animal/h
		Sediment Sector	
Sediment Conc (C_s)	Compartment	Concentration of contaminant in sediments	µmol/g dry wt

Pore Water Conc (C_{pw})	Compartment	Concentration of contaminant in pore water	µmol/mL
desorbed from sed	Inflow/Outflow	Desorption of contaminant from sediments to pore water	µmol/mL/h
readsorption to seds	Inflow/Outflow	Readsorption of remaining contaminant (i.e., that not taken up by organism) from pore water to sediments	µmol/mL/h
back to system by elimination	Inflow	Parent compound re-entering sediments from elimination by organism	µmol/g dry sed/h
reduction by animals	Outflow	Reduction of contaminant from particles due to animal ingestion	µmol/g dry sed/h
removal by animals	Outflow	Reduction of contaminant from pore water due to animal uptake	µmol/mL/h
lost from system	Outflow	Concentration of pore water lost with the flow of pore water	µmol/mL/h
Kp (K_p)	Parameter	Sediment-to-pore water partition coefficient of the contaminant	mL/g dry sed
phi (ø)	Parameter	Fraction of pore water per volume of sediment	fractional value
rho (ρ)	Parameter	Density of organisms per volume of pore water	g wet animal/mL
rhos (ρ_s)	Parameter	Mean density of sediment particles	g/mL sed
kdes (k_{des})	Parameter	Rate of contaminant desorption from sediments	1/h
frac flowing out (q)	Parameter	Fractional rate of contaminated pore water flowing out (i.e. replaced by fresh pore water)	1/h

Table 6.2. Parameter estimates for modeling the bioaccumulation of sediment-associated fluoranthene (FLU), trifluralin (TF) and chlorobenzenes (CBs) by *Lumbriculus variegatus*.

	Value of Parameters in Simulations				
	Lab Verification		Field Validation		
Parameter	**FLU**	**TF**	**CBs**	**Units**	**Source**
			Organism Sector		
AE	0.26[a]	0.26[a]	0.26[a]	fraction	[1,2]
FR	0.01 - 0.08	0.01 - 0.08	0.01 - 0.08	g sed dw/g org ww/h	[1,3-4]
k_e	0.116 ± 0.006	0.103 ± 0.004	0.10 - 0.26[b]	1/h	FLU & TF, [5]; CBnz, [6-11]
k_u	154 ± 14	121 ± 8.2	0.07 - 36[b]	mL/g org ww/h	FLU & TF, [5]; CBnz, [6-11]
			Sediment Sector		
K_p	2688; 4704[c]	3247; 5898[c]	see Table 6.5	mL/g sed dw	calculated [12-14]
ø	0.89; 0.91[d]	0.89; 0.91[d]	0.42	fractional value	FLU & TF, [5]; CBnz, [15]
ρ	0.00104[e]	0.00104[e]	0.0237[e]	g org ww/mL	[5,15-17]
ρ_s	2.5	2.5	2.5	g/mL sed	[18]
k_{des}	0.0002 - 0.59	0.0002 - 0.70	0.000042 - 0.0055	1/h	FLU & TF [5]; CBnz, [19-22]
q	0 -1	0 -1	0 -1	1/h	

[a] Species-specific value for benzo[a]pyrene assimilation by *Lumbriculus variegatus*.
[b] For Di- and Tri- Chlorobenzene congeners.
[c] K_p values for sediments from Lake Erie (f_{oc}, 0.021) or Lake Huron (f_{oc}, 0.036). Order: Lake Erie; Lake Huron.
[d] Order: Lake Erie; Lake Huron.
[e] Value within the theoretical maximum (0.0006-0.0256 g/mL) for *L. variegatus* based on min and max observed field densities [16-17].

[1,2] Kukkonen and Landrum (1995); Schuler & Lydy (2001).
[1,3-4] Kukkonen and Landrum (1995); Leppänen and Kukkonen (1998b,c).
[5] FLU & TF values from experiments.
[6-11] Van Hoogen and Opperhuizen (1988); Gabric et al. (1990); Belfroid et al. (1993); Sijm et al. (1993); Sijm and van der Linde (1995); Legierse et al. (1998).
[12-14] Di Toro et al. (1991); Mackay et al. (1992,1997); see Equations 6.4-6.5
[15] Values for chlorobenzenes from field study, Greenberg et al. (2002).
[16-17] Krezoski and Robbins (1985); Robbins et al. (1989).
[18] Robbins (1980).
[19-22] Cornelissen et al. (1997c,2000); ten Hulscher et al. (1999); Kan et al. (2000).

Table 6.3. Initial sediment concentrations used in simulations of the laboratory exposures of *Lumbriculus variegatus* to sediment-associated fluoranthene and trifluralin. The values represent the mean measured concentrations determined during the experiments (see Chapter 2).

		C_s (µmol/g dry sediment)	
Sediment	**Chemical**	**100 mg/kg treatment**	**200 mg/kg treatment**
Lake Erie	Fluoranthene	0.337	0.659
	Trifluralin	0.173	0.352
Lake Huron	Fluoranthene	0.406	0.746
	Trifluralin	0.210	0.386

Table 6.4. Values of feeding rate (FR; g dry sediment/g wet organism/h) and desorption rate (k_{des}; 1/h) used to predict body burdens of fluoranthene and trifluralin in *Lumbriculus variegatus* exposed in laboratory tests. Each data set (*i.e.,* each sediment, compound, treatment concentration) was simulated three times. FR and k_{des} were varied in these simulations to test the ability of the model to describe the experimentally measured bioaccumulation of FLU and TF under different assumptions of the relative roles of uptake via the routes of ingestion of contaminated particles and direct uptake from pore water (see text).

Compound	Sediment Concentration (µmol/g dry wt)	Simulation	FR (g/g/h)	kdes (1/h)	
				Lake Erie Sediments	Lake Huron Sediments
Fluoranthene	100	1	0.08	0.0003	0.003
		2	0.04	0.0008	0.03
		3	0.01	0.003	0.6
	200	1	0.08	0.0003	0.0004
		2	0.04	0.0008	0.004
		3	0.01	0.003	0.6
Trifluralin	100	1	0.08	0.0003	0.02
		2	0.04	0.0005	0.6
		3	0.01	0.003	0.6
	200	1	0.08	0.0003	0.003
		2	0.04	0.0005	0.6
		3	0.01	0.003	0.6

Table 6.5. Estimated K_p values for five chlorobenzenes.

Compound	MW (g/mol)	log K_{ow} [a]	log K_{oc} [b]	K_p [b]
MonoCB	113	2.77	2.73	12.0
1,2-DiCB	147	3.46	3.40	57.2
1,3-DiCB	147	3.52	3.47	66.0
1,4-DiCB	147	3.45	3.39	56.0
1,2,4-TriCB	181	4.08	4.01	229

[a]Mean values from Mackay et al. (1992).
[b]Calculated using Equations 6.4 and 6.5 with a mean f_{oc} of 0.0226.

Table 6.6. Chlorobenzene concentrations (µmol/g dry wt) estimated in sediments from measured concentrations in pore water samples taken from mini-piezometers at 10-30 cm depth in the sediments of the East Sebasticook River, Corrina, Maine, USA.

	Site		
Compound	**5**	**18**	**23**
MonoCB	0.100 ± 0.075	0.123 ± 0.046	0.024 ± 0.023
1,2-DiCB	0.011 ± 0.007	0.378 ± 0.217	ND
1,3-DiCB	0.043 ± 0.045	0.088 ± 0.014	0.008
1,4-DiCB	0.096 ± 0.087	0.486 ± 0.224	0.177 ± 0.154
1,2,4-TriCB	3.09×10^{-4} [a]	2.42×10^{-4} [a]	1.16×10^{-4} [a]

[a] Measured value. 1,2,4-TriCB was the only congener measured in sediment samples.

Table 6.7. Analytical sensitivity coefficients calculated as a percentage change in model output associated with a ±2x (factor of two change) in the input parameter (unless otherwise noted). The model simulation was for a 96-h exposure of *Lumbriculus variegatus* to Lake Erie sediments spiked with 100 mg fluoranthene/kg dry wt.

	Body Burden		Pore Water		Sediment	
Model Parameter	- D	+D	- D	+D	- D	+D
AE[a]	-22.78	64.99	0.30	-0.73	0.31	-0.74
FR[b]	-33.79	39.57	0.46	-0.47	0.47	-0.48
k_e	99.67	-50.07	0.17	-0.11	0.18	-0.12
k_u	-22.94	27.45	23.52	-28.08	0.02	0.01
q[c]	-12.43	-44.73	-19.77	-71.13	-0.16	-0.56
K_p[d]	24.81	-26.58	39.60	-42.46	0.03	0.02
ø[e]	18.39	-5.77	29.28	-9.17	0.18	-0.11
ρ	12.38	-62.21	19.62	-91.60	0.31	-14.33
ρ_s	-17.93	15.36	-28.41	24.46	-0.47	0.16
k_{des}[f]	-14.39	40.07	-22.98	63.99	0.02	0.01

[a] AE values represented the range of literature values (0.10 to 0.72, fractional value). See Table 6.2 and text for citations.
[b] FR values represented the range of literature values (0.0071 to 0.17 g sediments/g wet animal/h). See Table 6.2 and text for citations.
[c] Fraction of porewater flowing out varies depending on the system. For this analysis, the default was 0. To test the sensitivity of this parameter, the value was set to 0.01 and 1.0 (*i.e.,* full range) and the percentage change evaluated.
[d] The calculated range over log K_{ow} = 4.94-5.3; TOC = 2.0-3.66 was used.
[e] The % water in sediments and wet volume to wet mass ratio as upper and lower values, respectively, were used.
[f] The measured range of k_{des} values measured for fluoranthene (0.00017 to 0.37/h) were used. See Chapter 4.

Table 6.8. Summary of the closest and furthest estimates of 96-h chlorobenzene concentrations (µmol/g wet wt) in the tissues of *in situ* exposed *Lumbriculus variegatus* predicted by the model. The measured body burdens are shown for comparison. The magnitude of the difference between predicted and measured body burdens is indicated by the factor. Chlorobenzene tissue data from the *in situ* bioaccumulation test conducted on the East Sebasticook River, Corinna, ME, USA was used in the model validation. Parameters that were varied for visual calibration of the model and their values are also shown[a].

			Closest estimate			Furthest estimate		
Compound	**Site**	**Measured body burden (µmol/g wet wt)**	**Predicted body burden (µmol/g wet wt)**	**Factor**	**Parameter and value**	**Predicted body burden (µmol/g wet wt)**	**Factor**	**Parameter and value**
MonoCB	5	1.42e-03	1.43e-03	1.00	k_e = 0.265 FR = 0.00 q = 0.50 k_{des} = 4.2e-05	2.19e-01	154	k_e = 0.099 FR = 0.08 q = 0.00 k_{des} = 5.6e-03
	18	1.78e-03	1.76e-03	1.01	k_e = 0.265 FR = 0.00 q = 0.50 k_{des} = 4.2e-05	2.81e-01	158	k_e = 0.099 FR = 0.08 q = 0.00 k_{des} = 5.6e-03
1,2-DiCB	18	1.91e-03	3.94e-03	1.01	k_e = 0.265 FR = 0.00	8.65e-01	454	k_e = 0.099 FR = 0.08

					q =	1.00			q =	0.00
					k_{des} =	4.2e-05			k_{des} =	5.6e-03
1,3-DiCB	5	3.27e-04	4.46e-04	1.37	k_e =	0.265	7.88e-02	241	k_e =	0.099
					FR =	0.00			FR =	0.08
					q =	1.00			q =	0.00
					k_{des} =	4.2e-05			k_{des} =	5.6e-03
	18	6.12e-04	9.20e-03	1.50	k_e =	0.265	1.66e-01	271	k_e =	0.099
					FR =	0.00			FR =	0.080
					q =	1.00			q =	0.00
					k_{des} =	4.2e-05			k_{des} =	5.6e-03
1,4-DiCB	5	1.02e-03	1.00e-03	1.02	k_e =	0.265	1.88e-01	184	k_e =	0.099
					FR =	0.00			FR =	0.08
					q =	1.00			q =	0.00
					k_{des} =	4.2e-05			k_{des} =	5.6e-03
	18	3.74e-03	5.07e-03	1.36	k_e =	0.265	1.20e+00	321	k_e =	0.099
					FR =	0.00			FR =	0.08
					q =	1.00			q =	0.00
					k_{des} =	4.2e-05			k_{des} =	5.6e-03
	23	2.31e-04	1.84e-03	7.98	k_e =	0.265	3.62e-01	1565	k_e =	0.099
					FR =	0.00			FR =	0.080

					q = 1.00 k_{des} = 4.2e-05			q = 0.00 k_{des} = 5.6e-03	
1,2,4-TriCB	5[b]	8.27e-04	3.61e-04	2.29	k_e = 0.099 FR = 0.08 q = 0.00 k_{des} = 5.6e-03	3.18e-06	260	k_e = 0.265 FR = 0.00 q = 1.00 k_{des} = 4.2e-05	

[a] Parameters and units: k_e (1/h), elimination rate constant of the chemical from the animal; FR (g dry sediment/g wet animal/h), animal feeding rate on sediments; q (1/h), rate of contaminated pore water flowing out of the sediments; kdes (1/h), desorption rate.

[b] Predictions based on the measured sediment concentration.

Figure 6.1. Diagram of the uptake and accumulation model of sediment-associated organic contaminants by benthic invertebrates. The large boxes divide the model into conceptual submodels. Rectangles represent compartments which are described by "flows" representing equations. The circles describe individual parameters used in model equations and arrows indicate the connections of parameters to equations or compartment values to parameters. Ghosted boxes and circles represent aliases to model compartments and parameters that are used in more than one model equation. Definitions of compartments, flows and parameters are provided in Table 6.1.

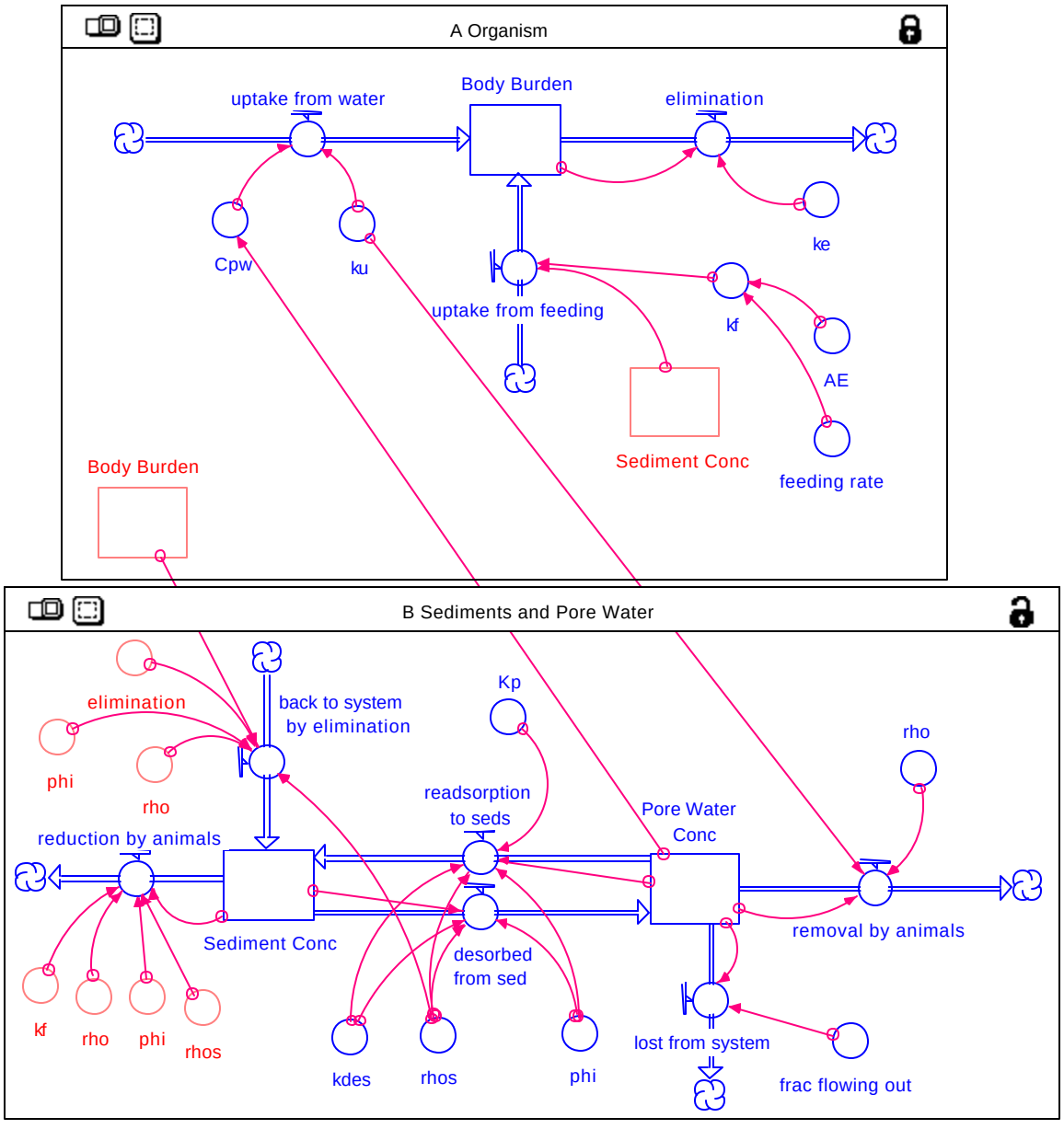


Figure 6.2. Example of a typical model output. The behavior of the model with the fractional flow of pore water (q, 1/h) set to zero (*i.e.,* no flow, stagnant system) is demonstrated. The exposure of *Lumbriculus variegatus* to 200 mg/kg of fluoranthene spiked onto Lake Erie sediments was simulated. The patterns of the body burden, sediment concentration, and pore water concentration are shown. Note that the scales for each of the three compartments on the graph are different.

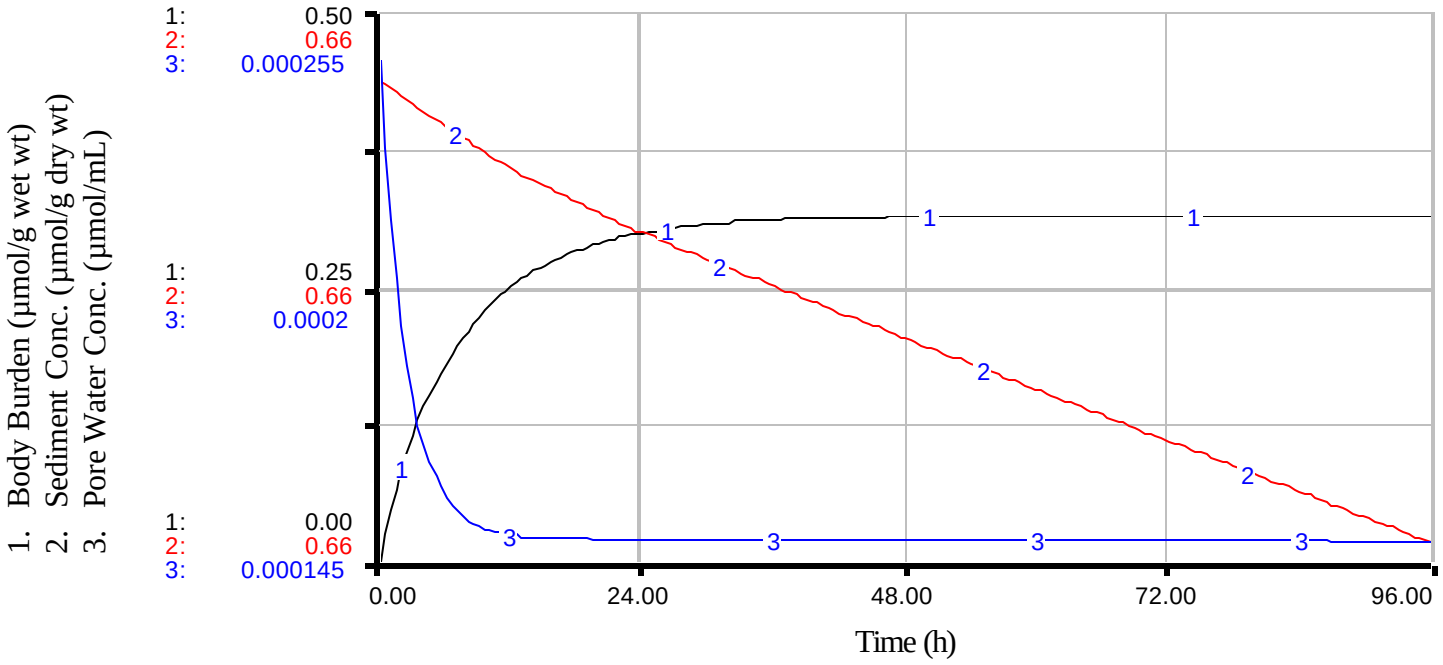


Figure 6.3. Example of a typical model output. This demonstrates the behavior of the model with the fractional flow of pore water (q, 1/h) set to 1.0 (*i.e.,* maximum flow, dynamic system). The exposure of *Lumbriculus variegatus* to 200 mg/kg of fluoranthene spiked onto Lake Erie sediments was simulated. The patterns of the body burden, sediment concentration, pore water concentration and the fraction desorbed are shown. Note that the scales for each of the four compartments on the graph are different.

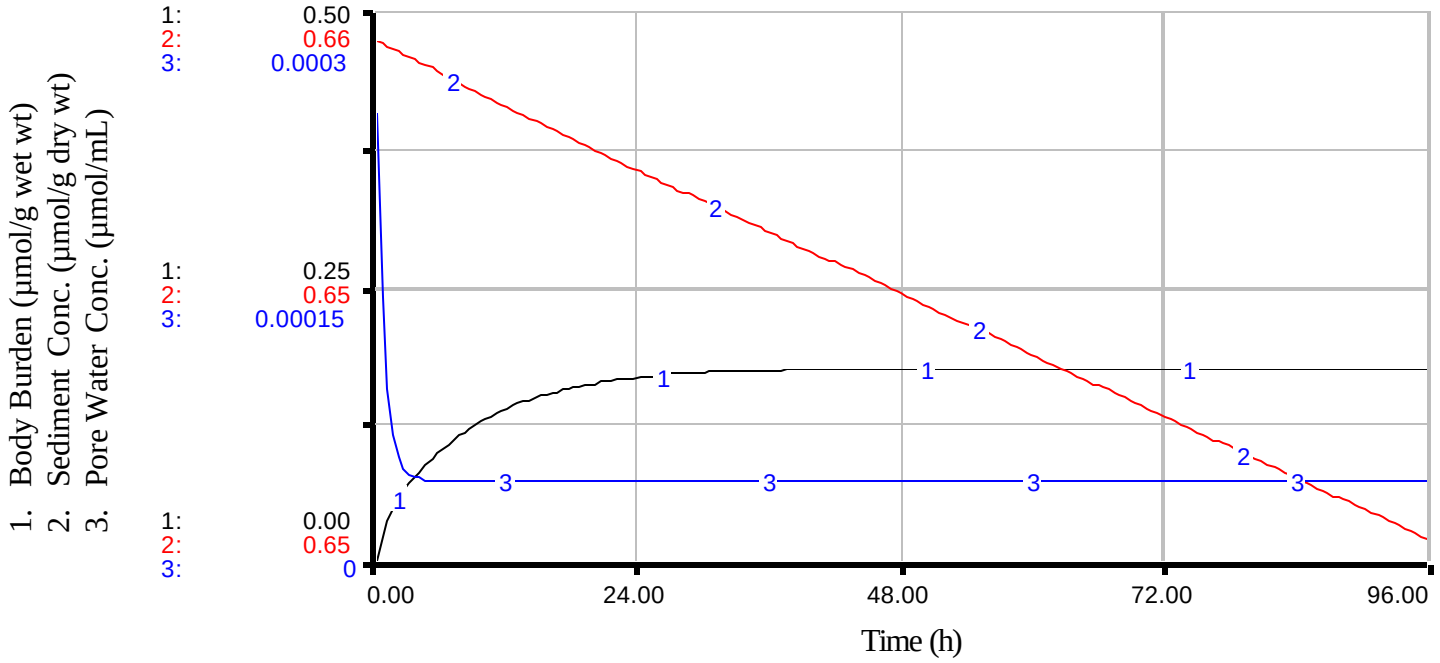


Figure 6.4. Comparison of model predictions (lines) and experimental tissue concentrations (symbols) of **(A)** fluoranthene and **(B)** trifluralin in *Lumbriculus variegatus* exposed to 100 mg/kg (solid circles) and 200 mg/kg (solid triangles) of the test chemicals spiked onto sediments from Lake Erie. Each data point represents the mean (± SD) of three samples. Values for the feeding rate (FR) and desorption rate (k_{des}) in simulations 1-3 are given in Table 6.4.

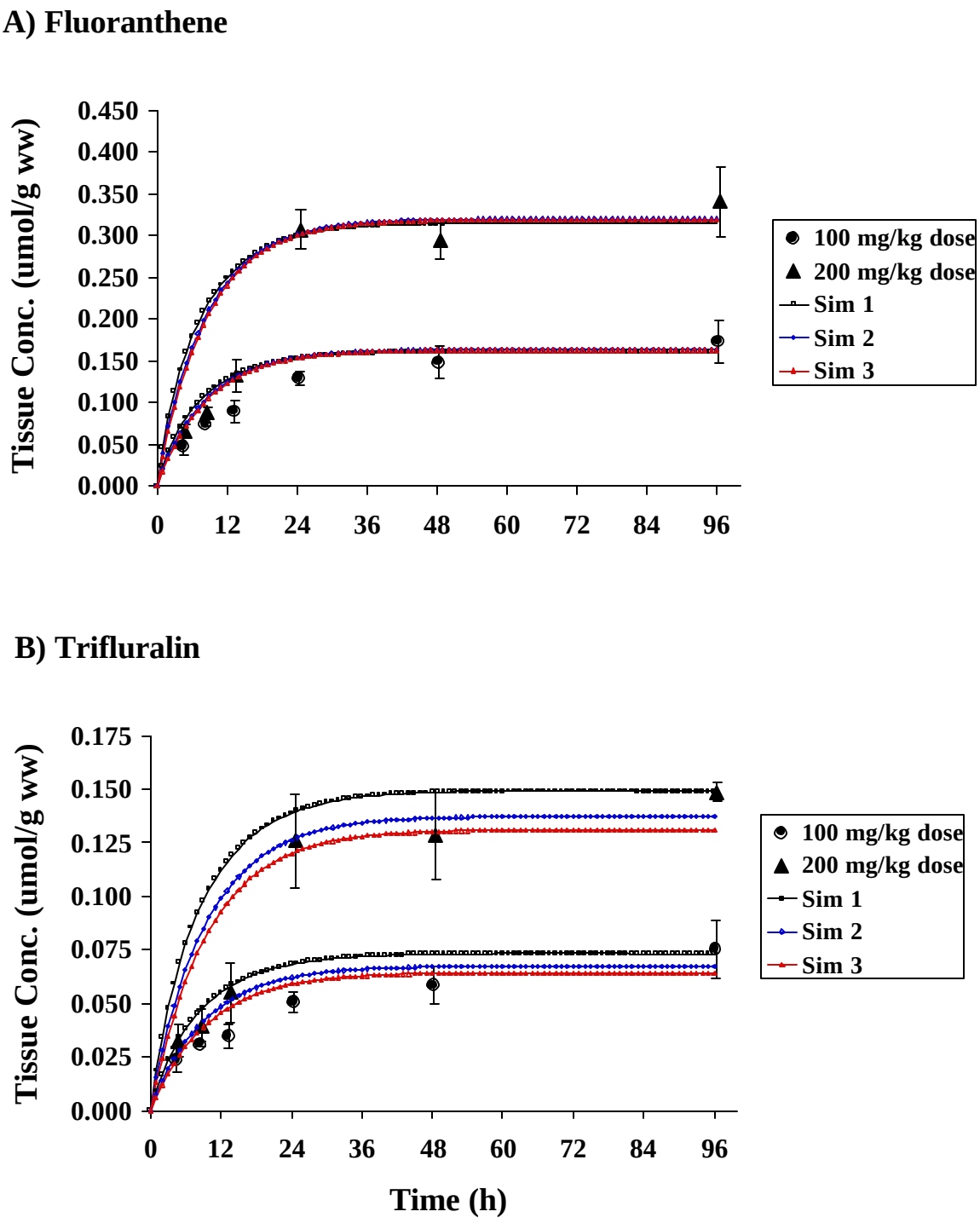
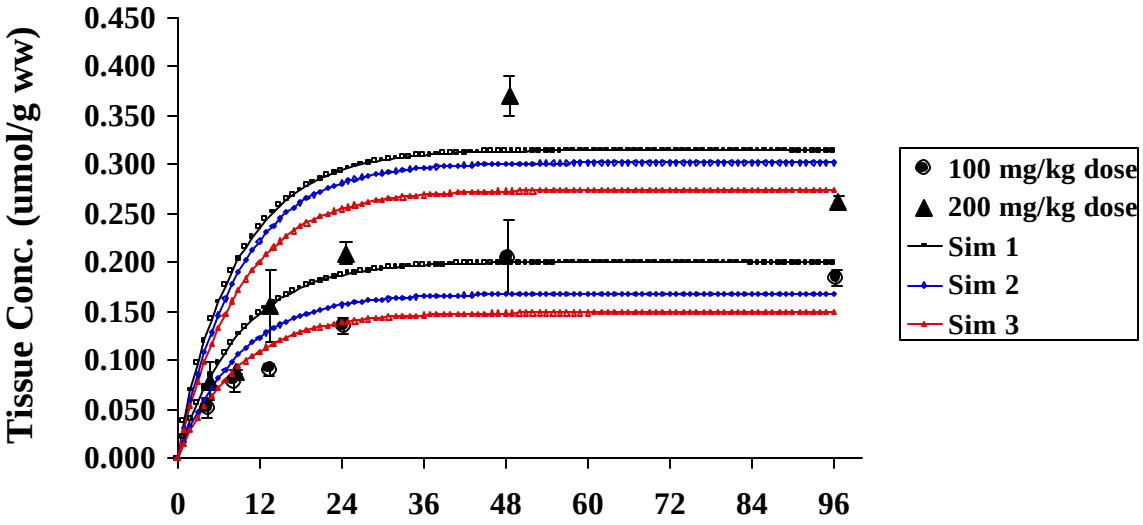
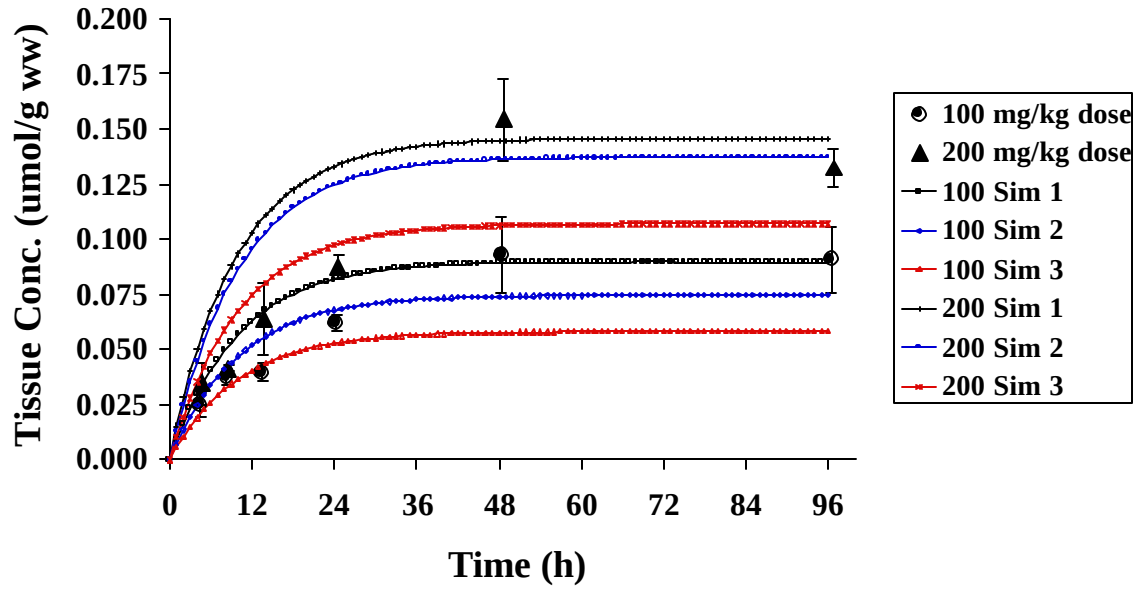


Figure 6.5. Comparison of model predictions (lines) and experimental tissue concentrations (symbols) of **(A)** fluoranthene and **(B)** trifluralin in *Lumbriculus variegatus* exposed to 100 mg/kg (solid circles) and 200 mg/kg (solid triangles) of the test chemicals spiked onto sediments from Lake Huron. Each data point represents the mean (± SD) of three samples. Values for the feeding rate (FR) and desorption rate (k_{des}) in simulations 1-3 are given in Table 6.4.

A) Fluoranthene



B) Trifluralin



CHAPTER 7

General Discussion and Conclusions

In this study, the original hypothesis was that factors such as the total organic carbon contents and interactions between groundwater and surface water in the sediments can affect chemical desorption, bioavailability and organism exposure in freshwater stream systems. The results of the experiments generally supported the hypothesis. The impact of upwelling and downwelling on exposure, effects and bioaccumulation were demonstrated using *in situ* testing methods and in simulations of pore water flow in the sediments using a bioaccumulation model.

The role of TOC in the bioavailability of sediment-associated FLU and TF was demonstrated in exposures of *L. variegatus* and *H. azteca* to sediments spiked with the test compounds and in studies of the desorption of the contaminants from the sediments. In the bioaccumulation tests, TOC affected the bioavailability of contaminants as indicated by: 1) the toxicity observed in exposures of *L. variegatus* to the 200 mg/kg sediments from Lake Erie, 2) the observed lack of feeding by *L. variegatus* and behavioral avoidance of *H. azteca* in the 200 mg/kg treatment from Lake Erie, and 3) the statistical differences in the estimated elimination rates of FLU and TF between Lakes Huron and Erie sediments for *H. azteca*. The organic contents of the sediments

from Lake Erie (~2.1%) were lower than in the Lake Huron sediments (~3.6%), and the contaminants were spiked on the basis of sediment dry weight. Thus, the higher TOC in the Lake Huron sediments would favor faster elimination as it would act as a reverse sink for contaminants from the organism back to the sediments during feeding (Landum and Scavia, 1983; Kukkonen and Landrum, 1994) or more simply by passive diffusion (Lotufo and Landrum, 2002). In addition, the partitioning of FLU and TF between sediment organic matter and pore water would result in higher exposure concentrations of the compounds in the pore water of the Lake Erie sediments (Di Toro et al., 1991). It was concluded that higher pore water concentrations in the lower TOC sediments combined with the mixture of two different compounds led to the observations of toxicity and sediment avoidance by the test species.

The desorption of FLU and TF from the Lake Huron sediments was higher than desorption from Lake Erie bottom sediments. This was interesting because it was contrary to the expected inverse relationship between desorption rates and TOC (Karickhoff et al., 1979; Wu and Gschwend, 1986; Kan et al., 1998; Celis et al., 1999; Chen et al., 2000). Evaluations of other measured sediment parameters including particle size distribution and polarity (*i.e.*, C:N ratios) of the sediments failed to explain the observation. Therefore, speculation as to the reasons for this difference included that the type, age and quality of the organic matter may have been responsible for the observed differences in desorption rates. Carbon structures which are analogous to flexible or glassy polymers have been theorized to control the amounts of rapidly (soft, flexible carbon) and

slowly (hard. glassy carbon) desorbing sites within the sediment organic matrix (Huang et al., 1997; Leboef and Weber, 1997, 1999; Cornelissen et al., 2000). Future research focused on these specific aspects of organic carbon and their roles in desorption will provide important details for determining bioavailability at contaminated sites.

The data collected on the desorption of FLU and TF appeared to have supported the generalization that the rapidly desorbing fraction (F_{rap}) of sediment-associated compounds are bioavailable (van Noort et al., 1999; Kraaij et al., 2000). The bioavailable concentrations (µmol/g dry wt) of FLU and TF were predicted from the bulk concentrations of the contaminants in Lake Huron and Lake Erie sediments (Cs, µmol/g dry wt):

$$\text{Bioavailable Concentration} = F_{rap} * C_s . \quad (7.1)$$

This value was then compared to the observed steady state tissue concentrations for *L. variegatus* (C_{ss}) and predicted C_{ss} levels using the toxicokinetic rates estimated for the exposed worms (see Chapter 2):

$$C_{ss}, = (k_s / k_e) * C_s , \quad (7.2)$$

where k_s is the conditional uptake clearance rate of a compound from sediments and pore water (g dry sediment/g wet wt organism/h) and k_e is the elimination rate (1/h). The results of this analysis are shown in Table 7.1.

There was very close correspondence between the calculated bioavailable concentrations of FLU and TF and both observed and predicted C_{ss} levels (Table 7.1). The ratios between dose for the predicted bioavailable and C_{ss} concentrations are also in very close agreement (Table 7.1). This approach was

successful for *L. variegatus* because the kinetic rates were not significantly different for the worms between the 100 and 200 mg/kg treatments. Also, this approach seems to be most applicable to infaunal species, such as *L. variegatus*, that are exposed to both pore water and contaminated particles. Interestingly, the dry weight concentrations of contaminants were very close to the wet weight concentrations in the worms. It is proposed that if enough parameters are measured (*i.e.,* F_{rap}, k_s, k_e) for a chemical of concern, this approach could be useful for estimating tissue concentrations of benthic invertebrates in screening level assessments of contaminated sediments. Even if kinetic rates of accumulation changed over a range of environmentally relevant contamination levels, such information would be useful because confidence limits could be determined for the predictions of body burden.

Toxicokinetic parameters were derived for *L. variegatus* and *H. azteca* from both sediment exposures (bioaccumulation tests) and water-only exposures (bioconcentration tests) to FLU and TF. Following the waterborne exposure of the organisms, the elimination rate ($k_{e(m)}$) was directly measured in the presence of uncontaminated sediments and water. One may argue that the value of k_s from the sediment test could have been more accurately estimated by re-fitting the bioaccumulation data to the two-compartment model (Equation 2.2) and using the experimentally measured value of $k_{e(m)}$ as an input. However, this was not done because the condition of the organisms, as indicated by their lipid contents was different between experiments. The mean lipid content (% of wet wt) of the worms ranged from 0.95 to 1.48% in the sediment exposures and were

1.69% in the water-only exposures. For *H. azteca* mean lipids were 1.48-2.14% and 3.15 in the sediment and waterborne experiments, respectively. The size of the lipid pools in organisms are important to both storage and elimination, with higher lipid concentrations leading to slower rates of elimination (Lotufo et al., 2000). Therefore, it was inappropriate to estimate k_s using the conditional elimination rate constant measured in the separate water-only study.

The novel bioaccumulation model that was developed from this research was capable of predicting body burdens for exposures in both the laboratory and in the field. A key assumption was that uptake from pore water was the dominant route of uptake over the exposure durations used in the experiments. Therefore, the uptake rate coefficients measured in the water-only exposures of *L. variegatus* and *H. azteca* were used to describe accumulation from pore water. Uptake from ingestion was based on reported feeding and chemical assimilation rates, and not from the uptake clearance rates of the compounds from sediments (k_s values) that were estimated from laboratory bioaccumulation tests. The decision to model uptake in this way was made because the k_s estimate integrates all of the processes that were separately modeled (*e.g.,* uptake from pore water and ingestion and desorption, adsorption and partitioning of contaminants in the sediments). The advantage to separately modeling pore water uptake and uptake by ingestion was that it allowed for the evaluation of the importance of both: 1) ingestion by the organisms; and, 2) desorption of the chemicals from sediments during an exposure. Therefore, future laboratory and *in situ* studies should investigate the relationships between desorption and

bioaccumulation in order to more accurately predict the tissue concentrations of indigenous benthic organisms.

Sediment toxicity assessment using both laboratory and field experiments offers a high degree of both control and environmental relevance. Hypothesis testing of sediment-specific factors such as TOC were addressed via laboratory investigations, while the system dynamics associated with GSI were evaluated with *in situ* exposures of benthic invertebrates. These dual approaches led to the conceptualization and development of a bioaccumulation model that was capable of representing exposure conditions in the field. This model was merely a first step; however, it represents a significant advancement because the kinetics of contaminants in organisms and sediments were simultaneously considered. Further refinement of the model should consider improving the descriptions of GSI beyond a simple expression of pore water flow. With such improvements, then contaminant fate and transport in the sediments, groundwater and surface water can be modeled, and a number of scenarios in which any of these compartments serves as the source of exposure to receptors of interest *(e.g.,* benthic invertebrates) can be simulated. This would provide a powerful tool that could be applied to predict the fate and effects of groundwater plumes, discharges to surface waters via accidental releases or effluents, and historically contaminated sediments that undergo weathering and aging.

Table 7.1. Relationship between the rapidly desorbing fraction (F_{rap}), bioavailability and the bioaccumulation of fluoranthene (FLU) and trifluralin (TF) from Lakes Huron (LH) and Erie (LE) sediments by *Lumbriculus variegatus*. The observed tissue concentrations at steady state (C_{ss}) are also shown for comparison to the calculations.

Sediment	Compound	Treatment	C_s (µmol/g dw)	F_{rap}	k_s/k_e [a]	"Bioavailable Amount" (µmol/g dw) [b]	C_{ss} calculated (µmol/g ww) [c]	C_{ss} observed (µmol/g ww)
LH	FLU	100 ppm	0.406	0.474	0.485	0.192	0.197	0.194 ± 0.027
		200 ppm	0.746	0.450	0.440	0.336	0.328	0.326 ± 0.062
					ratio	*1.75*	*1.67*	
	TF	100 ppm	0.210	0.485	0.450	0.102	0.094	0.092 ± 0.015
		200 ppm	0.386	0.508	0.392	0.196	0.151	0.145 ± 0.018
					ratio	*1.93*	*1.60*	
LE	FLU	100 ppm	0.337	0.438	0.491	0.147	0.165	0.161 ± 0.024
		200 ppm	0.659	0.379	0.401	0.250	0.265	0.312 ± 0.037
					ratio	*1.69*	*1.60*	
	TF	100 ppm	0.173	0.549	0.529	0.095	0.091	0.067 ± 0.014
		200 ppm	0.352	0.440	0.431	0.155	0.152	0.137 ± 0.018
					ratio	*1.63*	*1.66*	

[a] The ratio of the conditional uptake clearance rate of a compound from sediments and pore water (k_s; g dry sediment/g wet wt organism/h) to the elimination rate (k_e; 1/h). Also known as the kinetic maximum BSAF (Kraaij et al., 2001).

[b] The bioavailable amount was calculated with Equation 7.1.

c The calculated steady state tissue concentrations was determined by Equation 7.2.

APPENDIX A

Statistical Comparisons of the Desorption Curves Between Lake Erie and Lake Huron Sediments with Respect to Treatment Concentration

These additional statistical analyses are supplements to Chapter 4: *Desorption Kinetics of Fluoranthene and Trifluralin from Lake Huron and Lake Erie Sediments*. Pairwise comparisons of the curves were performed between sediments with respect to dose (i.e., 10 mg/kg Lake Huron versus 10 mg/kg Lake Erie). Tables begin on the next page.

Appendix A.1. Comparison of the fits for fluoranthene desorption at 10 mg/kg dry wt from the Lakes Erie and Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe desorption at this concentration for both sediments. All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	11	78	67	0.00832	
(G)	Common F_{vs}	11	78	67	0.01259	
(F)	Common k_{slow}	11	78	67	0.00817	
(E)	Common F_{slow}	11	78	67	0.00815	
(D)	Common k_{rap}	11	78	67	0.00918	
(C)	Common F_{rap}	11	78	67	0.00885	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	78	72	0.04440	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	12	78	66	0.00810	0.00012

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	0.03630	0.00605	49.29630	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	1	0.00075	0.00075	6.11111	0.01598	*
(D) - (A)	test of invariant k_{rap}	1	0.00108	0.00108	8.80000	0.00417	*
(E) - (A)	test of invariant F_{slow}	1	0.00005	0.00005	0.40741	0.52546	ns
(F) - (A)	test of invariant k_{slow}	1	0.00007	0.00007	0.57037	0.45276	ns
(G) - (A)	test of invariant F_{vs}	1	0.00449	0.00449	36.58519	<0.00001	*
(H) - (A)	test of invariant k_{vs}	1	0.00022	0.00022	1.79259	0.18514	ns

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),6,72} = 2.236$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),1,67} = 3.991$

Reject H_0 if $F > F_{crit}$

Appendix A.2. Comparison of the fits for trifluralin desorption at 10 mg/kg dry wt from the Lakes Erie and Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe desorption at this concentration for both sediments. All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	11	78	67	0.00645	
(G)	Common F_{vs}	11	78	67	0.00557	
(F)	Common k_{slow}	11	78	67	0.00577	
(E)	Common F_{slow}	11	78	67	0.00589	
(D)	Common k_{rap}	11	78	67	0.00707	
(C)	Common F_{rap}	11	78	67	0.00592	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	78	72	0.01208	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	12	78	66	0.00542	0.00008

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	0.00666	0.00111	13.51661	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	1	0.00050	0.00050	6.08856	0.01617	*
(D) - (A)	test of invariant k_{rap}	1	0.00165	0.00165	20.09225	0.00003	*
(E) - (A)	test of invariant F_{slow}	1	0.00047	0.00047	5.72325	0.01955	*
(F) - (A)	test of invariant k_{slow}	1	0.00035	0.00035	4.26199	0.04285	*
(G) - (A)	test of invariant F_{vs}	1	0.00015	0.00015	1.82657	0.18108	ns
(H) - (A)	test of invariant k_{vs}	1	0.00103	0.00103	12.54244	0.00073	*

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),6,72} = 2.236$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),1,67} = 3.991$

Reject H_0 if $F > F_{crit}$

Appendix A.3. Comparison of the fits for fluoranthene desorption at 40 mg/kg dry wt from the Lakes Erie and Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe desorption at this concentration for both sediments. All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	11	70	59	0.00264	
(G)	Common F_{vs}	11	70	59	0.00346	
(F)	Common k_{slow}	11	70	59	0.01419	
(E)	Common F_{slow}	11	70	59	0.00260	
(D)	Common k_{rap}	11	70	59	0.00377	
(C)	Common F_{rap}	11	70	59	0.00285	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	70	64	0.00936	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	12	70	58	0.00256	0.00004

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	0.00680	0.00113	25.67708	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	1	0.00029	0.00029	6.57031	0.01294	*
(D) - (A)	test of invariant k_{rap}	1	0.00121	0.00121	27.41406	<0.00001	*
(E) - (A)	test of invariant F_{slow}	1	0.00004	0.00004	0.90625	0.34500	ns
(F) - (A)	test of invariant k_{slow}	1	0.01163	0.01163	263.49219	<0.00001	*
(G) - (A)	test of invariant F_{vs}	1	0.00090	0.00090	20.39063	0.00003	*
(H) - (A)	test of invariant k_{vs}	1	0.00008	0.00008	1.81250	0.18336	ns

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),6,64} = 2.245$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),1,59} = 4.004$

Reject H_0 if $F > F_{crit}$

Appendix A.4. Comparison of the fits for trifluralin desorption at 40 mg/kg dry wt from the Lakes Erie and Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe desorption at this concentration for both sediments. All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	11	70	59	0.00466	
(G)	Common F_{vs}	11	70	59	0.00759	
(F)	Common k_{slow}	11	70	59	0.00469	
(E)	Common F_{slow}	11	70	59	0.00509	
(D)	Common k_{rap}	11	70	59	0.00635	
(C)	Common F_{rap}	11	70	59	0.00688	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	70	64	0.04405	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	12	70	58	0.00457	0.00008

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	0.03948	0.00658	83.50985	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	1	0.00231	0.00231	29.31729	<0.00001	*
(D) - (A)	test of invariant k_{rap}	1	0.00178	0.00178	22.59081	0.00001	*
(E) - (A)	test of invariant F_{slow}	1	0.00052	0.00052	6.59956	0.01275	*
(F) - (A)	test of invariant k_{slow}	1	0.00012	0.00012	1.52298	0.22206	ns
(G) - (A)	test of invariant F_{vs}	1	0.00302	0.00302	38.32823	<0.00001	*
(H) - (A)	test of invariant k_{vs}	1	0.00009	0.00009	1.14223	0.28953	ns

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),6,64} = 2.245$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),1,59} = 4.004$

Reject H_0 if $F > F_{crit}$

Appendix A.5. Comparison of the fits for fluoranthene desorption at 100 mg/kg dry wt from the Lakes Erie and Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe desorption at this concentration for both sediments. All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	11	78	67	0.00689	
(G)	Common F_{vs}	11	78	67	0.00838	
(F)	Common k_{slow}	11	78	67	0.00721	
(E)	Common F_{slow}	11	78	67	0.00760	
(D)	Common k_{rap}	11	78	67	0.00829	
(C)	Common F_{rap}	11	78	67	0.00768	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	78	72	0.04413	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	12	78	66	0.00667	0.00010

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	0.03746	0.00624	61.77811	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	1	0.00101	0.00101	9.99400	0.00236	*
(D) - (A)	test of invariant k_{rap}	1	0.00162	0.00162	16.02999	0.00016	*
(E) - (A)	test of invariant F_{slow}	1	0.00093	0.00093	9.20240	0.00344	*
(F) - (A)	test of invariant k_{slow}	1	0.00054	0.00054	5.34333	0.02388	*
(G) - (A)	test of invariant F_{vs}	1	0.00171	0.00171	16.92054	0.00011	*
(H) - (A)	test of invariant k_{vs}	1	0.00022	0.00022	2.17691	0.14478	ns

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),6,72} = 2.236$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),1,67} = 3.991$

Reject H_0 if $F > F_{crit}$

Appendix A.6. Comparison of the fits for trifluralin desorption at 100 mg/kg dry wt from the Lakes Erie and Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe desorption at this concentration for both sediments. All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	11	78	67	0.01224	
(G)	Common F_{vs}	11	78	67	0.01366	
(F)	Common k_{slow}	11	78	67	0.01207	
(E)	Common F_{slow}	11	78	67	0.01234	
(D)	Common k_{rap}	11	78	67	0.01242	
(C)	Common F_{rap}	11	78	67	0.01361	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	78	72	0.04031	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	12	78	66	0.01203	0.00018

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	0.02828	0.00471	25.85869	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	1	0.00158	0.00158	8.66833	0.00445	*
(D) - (A)	test of invariant k_{rap}	1	0.00039	0.00039	2.13965	0.14821	ns
(E) - (A)	test of invariant F_{slow}	1	0.00031	0.00031	1.70075	0.19665	ns
(F) - (A)	test of invariant k_{slow}	1	0.00004	0.00004	0.21945	0.64098	ns
(G) - (A)	test of invariant F_{vs}	1	0.00163	0.00163	8.94264	0.00389	*
(H) - (A)	test of invariant k_{vs}	1	0.00021	0.00021	1.15212	0.28696	ns

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),6,72} = 2.236$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),1,67} = 3.991$

Reject H_0 if $F > F_{crit}$

Appendix A.7. Comparison of the fits for fluoranthene desorption at 200 mg/kg dry wt from the Lakes Erie and Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe desorption at this concentration for both sediments. All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	11	78	67	0.00336	
(G)	Common F_{vs}	11	78	67	0.00417	
(F)	Common k_{slow}	11	78	67	0.00397	
(E)	Common F_{slow}	11	78	67	0.00414	
(D)	Common k_{rap}	11	78	67	0.00469	
(C)	Common F_{rap}	11	78	67	0.00439	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	78	72	0.06743	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	12	78	66	0.00327	0.00005

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	0.06416	0.01069	215.82875	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	1	0.00112	0.00112	22.60550	0.00001	*
(D) - (A)	test of invariant k_{rap}	1	0.00142	0.00142	28.66055	<0.00001	*
(E) - (A)	test of invariant F_{slow}	1	0.00087	0.00087	17.55963	0.00008	*
(F) - (A)	test of invariant k_{slow}	1	0.00070	0.00070	14.12844	0.00036	*
(G) - (A)	test of invariant F_{vs}	1	0.00090	0.00090	18.16514	0.00006	*
(H) - (A)	test of invariant k_{vs}	1	0.00009	0.00009	1.81651	0.18227	ns

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),6,72} = 2.236$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),1,67} = 3.991$

Reject H_0 if $F > F_{crit}$

Appendix A.8. Comparison of the fits for trifluralin desorption at 200 mg/kg dry wt from the Lakes Erie and Huron sediments. An *F*-test was used to test the hypothesis that common parameter estimates were sufficient to describe desorption at this concentration for both sediments. All comparisons were performed at α =0.05 with a one-tailed test. Abbreviations are as in Table 4.4. Reject H_0 if $F > F_{crit}$.

	Description of Fit or Test	No. param. (p)	No. obs. (n)	*df* (n-p)	RSS	RMS (=RSS/df)
(H)	Common k_{vs}	11	78	67	0.00393	
(G)	Common F_{vs}	11	78	67	0.00406	
(F)	Common k_{slow}	11	78	67	0.00493	
(E)	Common F_{slow}	11	78	67	0.00568	
(D)	Common k_{rap}	11	78	67	0.00654	
(C)	Common F_{rap}	11	78	67	0.00538	
(B)	Common F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	78	72	0.07404	
(A)	Individual F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	12	78	66	0.00365	0.00006

		df	DRSS	MS (=DRSS/df)	Var. Ratio, F (=MS/RMS)	*p*	Signif.
(B) - (A)[a]	test of invariant F_{rap}, k_{rap}, F_{slow}, k_{slow}, F_{vs}, k_{vs}	6	0.07039	0.01173	212.13425	<0.00001	*
(C) - (A)[b]	test of invariant F_{rap}	1	0.00173	0.00173	31.28219	<0.00001	*
(D) - (A)	test of invariant k_{rap}	1	0.00289	0.00289	52.25753	<0.00001	*
(E) - (A)	test of invariant F_{slow}	1	0.00203	0.00203	36.70685	<0.00001	*
(F) - (A)	test of invariant k_{slow}	1	0.00128	0.00128	23.14521	0.00001	*
(G) - (A)	test of invariant F_{vs}	1	0.00041	0.00041	7.41370	0.00824	*
(H) - (A)	test of invariant k_{vs}	1	0.00028	0.00028	5.06301	0.02773	*

[a]Critical F value, 1-tailed hypothesis test $F_{0.05(1),6,72} = 2.236$

[b]Critical F value, 1-tailed hypothesis test $F_{0.05(1),1,67} = 3.991$

APPENDIX B

Bioaccumulation model code from STELLA for Research

This model code was printed from simulations of the bioaccumulation of fluoranthene from Lake Erie sediments by *Lumbriculus variegatus.* The exposure concentration was 200 mg/kg.

A Organism Sector

Body_Burden(t) = Body_Burden(t - dt) + (uptake_from_water +

uptake_from_feeding - elimination) * dt

INIT Body_Burden = 0.000

INFLOWS:

uptake_from_water = Cpw*ku

uptake_from_feeding = kf*Sediment_Conc

OUTFLOWS:

elimination = Body_Burden*ke

AE = 0.26

Cpw = Pore_Water_Conc

feeding_rate = 0.08

ke = 0.11613

kf = feeding_rate*AE

ku = 153.70

B Sediments and Pore Water Sector

Pore_Water_Conc(t) = Pore_Water_Conc(t - dt) + (desorbed_from_sed - readsorption_to_seds - removal_by_animals - lost_from_system) * dt

INIT Pore_Water_Conc = Sediment_Conc/Kp

INFLOWS:

desorbed_from_sed = (kdes*Sediment_Conc*rhos*(1-phi))/phi

OUTFLOWS:

readsorption_to_seds = (Kp*kdes*Pore_Water_Conc*rhos*(1-phi))/phi

removal_by_animals = ku*rho*Pore_Water_Conc

lost_from_system = Pore_Water_Conc*frac_flowing_out

Sediment_Conc(t) = Sediment_Conc(t - dt) + (readsorption_to_seds + back_to_system_by_elimination - desorbed_from_sed - reduction_by_animals) * dt

INIT Sediment_Conc = 0.659

INFLOWS:

readsorption_to_seds = (Kp*kdes*Pore_Water_Conc*rhos*(1-phi))/phi

back_to_system_by_elimination = (elimination*rho*phi*Body_Burden)/(rhos*(1-phi))

OUTFLOWS:

desorbed_from_sed = (kdes*Sediment_Conc*rhos*(1-phi))/phi

reduction_by_animals = Sediment_Conc*((kf*rho*phi)/(rhos*(1-phi)))

frac_flowing_out = 0

kdes = 0.0003

Kp = 2687.56

phi = 0.8899

rho = 0.0010399

rhos = 2.5

REFERENCES

Abbot, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.

Adams, S. M. and M. S. Greeley. 2000. Ecotoxicological indicators of water quality: Using multi-response indicators to assess the health of aquatic ecosystems. *Water Air and Soil Pollution* 123(1-4): 103-115.

Ahokas, J. T. 1979. Cytochrome P-450 in fish liver microsomes and carcinogen activation: Amer. Chem. Soc. Symposium Series 99. pp 279-295.

Albanis, T. A., D. G. Hela, T. Sakellarides and I. K. Konstantinou. 1998. Monitoring of pesticide residues and their metabolites in surface and underground waters of Imathia (N. Greece) by means of solid-phase extraction disks and gas chromatography. *J. Chromatog. A* 823(1-2): 59-71.

Alexander, M. 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ. Sci. Technol.* 34(20): 4259-4265.

Ankley, G. T., D. J. Call, J. S. Cox, M. D. Kahl, R. A. Hoke and P. A. Kosian. 1994. Organic carbon partitioning as a basis for predicting the toxicity of chlorpyrifos in sediments. *Environ. Toxicol. Chem.* 13(4): 621-626.

Ankley, G. T., R. J. Erickson, G. L. Phipps, V. R. Mattson, P. A. Kosian, B. R. Sheedy and J. S. Cox. 1995. Effects of light intensity on the phototoxicity of fluoranthene to a benthic macroinvertebrate. *Environ. Sci. Technol.* 29(11): 2828-2833.

Ankley, G. T., R. J. Erickson, B. R. Sheedy, P. A. Kosian, V. R. Mattson and J. S. Cox. 1997. Evaluation of models for predicting the phototoxic potency of polycyclic aromatic hydrocarbons. *Aquat. Toxicol.* 37(1): 37-50.

APHA. 1985. *Standard methods for the examination of water and wastewater: 16th Edition*. M. A. Franson (ed.). American Public Health Association. pp 92-100.

ASTM. 1995a. *Standard guide for determination of the bioaccumulation of sediment-associated contaminants by benthic invertebrates*. E 1688-95. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1995b. *Standard test methods for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates*. E 1706-95a. American Society for Testing and Materials, Philadelphia, PA

ATSDR. 1995. *Toxicological profile for polycyclic aromatic hydrocarbons (PAHs)*. TP-95-20. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA. 231 p.

Bailer, A. J. 1988. Testing for the equality of area under the curves when using destructive measurement techniques. *J. Pharmacokin. Biopharm.* 16(3): 303-309.

Bailer, A. J. 1992. *Regression Analysis*. Miami University. Department of Mathematics and Statistics, Oxford, OH. 55 p.

Bailer, A. J., S. E. Walker and K. J. Venis. 2000. Estimating and testing bioconcentration factors. *Environ. Toxicol. Chem.* 19(9): 2338-2340.

Ball, W. P. and P. V. Roberts. 1991. Long-term sorption of halogenated organic chemicals by aquifer material. 2. Intraparticle diffusion. *Environ. Sci. Technol.* 25: 1237 - 1249.

Baumard, P., H. Budzinski, P. Garrigues, J. C. Sorbe, T. Burgeot and J. Bellocq. 1998. Concentrations of PAHs (polycyclic aromatic hydrocarbons) in various marine organisms in relation to those in sediments and to trophic level. *Mar. Pollut. Bull.* 36(12): 951-960.

Belden, J. B. and M. J. Lydy. 2000. Impact of atrazine on organophosphate insecticide toxicity. *Environ. Toxicol. Chem.* 19(9): 2266-2274.

Belfroid, A., A. Van Wezel, M. Sikkenk, K. Vangestel, W. Seinen and J. Hermens. 1993. The toxicokinetic behavior of chlorobenzenes in earthworms (*Eisenia andrei*)-Experiments in water. *Ecotoxicol. Environ. Saf.* 25(2): 154-165.

Benner, S. G., E. W. Smart and J. N. Moore. 1995. Metal behavior during surface-groundwater interaction, Silver Bow Creek, Montana. *Environ. Sci. Technol.* 29:1789-1795

Bjorklund, E., S. Bowadt, L. Mathiasson and S. B. Hawthorne. 1999. Determining PCB sorption desorption behavior on sediments using

selective supercritical fluid ertraction. 1. Desorption from historically contaminated samples. *Environ. Sci. Technol.* 33(13): 2193-2203.

Boese, B. L., H. Lee II, D. T. Specht, R. C. Randall and M. H. Winsor. 1990. Comparison of aqueous and solid-phase uptake for hexachlorobenzene in the tellinid clam *Macoma natuta* (Conrad): A mass balance approach. *Environ. Toxicol. Chem.* 9: 221-231.

Boese, B. L. and H. Lee II. 1992. *Synthesis of methods to predict bioaccumultion of sediment pollutants*. ERL-N/N232. U. S. EPA.

Boese, B. L., H. Lee and S. Echols. 1997. Evaluation of a first-order model for the prediction of the bioaccumulation of PCBs and DDT from sediment into the marine deposit-feeding clam Macoma nasuta. *Environ. Toxicol. Chem.* 16(7): 1545-1553.

Borgmann, U. 1996. Systematic analysis of aqueous ion requirements of *Hyalella azteca*: A standard artificial medium including the essential bromide ion. *Arch. Environ. Contam. Toxicol.* 30(3): 356-363.

Boulton, A. J. 1993. Stream ecology and surface-hyproheic hydrologic exchange: Implications, techniques and limitations. *Aust. J. Mar. Freshw. Res.* 44:553-564

Bremle, G. and G. Ewald. 1995. Bioconcentration of polychlorinated biphenyls (PCBs) in chironomid larvae, oligochaete worms and fish from contaminated lake sediment. *Mar. Freshw. Res.* 46(1): 267-273.

Brick, C. M. and J. N. Moore. 1996. Diel variation of trace metals in the Upper Clark Fork River, Montana. *Environ. Sci. Technol.* 30(6): 1953-1960.

Brinkhurst, R. O. and G. M. Jamieson. 1971. *Aquatic Oligochaeta of the World*. Oliver & Boyd, Edinburgh, UK.

Brooks, S. S., M. A. Palmer, B. J. Cardinale, C. M. Swan and S. Ribblett. 2002. Assessing stream ecosystem rehabilitation: Limitations of community structure data. *Restoration Ecology* 10(1): 156-168.

Bruner, K. A., S. W. Fisher and P. F. Landrum. 1994. The role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling .1. The effect of body size and lipid content on the bioconcentration of PCBs and PAHs. *J. Gt. Lakes Res.* 20(4): 725-734.

Brunson, E. L., T. J. Canfield, F. J. Dwyer, C. G. Ingersoll and N. E. Kemble. 1998. Assessing the bioaccumulation of contaminants from sediments of the upper Mississippi River using field-collected oligochaetes and

laboratory-exposed *Lumbriculus variegatus*. *Arch. Environ. Contam. Toxicol.* 35(2): 191-201.

Burgess, R. M. and K. J. Scott. 1992. The signficance of in-place contaminated marine sediments on the water column: Processes and effects. In *Sediment Toxicity Assessment*. G. A. Burton, Jr. (ed.). Lewis Publishers, Boca Raton, FL. pp 129-165.

Burgess, R. M., R. A. McKinney and W. A. Brown. 1996. Enrichment of marine sediment colloids with polychlorinated biphenyls: Trends resulting from PCB solubility and chlorination. *Environ. Sci. Technol.* 30(8): 2556-2566.

Burton, G. A., Jr. 1991. Assessing the toxicity of freshwater sediments. *Environ. Toxicol. Chem.* 10: 1585-1627.

Burton, G. A., Jr. 1995. Critical Issues in sediment bioassays and toxicity testing. *J. Aquat. Ecosys. Health.* 4: 151-156.

Burton, G. A., Jr. 1999. Realistic assessments of ecotoxicity using traditional and novel approaches. *Aquat. Ecosyst. Health Manage.* 2: 1-8.

Burton, G. A., R. Pitt and S. Clark. 2000. The role of traditional and novel toxicity test methods in assessing stormwater and sediment contamination. *Critical Reviews in Environmental Science and Technology* 30(4): 413-447.

Burton, G. A., Jr., M. S. Greenberg and others. 2001. *Assessment of In Situ Stressors and Sediment Toxicity in the Lower Housatonic River*. Final Report to The Lower Housatonic River Project, Pittsfield, MA. Wright State University. Institute for Environmental Quality, Dayton, OH.

Burton, G. A., Jr., M. S. Greenberg, C. Irvine, J. Johnson and C. D. Rowland. *in review. Standard Guide for Assessing Freshwater Ecosystem Impairment Using Caged Fish and Invertebrate Assays*. American Society for Testing and Materials (ASTM). Committee E 47, Philadelphia, PA.

Burton G. A., Jr., C. D. Rowland, M. S. Greenberg, D. R. Lavoie, J. F. Nordstrom and L. M. Eggert. *in press*. A tiered, weight-of-evidence approach for evaluating aquatic ecosystems. In *Sediment Quality Assessment: Approaches, Insights and Technology for the 21st century*, M. Munawar, (ed.). Ecovision World Monograph Series. Backhuys Publishers, Leiden, The Netherlands.

Camper, N. D., K. Stralka and H. D. Skipper. 1980. Aerobic and anaerobic degradation of profluralin and trifluralin. *J. Environ. Sci. Health Part B-Pestic. Contam. Agric. Wastes* 15(5): 457-473.

Carroll, K. M., M. R. Harness, A. A. Bracco and R. R. Balcarcel. 1994. Application of a permeant/polymer diffusion model to the desorption of PCBs from Husdon River sediments. *Environ. Sci. Technol.* 28: 253-258.

Cauchie, H. M., M. F. Jaspar-Versali, L. Hoffmann and J. P. Thome. 1999. Analysis of the seasonal variation in biochemical composition of *Daphnia magna* Straus (Crustacea : Branchiopoda : Anomopoda) from an aerated wastewater stabilisation pond. *Annal. Limnol.-Int. J. Limnol.* 35(4): 223-231.

Celis, R., W. C. Koskinen, M. C. Hermosin and J. Cornejo. 1999. Sorption and desorption of triadimefon by soils and model soil colloids. *J. Agric. Food Chem.* 47: 776-781.

Chaisuksant, Y., Q. Yu and D. Connell. 1997. Internal lethal concentrations of halobenzenes with fish (*Gambusia affinis*). *Ecotoxicol. Environ. Saf.* 37: 66-75.

Chapman, P. M. 1990. The sediment quality triad approach to determining pollution-induced degradation. *Sci. Tot. Environ.* 97/98:815-825.

Chappie, D. J. and G. A. J. Burton. 1997. Optimization of *in situ* bioassays with *Hyalella azteca* and *Chironomus tentans*. *Environ. Toxicol. Chem.* 16: 559-564.

Chen, W., A. T. Kan and M. B. Tomson. 2000. Irreversible adsorption of chlorinated benzenes to natural sediments: Implications for sediment anality criteria. *Environ. Sci. Technol.* 34(3): 385-392.

Chung, N. H. and M. Alexander. 1998. Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils. *Environ. Sci. Technol.* 32(7): 855-860.

Ciarelli, S., B. J. Kater and N. M. van Straalen. 2000. Influence of bioturbation by the amphipod Corophium volutator on fluoranthene uptake in the marine polychaete Nereis virens. *Environ. Toxicol. Chem.* 19(6): 1575-1581.

Colombo, J. C., C. Bilos, M. Campanaho, M. J. R. Presa and J. A. Catoggio. 1995. Bioaccumulation of polychlorinated biphenyls and chlorinated pesticides by the asiatic clam *Corbicula-Fluminea* - Its use as sentinel organism in the Rio De La Plata Estuary, Argentina. *Environ. Sci. Technol.* 29(4): 914-927.

Cook, D. G. 1969. Observation on the life history and ecology of some lumbriculidae (Annelida, Oligochaeta). *Hydrobiologia* 34: 561-574.

Cornelissen, G., P. C. M. vanNoort and H. A. J. Govers. 1997a. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: Sediment extraction with Tenax(R) and effects of contact time and solute hydrophobicity. *Environ. Toxicol. Chem.* 16(7): 1351-1357.

Cornelissen, G., P. C. M. VanNoort, J. R. Parsons and H. A. J. Govers. 1997b. Temperature dependence of slow adsorption and desorption kinetics of organic compounds in sediments. *Environ. Sci. Technol.* 31(2): 454-460.

Cornelissen, G., H. Rigterink, B. A. Vrind, T. E. M. tenHulscher, M. M. A. Ferdinandy and P. C. M. vanNoort. 1997c. Two-stage desorption kinetics and in situ partitioning of hexachlorobenzene and dichlorobenzenes in a contaminated sediment. *Chemosphere* 35(10): 2405-2416.

Cornelissen, G., H. Rigterink, M. M. A. Ferdinandy and P. C. M. Van Noort. 1998. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. *Environ. Sci. Technol.* 32(7): 966-970.

Cornelissen, G., H. van Zuilen and P. C. M. van Noort. 1999a. Particle size dependence of slow desorption of in situ PAHs from sediments. *Chemosphere* 38(10): 2369-2380.

Cornelissen, G. 1999b. *Mechanism and consequences of slow desorption of organic compounds from sediments*. Ph.D. Thesis. University of Amsterdam, Amsterdam, The Netherlands. 297 p.

Cornelissen, G., H. Rigterink, P. C. M. van Noort and H. A. J. Govers. 2000. Slowly and very slowly desorbing organic compounds in sediments exhibit Langmuir-type sorption. *Environ. Toxicol. Chem.* 19(6): 1532-1539.

Cornelissen, G., H. Rigterink, D. E. M. ten Hulscher, B. A. Vrind and P. C. M. van Noort. 2001. A simple Tenax(R) extraction method to determine the availability of sediment-sorbed organic compounds. *Environ. Toxicol. Chem.* 20(4): 706-711.

Cox, L., W. C. Koskinen and P. Y. Yen. 1997. Sorption-desorption of imidacloprid and its metabolites in soils. *J. Agric. Food Chem.* 45: 1468-1472.

Dabrowska, H. and S. W. Fisher. 1993. Environmental factors affecting the accumulation of sediment-sorbed hexachlorobiphenyls by channel catfish. *Aquat. Toxicol.* 27(1-2): 179-198.

Dahm, C. N., N. B. Grimm, P. Marmonier, H. M. Valett and P. Vervier. 1998. Nutrient dynamics at the interface between surface waters and groundwaters. *Freshwater Biology* 40(3): 427-451.

de March, B. G. E. 1981. *Hyalella azteca* (Saussure). In *Manual for the culture of selected freshwater invertebrates*. S. G. Lawrence (ed.). Can. Spec. Pub. fish. Aquat. Sci. No. 54. Department of Fisheries and Oceans.

Dewitt, T. H., R. J. Ozretich, R. C. Swartz, J. O. Lamberson, D. W. Schults, G. R. Ditsworth, J. K. P. Jones, L. Hoselton and L. M. Smith. 1992. The influence of organic matter quality on the toxicity and partitioning of sediment-associated fluoranthene. *Environ. Toxicol. Chem.* 11(2): 197-208.

Di Giulio, R. T., W. H. Benson, B. M. Sanders and P. A. Van Veld. 1995. Biochemical mechanisms: metabolism, adaptation, and toxicity. In *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment*. G. M. Rand (ed.). Taylor & Francis, Washington, D.C. pp 523-562.

Di Toro, D. M., C. S. Zarba, et al. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* 10(12): 1541-1583.

Diaz, R. D., C. Gaggi, J. C. Sanchezhernandez and E. Bacci. 1995. The role of soil and active ingredient properties in degradation of pesticides-A preliminary sssessment. *Chemosphere* 30(12): 2375-2386.

Ditsworth, G. R., D. W. Schults and J. K. P. Jones. 1990. Preparation of benthic substrates for sediment toxicity testing. *Environ. Toxicol. Chem.* 9: 1523-1529.

Dzantor, E. K. and A. S. Felsot. 1991. Microbial responses to large concentrations of herbicides in soil. *Environ. Toxicol. Chem.* 10(5): 649-655.

Eadie, B. J., N. R. Morehead and P. F. Landrum. 1990. Three-phase partitioning of hydrophobic organic compounds in great lakes waters. *Chemosphere* 20(1-2): 161-178.

Ellersieck, M. R. and T. W. La Point. 1995. Statistical analysis. In *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and*

Risk Assessment. G. M. Rand (ed.). Taylor & Francis, Washington, DC. pp 307-343.

Erkog, F. U. and R. E. Menzer. 1985. Metabolism of trifluralin in rats. *J. Agric. Food Chem.* 33(6): 1061-1070.

Farrell, J. and M. Reinhard. 1994. Desorption of halogenated organics from model solids, sediments, and soil under unsaturated conditions: 1. Isotherms. *Environ. Sci. Technol.* 28(1): 53-62.

Fay, A. A., B. J. Brownawell, A. A. Elskus and A. E. McElroy. 2000. Critical body residues in the marine amphipod *Ampelisca abdita*: Sediment exposures with nonionic organic contaminants. *Environ. Toxicol. Chem.* 19(4): 1028-1035.

Ferraro, S., I. H. Lee, L. Smith, R. Ozretich and D. T. Specht. 1990a. Accumulation factors for eleven polychlorinated biphenyl congeners. *Bull. Environ. Contam. Toxicol.* 46: 276-283.

Ferraro, S. P., H. Lee, II, R. J. Ozretich and D. T. Specht. 1990b. Predicting bioaccumulation potential a test of a fugacity-based model. *Arch. Environ. Contam. Toxicol.* 19(3): 386-394.

Fisher, S. W., S. W. Chordas and P. F. Landrum. 1999. Lethal and sublethal body residues for PCB intoxication in the oligochaete, *Lumbriculus variegatus*. *Aquat. Toxicol.* 45(2-3): 115-126.

Fisk, A. T., S. C. Wiens, G. R. B. Webster, W. Bergman and D. C. G. Muir. 1998. Accumulation and depuration of sediment-sorbed C-12- and C-16-polychlorinated alkanes by oligochaetes (*Lumbriculus variegatus*). *Environ. Toxicol. Chem.* 17(10): 2019-2026.

Fitzgerald, D. G., K. A. Warner, R. P. Lanno and D. G. Dixon. 1996. Assessing the effects of modifying factors on pentachlorophenol toxicity to earthworms: Applications of body residues. *Environ. Toxicol. Chem.* 15(12): 2299-2304.

Forbes, T. L., V. E. Forbes, A. Giessing, R. Hansen and L. K. Kure. 1998. Relative role of pore water versus ingested sediment in bioavailability of organic contaminants in marine sediments. *Environ. Toxicol. Chem.* 17(12): 2453-2462.

Forbes, V. E., M. S. H. Andreassen and L. Christensen. 2001. Metabolism of the polycyclic aromatic hydrocarbon fluoranthene by the polychaete *Capitella capitata* species I. *Environ. Toxicol. Chem.* 20(5): 1012-1021.

Franke, C. 1996. How meaningful is the bioconcentration factor for risk assessment? *Chemosphere* 32(10): 1897-1905.

Franke, C., G. Studinger, G. Berger, S. Bohling, U. Bruckmann, D. Cohorsfresenborg and U. Johncke. 1994. The assessment of bioaccumulation. *Chemosphere* 29(7): 1501-1514.

Fuchsman, P. C., D. J. Duda and T. R. Barber. 1999. A model to predict threshold concentrations for toxic effects of chlorinated benzenes in sediment. *Environ. Toxicol. Chem.* 18(9): 2100-2109.

Gabric, A. J., D. W. Connell and P. R. F. Bell. 1990. A kinetic model for bioconcentration of lipophilic compounds By oligochaetes. *Wat. Res.* 24(10): 1225-1231.

Gardner, W. S., W. A. Frez, E. A. Cichocki and C. C. Parrish. 1985. Micromethod For lipids in aquatic invertebrates. *Limnol. Oceanogr.* 30(5): 1099-1105.

George, S. G. and P. Young. 1988. Purification and Properties of Plaice Liver Cytosolic Glutathione-S-Transferases. *Mar. Environ. Res.* 24(1-4): 93-96.

Gerould, S. P., P. F. Landrum and J. P. Giesy. 1983. Anthracene bioconcentration and biotransformation in chironomids: effects of temperature and concentration. *Environ. Pollut. (Ser. A)* 30: 175-188.

Ghosh, U., J. S. Gillette, R. G. Luthy and R. N. Zare. 2000. Microscale location, characterization, and association of polycyclic aromatic hydrocarbons on harbor sediment particles. *Environ. Sci. Technol.* 34(9): 1729-1736.

Gobas, F. A. P. C., A. Opperhuizen and O. Hutzinger. 1986. Bioconcentration of hydrophobic chemicals in fish: Relationship with membrane permeation. *Environ. Toxicol. Chem.* 5: 637-646.

Gobas, F. A. P. C., D. C. Bedard, J. J. H. Ciborowski and G. D. Haffner. 1989. Bioaccumulation of chlorinated hydrocarbons by the mayfly (*Hexagenia limbata*) in Lake St. Clair. *J. Great Lakes Res.* 15(4): 581-588.

Gobas, F. A. P. C. 1992. Modeling the accumulation and toxicity of organic chemicals in aquatic food chains. In *Chemical Dynamics in Fresh Water Ecoystems.* F. A. P. C. Gobas and J. A. McCorquodale (eds.). Lewis Publishers, Boca Raton, FL. pp 129-151.

Golab, T. and J. L. Occolowitz. 1979. Soil degradation of trifluralin: Mass spectrometry of products and potential products. *Biomed. Mass Spectrom.* 6(1): 1-9.

Gossiaux, D. C., P. F. Landrum and V. N. Tsymbal. 1993. A survey of Saginaw River and Saginaw Bay, Lake Huron, sediments using two bioassays with the amphipod *Diporeia* spp. *J. Great Lakes Res.* 19(2): 322-332.

Graper, L. and D. Rainey. 1988. *Laboratory studies of carbon-14 trifluralin accumulation in bluegill sunfish*. Project ID: ABC-0372:ABC-0376. Lilly Research Laboratories, Greenfield, IN, USA. 63 p.

Grathwohl, P. 1990. Influence of organic matter from soils and sediments from various origin on the sorption of some chlorinated aliphatic hydrocarbons: Implications on K_{oc} correlations. *Environ. Sci. Technol.* 24(11): 1687-1693.

Greenberg, M. S., G. A. Burton, Jr. and C. D. Rowland. 2002. Optimizing interpretation of *in situ* effects of riverine pollutants: Impact of upwelling and downwelling. *Environ. Toxicol. Chem.* 21(2): 289-297.

Hamer, M. J. and F. Heimback. 1996. Ring testing a standardised test method for a toxicity test on sediment-dwelling *Chrionomus riparius* by spiking the overlying water. In *Development and Progress in Sediment Quality Assessment: Rationale, Challenges, Techniques & Strategies*. M. Munawar and G. Dave (eds.). Ecovision World Monograph Series. SPB Academic Publishing, Amsterdam, The Netherlands.

Hargrave, B. T. 1970. The utilization of benthic microflora by *Hyalella azteca*. *J. Animal Ecology* 39: 427-437.

Harkey, G. A., P. F. Landrum and S. J. Klaine. 1994. Comparison of whole-sediment, elutriate and pore water exposures for use in assessing sediment-associated organic contaminants in bioassays. *Environ. Toxicol. Chem.* 13(8): 1315-1329.

Harkey, G. A., P. L. Vanhoof and P. F. Landrum. 1995. Bioavailability of polycyclic aromatic hydrocarbons from a historically contaminated sediment core. *Environ. Toxicol. Chem.* 14(9): 1551-1560.

Harkey, G. A., S. K. Driscoll and P. F. Landrum. 1997. Effect of feeding in 30-day bioaccumulation assays using *Hyalella azteca* in fluoranthene-dosed sediment. *Environ. Toxicol. Chem.* 16(4): 762-769.

Hatch, A. C. and G. A. Burton. 1999. Photo-induced toxicity of PAHs to Hyalella azteca and Chironomus tentans: effects of mixtures and behavior. *Environ. Pollut.* 106(2): 157-167.

Hatzinger, P. B. and M. Alexander. 1995. Effect of aging of chemicals in soil on their biodegradability and extractability. *Environ. Sci. Technol.* 29(2): 537-545.

Hendricks, S. P. and D. S. White. 1995. Seasonal biogeochemical patterns in surface water, subsurface hyporheic, and riparian ground water in a temperate stream ecosystem. *Arch. Hydrobiol.* 134(4): 459-490.

Hendriks, A. J., A. van der Linde, G. Cornelissen and D. Sijm. 2001. The power of size. 1. Rate constants and equilibrium ratios for accumulation of organic substances related to octanol-water partition ratio and species weight. *Environ. Toxicol. Chem.* 20(7): 1399-1420.

Huang, W. L., T. M. Young, M. A. Schlautman, H. Yu and W. J. Weber. 1997. A distributed reactivity model for sorption by soils and sediments .9. General isotherm nonlinearity and applicability of the dual reactive domain model. *Environ. Sci. Technol.* 31(6): 1703-1710.

Hudiburgh, G. W., Jr. 1995. The Clean Water Act. In *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment*. G. M. Rand (ed.). Taylor & Francis, Washington, DC. pp 717-734.

Ingersoll, C. G. 1995. Sediment Tests. In *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment*. G. M. Rand (ed.). Taylor & Francis, Washington, DC. pp 231-255.

Ingersoll, C. G., F. J. Dwyer, S. A. Burch, M. K. Nelson, D. R. Buckler and J. B. Hunn. 1992. The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. *Environ. Toxicol. Chem.* 11(4): 503-511.

Ingersoll, C. G., G. T. Ankley, et al. 1995. Toxicity and bioaccumulation of sediment-associated contaminants using freshwater invertebrates - A review of methods and applications. *Environ. Toxicol. Chem.* 14(11): 1885-1894.

Ingersoll, C. G., P. S. Haverland, E. L. Brunson, T. J. Canfield, F. J. Dwyer, C. E. Henke, N. E. Kemble, D. R. Mount and R. G. Fox. 1996. Calculation and evaluation of sediment effect concentrations for the amphipod *Hyalella azteca* and the midge *Chironomus riparius*. *J. of Great Lakes Res.* 22(3): 602-623.

Ireland, D. S., G. A. J. Burton and G. G. Hess. 1996. *In situ* toxicity evaluations of turbidity and photoinduction of polycyclic aromatic hydrocarbons. *Environ. Toxicol. Chem.* 15: 574-581.

James, M. O. 1989. Biotransformation and disposition of PAH in aquatic invertebrates. In *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment.* U. Varanasi (ed.). CRC Press, Boca Raton, LF. pp 69-91.

James, M. O. and S. M. Boyle. 1998. Cytochromes P450 in crustacea. *Comp. Biochem. Physiol. C* 121: 157-172.

Jin-Clark, Y., M. J. Lydy and K. Y. Zhu. 2002. Effects of atrazine and cyanazine on chlorpyrifos toxicity in *Chironomus tentans* (Diptera : Chironomidae). *Environ. Toxicol. Chem.* 21(3): 598-603.

Johnson, S. E., J. S. Herman, A. L. Mills and G. M. Hornberger. 1999. Bioavailability and desorption characteristics of aged, nonextractable atrazine in soil. *Environ. Toxicol. Chem.* 18(8): 1747-1754.

Johnson, W. W. and M. T. Finley. 1980. *Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Summaries of toxicity tests conducted at Columbia National Fisheries Research Laboratory, 1965-78*. Resource Publication 137. U.S. Dept. of the Interior. Fish and Wildlife Service, Washington, DC.

Johnston, G., C. H. Walker and A. Dawson. 1994. Interactive effects between EBI fungicides (prochloraz, propiconazole and penconazole) and OP insecticides (dimethoate, chlorpyrifos, diazinon and malathion) in the hybrid red-legged partridge. *Environ. Toxicol. Chem.* 13(4): 615-620.

Jones, J. B., Jr., S. G. Fisher and N. B. Grimm. 1995. Vertical hydrologic exchange and ecosystem metabolism in a Sonoran Desert stream. *Ecology* 76(3): 942-952.

Kan, A. T., G. Fu, M. Hunter, W. Chen, C. H. Ward and M. B. Tomson. 1998. Irreversible sorption of neutral hydrocarbons to sediments: Experimental observations and model predictions. *Environ. Sci. Technol.* 32(7): 892-902.

Kan, A. T., W. Chen and M. B. Tomson. 2000. Desorption kinetics of neutral hydrophobic organic compounds from field-contaminated sediment. *Environ. Pollut.* 108(1): 81-89.

Kane Driscoll, S. and P. F. Landrum. 1997. A comparison of equilibrium partitioning and critical body residue approaches for predicting toxicity of sediment- associated fluoranthene to freshwater amphipods. *Environ. Toxicol. Chem.* 16(10): 2179-2186.

Kane Driscoll, S. B. and A. E. McElroy. 1997. Elimination of sediment-associated benzo[a]pyrene and its metabolites by polychaete worms exposed to 3-methylcholanthrene. *Aquat. Toxicol.* 39(1): 77-91.

Kane Driscoll, S., G. A. Harkey and P. F. Landrum. 1997a. Accumulation and toxicokinetics of fluoranthene in sediment bioassays with freshwater amphipods. *Environ. Toxicol. Chem.* 16(4): 742-753.

Kane Driscoll, S., P. F. Landrum and E. Tigue. 1997b. Accumulation and toxicokinetics of fluoranthene in water-only exposures with freshwater amphipods. *Environ. Toxicol. Chem.* 16(4): 754-761.

Kane Driscoll, S. B., L. C. Schaffner and R. M. Dickhut. 1998. Toxicokinetics of fluoranthene to the amphipod, *Leptocheirus plumulosus*, in water-only and sediment exposure. *Mar. Environ. Res.* 45(3): 269-284.

Kao, L. M., C. F. Wilkinson and L. B. Brattsten. 1995. In-vivo effects of 2,4-D and atrazine on cytochrome P450 and insecticide toxicity in southern armyworm (*Spodoptera eridania*) larvae. *Pestic. Sci.* 45: 331-334.

Karapanagioti, H. K., S. Kleineidam, D. A. Sabatini, P. Grathwohl and B. Ligouis. 2000. Impacts of heterogeneous organic matter on phenanthrene sorption: Equilibrium and kinetic studies with aquifer material. *Environ. Sci. Technol.* 34(3): 406-414.

Karickhoff, S. W. 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. *Chemosphere* 10(8): 833-846.

Karickhoff, S. W., D. S. Brown and T. A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. *Wat. Res.* 13: 241-248.

Karickhoff, S. W. and K. R. Morris. 1985a. Impact of tubificid oligochaetes on pollutant transport in bottom sediments. *Environ. Sci. Technol.* 19: 51-56.

Karickhoff, S. W. and K. R. Morris. 1985b. Sorption dynamics of hydrophobic pollutants in sediment suspensions. *Environ. Toxicol. Chem.* 4: 469-479.

Keilty, T. J., D. S. White and P. F. Landrum. 1988a. Sublethal responses to endrin in sediment by *Limnodrilus hoffmeisteri* (Tubificidae), and in mixed-culture with *Stylodrilus heringianus* (Lumbriculidae). *Aquat. Toxicol.* 13(3): 227-250.

Keilty, T. J., D. S. White and P. F. Landrum. 1988b. Sublethal responses to endrin in sediment by *Stylodrilus heringianus* (Lumbriculidae) as measured by a 137 cesium marker layer technique. *Aquat. Toxicol.* 13(3): 251-270.

Knezovitch, J. P., F. L. Harrison and R. G. Wilhelm. 1987. The bioavailability of sediment-sorbed organic chemicals: A review. *Water Air Soil Pollut* 32: 233-245.

Koelmans, A. A. and C. S. Jimenez. 1994. Temperature dependency of chlorobenzene bioaccumulation in phytoplankton. *Chemosphere* 28(12): 2041-2048.

Kosian, P. A., C. W. West, M. S. Pasha, J. S. Cox, D. R. Mount, R. H. Huggett and G. T. Ankley. 1999. Use of nonpolar resin for reduction of fluoranthene bioavailability in sediment. *Environ. Toxicol. Chem.* 18(2): 201-206.

Kraaij, R. H., S. Ciarelli, J. Tolls, B. J. Kater and A. Belfroid. 2001. Bioavailability of lab-contaminated and native polycyclic aromatic hydrocarbons to the amphipod *Corophium volutator* relates to chemical desorption. *Environ. Toxicol. Chem.* 20(8): 1716-1724.

Kraaij, R. H., J. Tolls, D. Sijm, G. Cornelissen, A. Heikens and A. Belfroid. 2002. Effects of contact time on the sequestration and bioavailability of different classes of hydrophobic organic chemicals to benthic oligochaetes (Tubificidae). *Environ. Toxicol. Chem.* 21(4): 752-759.

Kravitz, M. J., J. O. Lamberson, S. P. Ferraro, R. C. Swartz, B. L. Boese and D. T. Specht. 1999. Avoidance response of the estuarine amphipod *Eohaustorius estuarius* to polycyclic aromatic hydrocarbon-contaminated, field-collected sediments. *Environ. Toxicol. Chem.* 18(6): 1232-1235.

Krezoski, J. R. and J. A. Robbins. 1985. The vertical distribution of feeding and particle-selective transport of 137Cs in lake sediments by lumbriculid oligochaetes. *J. Geophys. Res.* 90: 11999-12006.

Kucklick, J. R., H. R. Harvey, P. H. Ostrom, N. E. Ostrom and J. E. Baker. 1996. Organochlorine dynamics in the pelagic food web of Lake Baikal. *Environ. Toxicol. Chem.* 15(8): 1388-1400.

Kukkonen, J. and P. F. Landrum. 1994. Toxicokinetics and toxicity of sediment-associated pyrene to *Lumbriculus variegatus* (Oligochaeta). *Environ. Toxicol. Chem.* 13(9): 1457-1468.

Kukkonen, J. and P. F. Landrum. 1995. Measuring assimilation efficiencies for sediment-bound PAH and PCB congeners by benthic organisms. *Aquat. Toxicol.* 32(1): 75-92.

Kukkonen, J. and P. F. Landrum. 1996. Distribution of organic carbon and organic xenobiotics among different particle-size fractions in sediments. *Chemosphere* 32(6): 1063-1076.

Kulkarni, A. P. and E. Hodgson. 1980. Hepatotoxicity. In *Introduction to Biochemical Toxicology*. E. Hodgson and F. E. Guthrie (eds.). Elsevier, New York, NY. pp 341-356.

L'Annunziata, M. F. and M. J. Kessler. 1998. Radiotracer liquid scintillation analysis. In *Handbook of radioactivity analysis*. M. F. L'Annunziata (ed.). Academic Press, San Diego, CA. pp 209-329.

Labrot, F., J. F. Narbonne, P. Ville, M. Saint Denis and D. Ribera. 1999. Acute toxicity, toxicokinetics, and tissue target of lead and uranium in the clam *Corbicula fluminea* and the worm *Eisenia fetida*: Comparison with the fish *Brachydanio rerio*. *Arch. Environ. Contam. Toxicol.* 36(2): 167-178.

Laflamme, R. E. and R. Hites. 1978. The global distribution of polycyclic aromatic hydrocarbons in recent sediments. *Geochim. Cosmochim* 42: 289-303.

Lake, J. L., N. I. Rubenstein and S. Pavignano. 1987. Predicting bioaccumulation: Development of a partitioning model for use as a screening tool in regulating ocean disposal of wastes. In *Fate and effects of sediment-bound chemicals in aquatic systems*. K. L. Dickson, A. W. Maki and W. A. Brungs (eds.). Pergammon Press, New York, NY. pp 151-166.

Landrum, P. F. 1988. Toxicokinetics of organic xenobiotics in the amphipod *PontoporeiA hoyi*: Role of physiological and environmental variables. *Aquat. Toxicol.* 12(3): 245-272.

Landrum, P. F. 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. *Environ. Sci. Technol.* 23(5): 588-595.

Landrum, P. F. and D. G. Crosby. 1981. The disposition of *p*-nitroanisole by the sea urchin, *Strongylocentrotus purpuratus* .1. Kinetics. *Ecotoxicol. Environ. Saf.* 5(2): 225-239.

Landrum, P. F. and D. Scavia. 1983. Influence of sediment on anthracene uptake, depuration, and biotransformation by the amphipod *Hyalella azteca*. *Can. J. Fish. Aquat. Sci.* 40(3): 298-305.

Landrum, P. F. and J. A. Robbins. 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. In *Sediments: Chemistry and*

Toxicity of In-Place Pollutants. R. Baudo, J. P. Giesy and H. Muntau (eds.). Lewis Publishers, Chelsea, MI. pp 237-263.

Landrum, P. F. and C. R. Stubblefield. 1991. Role of respiration in the accumulation of organic xenobiotics by the amphipod *Diporeia* Sp. *Environ. Toxicol. Chem.* 10(8): 1019-1028.

Landrum, P. F. and S. W. Fisher. 1998. Influence of lipids on the bioaccumulation and trophic transfer of organic contaminants in aquatic organisms. In *Lipids in Freshwater Ecosystems*. M. Arts and B. Wainman (eds.). Springer-Verlag, New York, NY. pp 203-234.

Landrum, P. F. and J. G. A. Burton. Polluted sediments: The continuing threat. *Unpublished manuscript* .

Landrum, P.F., W. R. Faust and B. J. Eadie. 1989. Bioavailability and toxicity of a mixture of sediment-associated chlorinated hydrocarbons to the amphipod, *Pontoporeia hoyi*. In *Aquatic Toxicology and Hazard Assessment: 12th Volume*. U. M. Cowgill and L. R. Williams (eds.). ASTM STP 1027. American Society for Testing and Materials, Philadelphia, PA. pp 315-329.

Landrum, P. F., B. J. Eadie and W. R. Faust. 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic-hydrocarbons to the amphipod *Diporeia* Sp. *Environ. Toxicol. Chem.* 10(1): 35-46.

Landrum, P. F., H. I. Lee and M. J. Lydy. 1992a. Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environ. Toxicol. Chem.* 11(12): 1709-1725.

Landrum, P. F., B. J. Eadie and W. R. Faust. 1992b. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. *Environ. Toxicol. Chem.* 11(8): 1197-1208.

Landrum, P. F., W. S. Dupuis and J. Kukkonen. 1994. Toxicokinetics and toxicity of sediment-associated pyrene and phenanthrene in *Diporeia* spp.: Examination of equilibrium-partitioning theory and residue-based effects for assessing hazard. *Environ. Toxicol. Chem.* 13(11): 1769-1780.

Landrum, P. F., D. C. Gossiaux and J. Kukkonen. 1997. Sediment characteristics influencing the bioavailability of nonpolar organic contaminants to *Diporeia* spp. *Chemical Speciation and Bioavailability* 9(2): 43-55.

Landrum, P. F., M. L. Gedeon, G. A. Burton, Jr., M. S. Greenberg and C. D. Rowland. 2002. Biological responses of *Lumbriculus variegatus* exposed to fluoranthene-spiked sediment. *Arch. Environ. Contam. Toxicol.* 42: 292-302.

La Point, T. W. and J. F. Fairchild. 1990. Evaluation of sediment contaminant toxicity: The use of freshwater community structure. In *Sediment Toxicity Assessment*. Burton, GA Jr., (ed.). Lewis Publishers, Chelsea, MI, USA, pp. 87-110.

Lavy, D., O. Nedved and H. A. Verhoef. 1997. Effects of starvation on body composition and cold tolerance in the collembolan *Orchesella cincta* and the isopod *Porcellio scaber*. *J. Insect Physiol.* 43(10): 973-978.

Lawrence, M. A. M., N. A. Davies, P. A. Edwards, M. G. Taylor and K. Simkiss. 2000. Can adsorption isotherms predict sediment bioavailability? *Chemosphere* 41(7): 1091-1100.

Leboeuf, E. J. and W. J. Weber, Jr. 1997. A distributed reactivity model for sorption by soils and sediments. 8. Sorbent organic domains: Discovery of a humic acid glass transition and an argument for a polymer-based model. *Environ. Sci. Technol.* 31(6): 1697-1702.

LeBoeuf, E. J. and W. J. Weber. 1999. Re-evaluation of general partitioning model for sorption of hydrophobic organic contaminants by soil and sediment organic matter. *Environ. Toxicol. Chem.* 18(8): 1617-1626.

Lee, D. R. and J. A. Cherry. 1978. A field exercise on groundwater flow using seepage meters and mini-piezometers. *J. Geol. Educ*. 27:6-10

Lee, H., II. 1992. Models, muddles, and mud: predicting bioaccumulation of sediment-associated pollutants. In *Sediment Toxicity Assessment*. G. A. Burton, Jr. (ed.). Lewis Publishers, Boca Raton, FL. pp 267-293.

Lee, R. F. 1998. Annelid cytochrome P-450. *Comp. Biochem. Physiol. C* 121: 173–179.

Legierse, K., D. Sijm, C. J. van Leeuwen, W. Seinen and J. L. M. Hermens. 1998. Bioconcentration kinetics of chlorobenzenes and the organophosphorus pesticide chlorthion in the pond snail *Lymnaea stagnalis* - a comparison with the guppy *Poecilia reticulata*. *Aquat. Toxicol.* 41(4): 301-323.

Lenihan, H. S., K. A. Kiest, K. E. Conlan, P. N. Slattery, B. H. Konar and J. S. Oliver. 1995. Patterns of survival and behavior in Antarctic benthic

invertebrates exposed to contaminated sediments: Field and laboratory bioassay experiments. *J. Exp. Mar. Biol. Ecol.* 192(2): 233-255.

Leppänen, M. T. 1999. *Bioaccumulation of sediment-associated polycyclic aromatic hydrocarbons in the freshwater oligochaete Lumbriculus variegatus (Muller)*. University of Joensuu, Joensuu, Finland. Ph.D. Thesis.

Leppänen, M. T. and J. V. K. Kukkonen. 1998a. Factors affecting feeding rate, reproduction and growth of an oligochaete *Lumbriculus variegatus* (Muller). *Hydrobiologia* 377: 183-194.

Leppänen, M. T. and J. V. K. Kukkonen. 1998b. Relative importance of ingested sediment and pore water as bioaccumulation routes for pyrene to oligochaete (*Lumbriculus variegatus*, Muller). *Environ. Sci. Technol.* 32(10): 1503-1508.

Leppänen, M. T. and J. V. K. Kukkonen. 1998c. Relationship between reproduction, sediment type, and feeding activity of *Lumbriculus variegatus* (Muller): Implications for sediment toxicity testing. *Environ. Toxicol. Chem.* 17(1): 2196-2202.

Leppänen, M. T. and J. V. K. Kukkonen. 2000. Fate of sediment-associated pyrene and benzo[a]pyrene in the freshwater oligochaete *Lumbriculus variegatus* (Muller). *Aquat. Toxicol.* 49(3): 199-212.

Leversee, G. J., J. P. Giesy, P. F. Landrum, S. Gerould, J. W. Bowling, T. E. Fannin, J. D. Haddock and S. M. Bartell. 1982. Kinetics and Biotransformation of Benzo(a)Pyrene in *Chironomus riparius*. *Arch. Environ. Contam. Toxicol.* 11(1): 25-31.

Levine, S. L. and J. T. Oris. 1999. Enhancement of acute parathion toxicity to fathead minnows following pre-exposure to propiconazole. *Pest. Biochem. Physiol.* 65(2): 102-109.

Liska, I. and K. Bilikova. 1998. Stability of polar pesticides on disposable solid-phase extraction precolumns. *J. Chromatog. A* 795: 61-69.

Loonen, H., D. C. G. Muir, J. R. Parsons and H. A. J. Govers. 1997. Bioaccumulation of polychlorinated dibenzo-p-dioxins in sediment by oligochaetes: Influence of exposure pathway and contact time. *Environ. Toxicol. Chem.* 16(7): 1518-1525.

Lotufo, G. R. 1997. Toxicity of sediment-associated PAHs to an estuarine copepod: Effects on survival, feeding, reproduction and behavior. *Mar. Environ. Res.* 44(2): 149-166.

Lotufo, G. R. 1998a. Lethal and sublethal toxicity of sediment-associated fluoranthene to benthic copepods: application of the critical body residue approach. *Aquat. Toxicol.* 44(1-2): 17-30.

Lotufo, G. R. 1998b. Bioaccumulation of sediment-associated fluoranthene in benthic copepods: Uptake, elimination and biotransformation. *Aquat. Toxicol.* 44(1-2): 1-15.

Lotufo, G. R., P. F. Landrum, M. L. Gedeon, E. A. Tigue and L. R. Herche. 2000. Comparative toxicity and toxicokinetics of DDT and its major metabolites in freshwater amphipods. *Environ. Toxicol. Chem.* 19(2): 368-379.

Lotufo, G. R. and P. F. Landrum. 2002. The influence of sediment and feeding on the elimination of polycyclic aromatic hydrocarbons in the freshwater amphipod, *Diporeia* spp. *Aquat. Toxicol.* 58(3-4): 137 - 149.

Luthy, R. G., G. R. Aiken, et al. 1997. Sequestration of hydrophobic organic contaminants by geosorbents. *Environ. Sci. Technol.* 31(12): 3341-3347.

Lydy, M. J., J. L. Lasater and P. F. Landrum. 2000. Toxicokinetics of DDE and 2-chlorobiphenyl in *Chironomus tentans*. *Arch. Environ. Contam. Toxicol.* 38(2): 163-168.

MacDonald, D. D., C. G. Ingersoll and T. A. Berger. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch. Environ. Contam. Toxicol.* 39:20-31.

Macek, K. J., M. A. Lindberg, S. Sauter, K. S. Buxton and P. A. Costa. 1976. *Toxicity of four pesticides to water fleas and fathead minnows* . EPA-600/3-76-099, Washington, DC. 58 p.

Mackay, D., W.-Y. Shiu and K.-C. Ma. 1992. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals: Volume I-Monoaromatic Hydrocarbons, Chlorobenzenes, and PCBs.* Lewis Publishers, Chelsea, MI. 704 p.

Mackay, D., W.-Y. Shiu and K.-C. Ma. 1997. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals: Volume V-Pesticide Chemicals*. Lewis Publishers, Boca Raton, FL. 812 p.

Maltby, L. 1999. Studying stress: The importance of organism-level responses. *Ecological Applications* 9(2): 431-440.

Maltby, L., S. A. Clayton, H. X. Yu, N. McLoughlin, R. M. Wood and D. Q. Yin. 2000. Using single-species toxicity tests, community-level responses and toxicity identification evaluations to investigate effluent impacts. *Environ. Toxicol. Chem.* 19(1): 151-157.

Malterre, F., G. Grebil, J. G. Pierre and M. Schiavon. 1997. Trifluralin behaviour in soil: A microlysimeter study. *Chemosphere* 34(3): 447-454.

Mayer, F. L., Jr. and M. R. Ellersieck. 1986. *Manual of Acute Toxicity: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater Animals*. Resource Publication 160. U.S. Dept. of the Interior. Fish and Wildlife Service, Washington, DC.

McCall, P. J. and G. L. Agin. 1985. Desorption kinetics of picloram as affected by residence time in the soil. *Environ. Toxicol. Chem.* 4: 37-44.

McCarty, L. S. and D. Mackay. 1993. Enhancing ecotoxicological modeling and assessment. *Environ. Sci. Technol.* 27(9): 1719-1728.

McCarty, L. S., D. Mackay, A. D. Smith, G. W. Ozburn and D. G. Dixon. 1992a. Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays - Neutral narcotic organics. *Environ. Toxicol. Chem.* 11(7): 917-930.

McCarty, L. S., G. W. Ozburn, A. D. Smith and D. G. Dixon. 1992b. Toxicokinetic modeling of mixtures of organic chemicals. *Environ. Toxicol. Chem.* 11(7): 1037-1047.

McElroy, A. E. and J. C. Means. 1988. Factors effecting the bioavailability of hexachlorobiphenyls to benthic organisms. In *Aquatic Toxicology and Hazard Assessment*. W. J. Adams, G. A. Chapman and W. G. Landis (eds.). American Society for Testing and Materials, Philadelphia, PA. Vol. 10 STP 971. pp 149-158.

McWilliam, R. A. and D. J. Baird. 2002. Postexposure feeding depresison: a new toxicity endpoint for use in laboratory studies with *Daphnia magna*. *Environ. Toxicol. Chem.* 21(6): 1198-1205.

Meador, J. P., E. Casillas, C. A. Sloan and U. Varanasi. 1995. Comparative bioaccumulation of polycyclic aromatic hydrocarbons from sediment by two infaunal invertebrates. *Mar. Ecol.-Prog. Ser.* 123(1-3): 107-124.

Meador, J. P., J. E. Stein, W. L. Reichert and U. Varanasi. 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Rev. Environ. Contam. Toxicol.* 143: 79-165.

Medina, H. S. G. 1986. The effect of diamino diphenyl sulfone on the embryonic development of eggs from the sea urchin (*Lytechinus Variegatus*). *Comp. Biochem. Physiol. C-Pharmacol. Toxicol. Endocrinol.* 83(2): 295-306.

Medina, H. S. G., M. E. Lopata and M. Bacila. 1994. The response of sea urchin egg embryogenesis towards the effect of some pesticides. *Arq. Biol. Tecnol.* 37(4): 895-906.

Merritt, R. W. and K. W. Cummins. 1996. Trophic relations of macroinvertebrates. In *Methods in Stream Ecology*. F. R. Hauer and G. A. Lamberti (eds.). Academic Press, San Diego, CA. pp 453-474.

Meylan, W. M., P. H. Howard, R. S. Boethling, D. Aronson, H. Printup and S. Gouchie. 1999. Improved method for estimating bioconcentration/ bioaccumulation factor from octanol/water partition coefficient. *Environ. Toxicol. Chem.* 18(4): 664-672.

Mihelcic, J. R., D. R. Lueking, R. J. Mitzell and J. M. Stapleton. 1993. Bioavailability of sorbed- and separate-phase chemicals. *Biodegradation* 4: 141-153.

Millward, R. N., J. W. Fleeger, D. D. Reible, K. A. Keteles, B. P. Cunningham and L. Zhang. 2001. Pyrene bioaccumulation, effects of pyrene exposure on particle- size selection, and fecal pyrene content in the oligochaete *Limnodrilus hoffmeisteri* (Tubificidae, Oligochaeta). *Environ. Toxicol. Chem.* 20(6): 1359-1366.

Montgomery, J. H. 1997. *Agrochemicals Desk Reference*. 2nd Edition. CRC Lewis Publishers, Boca Raton, FL. 656 p.

Morrison, H. A., F. Gobas, R. Lazar and G. D. Haffner. 1996. Development and verification of a bioaccumulation model for organic contaminants in benthic invertebrates. *Environ. Sci. Technol.* 30(11): 3377-3384.

Morrison, H. A., F. A. P. C. Gobas, R. Lazar, D. M. Whittle and G. D. Haffner. 1997. Development and verification of a benthic/pelagic food web bioaccumulation model for PCB congeners in Western Lake Erie. *Environ. Sci. Technol.* 31(11): 3267-3273.

Mortimer, M. R. and D. W. Connell. 1994. Critical internal and aqueous lethal concentrations of chlorobenzenes with the crab *Portunus pelagicus* (L). *Ecotoxicol. Environ. Saf.* 28(3): 298-312.

Motulsky, H. J. 1999. *Analyzing Data with GraphPad Prism*. GraphPad Software Inc., San Diego CA.

Mount, D. R., T. D. Dawson and L. P. Burkhard. 1999. Implications of gut purging for tissue residues determined in bioaccumulation testing of sediment with *Lumbriculus variegatus*. *Environ. Toxicol. Chem.* 18(6): 1244-1249.

Nebeker, A. V., S. T. Onjukka, D. G. Stevens, G. A. Chapman and S. E. Doninguez. 1992. Effects of low dissolved oxygen on survival, growth and reproduction of *Daphnia*, *Hyalella* and *Gammarus*. *Environ. Toxicol. Chem.* 11: 373-379.

Neff, J. 1984. Bioaccumulation of organic micropollutants from sediments and suspended particulates by aquatic animals. *Fresenius Z. Anal. Chem.* 319: 132-136.

Neff, J. M. 1979. *Polycyclic Aromatic Hydrocarbons in the Aquatic Environment*. Applied Science Publishers Ltd., London, England. 262 p.

Newman, M. C. 1995. *Quantitative methods in aquatic ecotoxicology*. CRC Press, Inc, Boca Raton, FL. 426 p.

NYSDEC. 1994. *Technical guidance for screening contaminated sediments.* New York State Department of Environmental Conservation, Division of Fish and Wildlife and Division of Marine Resources, Albany, NY.

Nitschke, L. and W. Schussler. 1998. Surface water pollution by herbicides from effluents of waste water treatment plants. *Chemosphere* 36(1): 35-41.

Norstrom, R. J., A. McKinnon and A. deFreitas. 1976. A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (*Perca flavescens*). *J. Fish. Res. Board Can.* 33: 248-267.

Northcott, G. L. and K. C. Jones. 2000. Spiking hydrophobic organic compounds into soil and sediment: A review and critique of adopted procedures. *Environ. Toxicol. Chem.* 19(10): 2418–2430.

Nowell, L. H., P. D. Capel and P. D. Dileanis. 1999. *Pesticides in Stream Sediment and Aquatic Biota: Distribution, Trends, and Governing Factors. Vol. 4 of the Series: Pesticides in the Hydrologic System*. Lewis Publishers, CRC Press, Boca Raton, FL. 1001 p.

Oliver, B. G. 1987. Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. *Environ. Sci. Technol.* 21: 785-790.

Opdyke, D. R. and R. C. Loehr. 1999. Determination of chemical release rates from soils: Experimental design. *Environ. Sci. Technol.* 33(8): 1193-1199.

Oris, J. T. and J. P. Giesy, Jr. 1986. Photoinduced toxicity of anthracene to juvenile bluegill sunfish (*Lepomis macrochirus* Rafinesque): Photoperiod effects and predictive hazard evaluation. *Environ. Toxicol. Chem.* 5: 761-768.

Pape-Lindstrom, P. A. and M. J. Lydy. 1997. Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. *Environ. Toxicol. Chem.* 16(11): 2415-2420.

Parrish, C. C. 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In *Lipids in freshwater ecosystems*. M. T. Arts and B. C. Wainman (eds.). Springer, New York, NY. pp 4-20.

Parrish, P. R., E. E. Dyar, J. M. Enos and W. G. Wilson. 1978. *Chronic Toxicity of chlordane, trifluralin, and pentachlorophenol to sheepshead minnows (Cyprinodon variegatus)*. EPA-600/3-78-010. U. S. EPA. ERL-ORD, Gulf Breeze, FL.

Pawlisz, A. V. and R. H. Peters. 1993. A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in *Daphnia magna*. *Environ. Sci. Technol.* 27:2795-2800.

Pawlisz, A. V. and R. H. Peters. 1995. Effects of sublethal exposure on lethal body burdens of narcotic organic chemicals in *Daphnia magna*. *Environ. Sci. Technol.* 29(3): 613-621.

Pennak, R. W. 1989. *Freshwater Invertebrates of the United States*. John Wiley and Sons, Inc, New York, NY. 628 p.

Phipps, G. L., G. T. Ankley, D. A. Benoit and V. R. Mattson. 1993. Use of the aquatic oligochaete *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants. *Environ. Toxicol. Chem.* 12: 269-279.

Pickard, D. P. and A. C. Benke. 1996. Production dynamics of *Hyalella azteca* (Amphipoda) among different habitats in a small wetland in the southeastern USA. *J. N. Am. Benthol. Soc.* 15(4): 537-550.

Pignatello, J. J. 1990. Slowly reversible sorption of aliphatic halocarbons in soils, I: Formation of residual fractions. *Environ. Toxicol. Chem.* 9: 1107-1115.

Pignatello, J. J., F. J. Ferrandino and L. Q. Huang. 1993. Elution of Aged and Freshly Added Herbicides From a Soil. *Environ. Sci. Technol.* 27(8): 1563-1571.

Pignatello, J. J. and B. Xing. 1996. Mechanisms of slow sorption of organic chemicals to natural particles. *Environ. Sci. Technol.* 30(1): 1-11.

Power, E. A. and P. M. Chapman. 1992. Assessing Sediment Quality. In *Sediment Toxicity Assessment*. G. A. Burton, Jr. (ed.). Lewis Publishers, Inc., Boca Raton, FL. pp 1-18.

Rand, G. M., P. G. Wells and L. S. McCarty. 1995. Introduction to aquatic toxicology. In *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment*. G. M. Rand (ed.). Taylor & Francis, Washington, DC. pp 3-67.

Randall, P. C., D. R. Young, H. Lee and S. F. Echols. 1998. Lipid methodology and pollutant normalization relationships for neutral nonpolar organic pollutants. *Environ. Toxicol. Chem.* 17:788-791

Ratkowsky, D. A. 1983. *Nonlinear regression modeling: A unified practical approach*. Marcel Dekker, New York, NY. 276 p.

Renner, R. 2002. The K_{ow} Controversy. *Environ. Sci. Technol.* 36(21): 411A-413A.

Reuber, B., D. Mackay, S. Paterson and P. Stokes. 1987. A discussion of the chemical equilibria and transport at the sediment-water interface. *Environ. Toxicol. Chem.* 6: 731-739.

Robbins, J. A. 1980. *Sediments of Southern Lake Huron: Elemental Composition and Accumulation Rates*. EPA-600/3-80-080. U. S. Environmental Protection Agency. Environmental Research Laboratory, Duluth, MN. 48 p.

Robbins, J. A., T. Keilty, D. S. White and D. N. Edgington. 1989. Relationship among tubificid abundances, sediment composition, and accumulation rates in Lake Erie. *Can. J. Fish. Aquat. Sci.* 46: 223-231.

Rodgers, J. H., K. L. Dickson, F. Y. Saleh and C. A. Staples. 1987. Bioavailability of sediment-bound chemicals to aquatic organisms–Some theory, evidence, and research needs. In *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems*. K. L. Dickson, A. W. Maki and W. A. Brungs (eds.). SETAC Special Publication Series. Pergamon Press, New York, NY. pp 219-244.

Rubenstein, N. I., J. L. Lake, R. J. Pruell, H. Lee, II, B. Taplin, J. Heltshe, R. Bowen and S. Pavignano. 1987. *Predicting bioaccumulation of sediment-associated organic contaminants: Development of a regulatory tool for dredged material evaluation*. U. S. EPA Report No. 600/x-87/368. ERL-Narragansett. 54 p.

Rutherford, D. W., C. T. Chiou and D. E. Kile. 1992. Influence of soil organic matter composition on the partition of organic compounds. *Environ. Sci. Technol.* 26: 336-340.

Salazar, M. H. and S. M. Salazar. *in press*. *Draft Standard Guide for Conducting Field Bioassays with Marine, Estuarine and Freshwater Bivalves*. American Society for Testing and Materials (ASTM). Committee E 47, Philadelphia.

Schlebaum, W., G. Schraa and W. H. Van Riemsdijk. 1999. Influence of nonlinear sorption kinetics on the slow-desorbing organic contaminant fraction in soil. *Environ. Sci. Technol.* 33(9): 1413-1417.

Schmitt, H., R. Altenburger, B. Jastorff and G. Schuurmann. 2000. Quantitative structure-activity analysis of the algae toxicity of nitroaromatic compounds. *Chem. Res. Toxicol.* 13(6): 441-450.

Schuler, L. J. and M. J. Lydy. 2001. Chemical and biological availability of sediment-sorbed benzo[a]pyrene and hexachlorobiphenyl. *Environ. Toxicol. Chem.* 20(9): 2014-2020.

Schultz, I. R. and W. L. Hayton. 1993. Toxicokinetics of trifluralin in rainbow trout. *Aquat. Toxicol.* 26(3-4): 287-306.

Schultz, I. R. and W. L. Hayton. 1994. Body size and the toxicokinetics of trifluralin in rainbow trout. *Toxicology and Applied Pharmacology* 129(1): 138-145.

Schultz, I. R. and W. L. Hayton. 1999. Interspecies scaling of the bioaccumulation of lipophilic xenobiotics in fish: An example using trifluralin. *Environ. Toxicol. Chem.* 18(7): 1440-1449.

Schuytema, G. S., D. F. Krawczyk, W. L. Griffis, A. V. Nebeker and M. L. Robideaux. 1990. Hexachlorobenzene uptake by fathead minnows and macroinvertebrates in recirculating sediment/water systems. *Arch. Environ. Contam. Toxicol.* 19: 1-9.

Schwarzenbach, R. P. and J. Westall. 1981. Transport of nonpolar organic compounds from surface water to groundwater. Laboratory sorption studies. *Environ. Sci. Technol.* 15(11): 1360-1370.

Schwarzenbach, R. P., W. Giger, E. Hoehn and J. K. Schneider. 1983. Behavior of organic compounds during infiltration of river water to groundwater. Field studies. *Environ. Sci. Technol.* 17(8): 472-479.

Segstro, M. D., D. C. G. Muir, M. R. Servos and G. R. B. Webster. 1995. Long-term fate and bioavailability of sediment-associated polychlorinated dibenzo-*p*-dioxins in aquatic mesocosms. *Environ. Toxicol. Chem.* 14(10): 1799-1807.

Sheedy, B., V. R. Mattson, J. S. Cox, P. A. Kosian, G. L. Phipps and G. T. Ankley. 1998. Bioconcentration of polycyclic aromatic hydrocarbons by the freshwater oligochaete *Lumbriculus variegatus*. *Chemosphere* 36(15): 3061-3070.

Shugart, L., J. McCarthy, B. Jimenez and J. Daniels. 1987. Analysis of adduct formation in the bluegill sunfish (*Lepomis macrochirus*) between benzo[a]pyrene and DNA of the liver and hemoglobin of the erythrocyte. *Aquat. Toxicol.* 9: 319-326.

Sijm, D., M. Schipper and A. Opperhuizen. 1993. Toxicokinetics of halogenated benzenes in fish-Lethal body burden as a toxicological end-point. *Environ. Toxicol. Chem.* 12(6): 1117-1127.

Sijm, D. T. H. M. and A. van der Linde. 1995. Size-dependent bioconcentration kinetics of hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric relationships. *Environ. Sci. Technol.* 29(11): 2769-2777.

Smith, A. E., A. J. Aubin and D. A. Derksen. 1988. Loss of trifluralin from clay and loam soils containing aged and freshly applied residues. *Bull. Environ. Contam. and Toxicol.* 41(4): 569-573.

Smyth, G. K. 2002. Nonlinear Regression. In *Encyclopedia of Environmetrics*. A. H. El-Shaarawi and W. W. Piegorsch (eds.). John H. Wiley & Sons, Chichester. Vol. 3. pp 1404-1411.

Snelgrove, P., T. H. Blackburn, et al. 1997. The importance of marine sediment biodiversity in ecosystem precesses. *Ambio* 26(8): 578-583.

Snyder, M. J. 2000. Cytochrome P450 enzymes in aquatic invertebrates: recent advances and future directions. *Aquat. Toxicol.* 48: 529-547.

Spacie, A. 1975. *The bioconcentration of trifluralin from a manufacturing effluent by fish in the Wabash River*. Ph.D. Thesis. Purdue University, West Lafayette, IN, USA. 149 p.

Spacie, A. and J. L. Hamelink. 1979. Dynamics of trifluralin accumulation in river fishes. *Environ. Sci. Technol.* 13(7): 817-822.

Sprague, J. B. 1963. Resistance of four freshwater crustaceans to lethal high temperature and low oxygen. *J. Fish. Res. Board Can.* 20: 387-415.

Staak, A., M. Frost and W. Kratz. 1998. Ecotoxicological studies on the impact of the herbicide trifluralin on *Porcellio scaber* (Isopoda). *Appl. Soil Ecol.* 9(1-3): 405-409.

Statsoft. 1997. *Statistica* version 5. Tulsa, OK, USA.

Steinberg, S. M., J. J. Pignatello and B. L. Sawhney. 1987. Persistence of 1,2-dibromoethane in soils-entrapment in intraparticle micropores. *Environ. Sci. Technol.* 21(12): 1201-1208.

Storey, RG, R. R. Fulthorpe and D. D. Williams. 1999. Perspectives and predictions on the microbial ecology of the hyporheic zone. *Freshw. Biol.* 41:119-130.

Suedel, B. C. and J. H. Rodgers. 1996. Toxicity of fluoranthene to *Daphnia magna, Hyalella azteca, Chironomus tentans*, and *Stylaria lacustris* in water-only and whole sediment exposures. *Bull. of Environ. Contam. Toxicol.* 57(1): 132-138.

Suedel, B. C., J. H. Rodgers and P. A. Clifford. 1993. Bioavailability of fluoranthene in fresh-water sediment toxicity tests. *Environ. Toxicol. Chem.* 12(1): 155-165.

Tarr, B. D., M. G. Barron and W. L. Hayton. 1990. Effect of body size on the uptake and bioconcentration of di-2-ethylhexyl phthalate in rainbow trout. *Environ. Toxicol. Chem.* 9(8): 989-995.

ten Hulscher, T. E. M. and G. Cornelissen. 1996. Effect of temperature on sorption equilibrium and sorption kinetics of organic micropollutants - A review. *Chemosphere* 32(4): 609-626.

ten Hulscher, T. E. M., B. A. Vrind, H. Van den Heuvel, L. E. Van der Velde, P. C. M. Van Noort, J. E. M. Beurskens and H. A. J. Govers. 1999. Triphasic desorption of highly resistant chlorobenzenes, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons in field contaminated sediment. *Environ. Sci. Technol.* 33(1): 126-132.

Thomann, R. V. and J. P. Connolly. 1984. Model of PCB in the Lake Michigan lake trout food chain. *Environ. Sci. Technol.* 18(2): 65-71.

Thomson, J. 1998. Sample Preparation techniques for liquid scintillation analysis. In *Handbook of radioactivity analysis*. M. F. L'Annunziata (ed.). Academic Press, San Diego, CA. pp 407-452.

Tippler, P. A. 1987. *College Physics*. Worth Publishers, New York, NY. 932 pp.

USEPA. 1987. *Quality Criteria for Water 1986, Update*. EPA/440/5-86-001. Office of Water Regulations and Standards, Washington, DC.

USEPA. 1989. *Guidance Manual: Bedded sediment bioaccumulation tests*. EPA/600/x-89/302, Washington, D.C.

USEPA. 1993. *Sediment Quality Criteria for the Protection of Benthic Organisms: Fluoranthene*. EPA-822-R-93-012. United States Environmental Protection Agency. Office of Water & Office of Research and Development, Washington, DC.

USEPA. 1994. *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms.* EPA/600/4-91/002. Environmental Monitoring Systems Laboratory, Cincinnati, OH.

USEPA. 1996. EcoUpdate: Ecotox Thresholds. EPA-540-F-95-083. Office of Solid Waste and Emergency Response, Washington, DC.

USEPA. 1997. *Method 1668: Toxic Polychlorinated Biphenyls by Isotope Dilution High Resolution Gas Chromatography/High Resolution Mass Spectrometry* [Draft]. EPA 821/R-97-001.

USEPA. 1998a. *EPA's Contaminated Sediment Management Strategy*. EPA/823/R-98/001. U. S. Environmental Protection Agency. Office of Water, Washington, DC.

USEPA. 1998b. *Test Methods for Evaluating Solid Waste.* Chapter 4. EPA 530/SW-846. Office of Water, Washington, DC.

USEPA. 2000a. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. Second Edition*. EPA/600/R-99/064. Office of Research and Development and Office of Water, Washington, D.C.

USEPA. 2000b. *Proceedings of the Ground-Water/Surface-Water Interactions Workshop*. EPA/542/R-00/007. Office of Solid Waste and Emergency Response, Washington, DC.

USGS. 1999. *The quality of our nation's waters-nutrients and pesticides*. Circular 1225. U. S. Geological Survey, Denver, CO. 82 p.

Valett, H. M., C. N. Dahm, M. E. Campana, J. A. Morrice, M. A. Baker and C. S. Fellows. 1997. Hydrologic influences on groundwater surface water ecotones: Heterogeneity in nutrient composition and retention. *J. N. Am. Benthol. Soc.* 16:239-247.

Van den Heuvel, M. R., L. S. McCarty, R. P. Lanno, B. E. Hickie and D. G. Dixon. 1991. Effect of total body lipid on the toxicity and toxicokinetics of pentachlorophenol in rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 20(4): 235-251.

van Hattum, B., M. J. Curto Pons and J. F. Cid Montanes. 1998. Polycyclic aromatic hydrocarbons in freshwater isopods and field-partitioning between abiotic phases. *Arch. Environ. Contam. Toxicol.* 35(2): 257-267.

Van Hoof, P. L., J. V. K. Kukkonen and P. F. Landrum. 2001. Impact of sediment manipulation on the bioaccumulation of polycyclic aromatic hydrocarbons from field-contaminated and laboratory-dosed sediments by an oligochaete. *Environ. Toxicol. Chem.* 20(8): 1752-1761.

van Metre, P. C., J. T. Wilson, E. Callender and C. C. Fuller. 1998. Similar rates of decrease of persistent, hydrophobic and particle-reactive contaminants in riverine systems. *Environ. Sci. Technol.* 32(21): 3312-3317.

van Noort, P. C. M., G. Cornelissen and D. ten Hulscher. 1999. Sediment quality criteria, bioavailability, and sorptive attenuation of organic compounds. *SETAC-Europe News* 10(3): 16-17.

van Wezel, A. P. and A. Opperhuizen. 1995. Narcosis due to environmental pollutants in aquatic organisms-Residue-based toxicity, mechanisms, and membrane burdens. *Crit. Rev. Toxicol.* 25(3): 255-279.

van Wezel, A. P., D. A. M. Devries, S. Kostense, D. Sijm and A. Opperhuizen. 1995. Intraspecies variation in lethal body burdens of narcotic compounds. *Aquat. Toxicol.* 33(3-4): 325-342.

Varanasi, U., J. E. Stein and M. Nishimoto. 1989. Biotransformation and disposition of polycyclic aromatic hydrocarbons (PAH) in fish. In *Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment*. U. Varanasi (ed.). CRC Press, Boca Raton, FL. pp 93-150.

Veith, G. D. and P. Kosian. 1983. Estimating bioconcentration potential from octanol/water partition coefficients. In *Physical Behavior of PCBs in the Great Lakes*. D. Mackay, et al. (ed.). Ann Arbor Science, Ann Arbor, MI, USA. pp 269-282.

Verrengia Guerrero, N. R., M. G. Taylor, N. A. Davies, M. A. M. Lawrence, P. A. Edwards, K. Simkiss and E. A. Wider. 2002. Evidence of differences in the biotransformation of organic contaminants in three species of freshwater invertebrates. *Environ. Pollut.* 117: 523-530.

Verschueren, K. 1983. *Handbook of Environmental Data on Organic Chemicals*. Second Edition. Van Nostrand Reinhold Co., New York, NY. 1310 p.

Voice, T. C. and W. J. Weber, Jr. 1983. Sorption of hydrophobic compounds by sediments, soils, and suspended solids-I: Theory and background. *Wat. Res.* 17(10): 1433-1441.

Von Hofe, E. H., K. L. Duncan, M. L. Beal, S. Mondal and H. W. Puffer. 1979. Hydrocarbon pollution of the oceans: secondary metabolites of a carcinogen in fish. *Proc. West. Pharmacol. Soc.* 22: 281-284.

Walker, W. W., C. R. Cripe, P. R. Pritchard and A. W. Bourquin. 1988. Biological and abiotic degradation of xenobiotic compounds in in vitro estuarine water and sediment/water systems. *Chemosphere* 17(12): 2255-2270.

Ward, J. V., G. Bretschko, M. Brunke, D. Danielopol, J. Gibert, T. Gonser and A. G. Hildrew. 1998. The boundaries of river systems: the metazoan perspective. *Freshwater Biology* 40(3): 531-569.

Weber, J. B. and C. T. Miller. 1989. Organic Chemical Movement over and through Soil. In *Reactions and Movement of Organic Chemicals in Soils*. B. L. Sawhney and B. K (eds.). SSSA Spec. Publ. 22; ASA, CSSA, and SSSA, Madison, WI. pp 305-334.

Weis, M., W. Sziegoleit and W. Forster. 1977. Dependence of pharmacokinetic parameters on the body weight. *Int. J. Clin. Pharmacol.* 15: 572-575.

WEST. 1994. *TOXSTAT*® version 3.4. Department of Zoology and Physiology. University of Wyoming. Laramie, WY, USA.

West, C. W., G. T. Ankley, J. W. Nichols, G. E. Elonen and D. E. Nessa. 1997. Toxicity and bioaccumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin in long-term tests with the freshwater benthic invertebrates *Chironomus tentans* and *Lumbriculus variegatus*. *Environ. Toxicol. Chem.* 16(6): 1287-1294.

Weston, D. P., D. L. Penry and L. K. Gulmann. 2000. The role of ingestion as a route of contaminant bioaccumulation in a deposit-feeding polychaete. *Arch. Environ. Contam. Toxicol.* 38(4): 446-454.

Whiteman, F. W., G. T. Ankley, M. D. Kahl, D. M. Rau and M. D. Balcer. 1996. Evaluation of interstitial water as a route of exposure for ammonia in sediment tests with benthic macroinvertebrates. *Environ. Toxicol. Chem.* 15(5): 794-801.

Wu, S. C. and P. M. Gschwend. 1986. Sorption kinetics of hydrophobic organic compounds to natural sediments and soils. *Environ. Sci. Technol.* 20(7): 717-725.

Xing, B., W. B. McGill and M. J. Dudas. 1994. Cross-correlation of polarity curves to predict partition coefficients of nonionic organic contaminants. *Environ. Sci. Technol.* 28(11): 1929-1933.

Ying, G. G. and B. Williams. 2000. Laboratory study on the interaction between herbicides and sediments in water systems. *Environ. Pollut.* 107(3): 399-405.

Yockim, R. S., A. R. Isensee and E. A. Walker. 1980. Behavior of trifluralin in aquatic model ecosystems. *Bull. of Environ. Contam.Toxicol.* 24(1): 134-141.

Zaleski, J., A. R. Steward and H. C. Sikka. 1991. Metabolism of benzo[a]pyrene and (-)-trans-benzo[a]pyrene-7,8-dihydrodiol by freshly isolated hepatocytes by mirror carp. *Carcinogenesis* 12: 167-174.

Zar, J. H. 1999. *Biostatistical analysis*. 4th Edition. Prentice-Hall, Upper Saddle River, NJ. 663 p.

Zolman, J. F. 1993. *Biostatistics : experimental design and statistical inference*. Oxford University Press, New York, NY. 343 p.

Zumwalt, D. C., F. J. Dwyer, I. E. Greer and C. G. Ingersoll. 1994. A water-renewal system that accurately delivers small volumes of water to exposure chambers. *Environ. Toxicol. Chem.* 13(8): 1311-1314.