NOAA Technical Memorandum GLERL-132

TOXICOKINETICS AND TISSUE DISTRIBUTIONS OF NON-POLAR CONTAMINANTS FROM AQUEOUS AND DIETARY EXPOSURES FOR THE CRAYFISH *PACIFASTACUS LENIUSCULUS*

Duane C. Gossiaux and Peter F. Landrum
NOAA, Great Lakes Environmental Research Laboratory, Ann Arbor, MI 48105

Great Lakes Environmental Research Laboratory
Ann Arbor, Michigan

March 2005

UNITED STATES
DEPARTMENT OF COMMERCE

Carlos M. Gutierrez
Secretary

NATIONAL OCEANIC AND
ATMOSPHERIC ADMINISTRATION

Conrad C. Lautenbacher, Jr.
Administrator

NOTICE

Mention of a commercial company or product does not constitute an endorsement by the NOAA. Use of information from this publication concerning proprietary products or the tests of such products for publicity or advertising purposes is not authorized. This is GLERL Contribution No. 1349.

This publication is available as a PDF file and can be downloaded from GLERL's web site: www.glerl.noaa.gov. Hard copies can be requested from GLERL, Information Services, 2205 Commonwealth Blvd., Ann Arbor, MI 48105. pubs.glerl@noaa.gov.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Toxicokinetics and tissue distributions of non-polar contaminants from aqueous and dietary exposures for the crayfish *Pacifastacus leniusculus.*

D.C. Gossiaux and P.F. Landrum

ABSTRACT. The crayfish, *Pacifastacus leniusculus,* was exposed to dissolved polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyls (PCB) congeners in short-term static and flow-thru water-only exposures. The uptake and elimination rate constants were determined for the total body mass and internal organ tissues. The uptake rate coefficient (k_u) for whole crayfish (1–2 g) from static water-only exposures ranged from 23.8 to 33.1 ml g^{-1} h^{-1} and was negatively correlated with log K_{ow}. Uptake rates varied between tissues and compounds. For example, the k_u from static aqueous exposures for the gill tissue ranged from 37.7 to 63.8 ml g^{-1} h^{-1} and generally increased with increasing log $K_{ow,}$ while k_u from static aqueous exposures for the hepatopancreas ranged from 357.1 to 37.8 ml g^{-1} h^{-1} and decreased with increasing log K_{ow}. The elimination rate constant (k_e) for whole crayfish ranged from 0.001 to 0.013 h^{-1} and decreased with increasing log K_{ow}. Similarly, the k_e values for other individual tissues decreased with increasing log K_{ow}. In addition to the aqueous exposures, crayfish were exposed via ingestion to zebra mussel, *Dreissena polymorpha,* tissue pre-exposed to radiolabeled contaminants. The percent absorption efficiency (%AE) ranged from 91.2 to 96.5 %, and the % AE increased with increasing log K_{ow}.

INTRODUCTION

Since the zebra mussel, *Dreissena polymorpha*, invasion into the Great Lakes (Nalepa *et al*. 1989), many biological and ecological changes have occurred (Nalepa and Schloesser 1993). Such ecological changes have been attributed to the ability of the zebra mussel to filter large volumes of water and compete for food that would normally supply the rest of the benthos (Gossiaux *et al.* 1997). As a result of the high filtering rates and relatively high lipid content, zebra mussels have a high bioaccumulation potential for organic contaminants from the suspended sediments, algae, and water (Gossiaux *et al.* 1997, Bruner *et al*. 1995, Fisher *et al*. 1993), and these contaminants become available for trophic transfer. The zebra mussels also have high absorption efficiencies for polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) congeners from algae (Bruner *et al.* 1995b). Since zebra mussels are very abundant in the Great Lakes (Nalepa *et al*. 1989), there is an excellent opportunity for predators of zebra mussels to be exposed to high levels of contaminants through trophic transfer. One such predator, the crayfish, was observed in laboratory studies (Love and Savino 1993) to consume large amounts of zebra mussel tissue. Thus, crayfish may be at greater risk of contaminant exposure than organisms that do not feed on zebra mussels. This work determined the contaminant uptake through aqueous exposures and the absorption of contaminants from ingestion of laboratory-dosed zebra mussel tissue by crayfish.

MATERIALS AND METHODS

Chemicals

The radiolabeled compounds were either purchased from the Sigma Chemical Company (St. Louis, MO, USA) or Chemsyn Science Laboratories (Lenexa, KS, USA) and included ^{3}H-pyrene (32.3 Ci/mMol), ^{3}H-chrysene (340 mCi/mMol), ^{14}C-benzo(a)pyrene (BaP, 26.6 mCi/mMol), ^{14}C-3,3',4,4'-tetrachlorophenyl (TCBP 37.1 mCi/mMol), and ^{14}C-2,2',4,4',5,5'-hexachlorobiphenyl (HCBP, 12.6 mCi/mMol). The radiopurity was determined by thin layer chromatography (TLC) using hexane:benzene (8:2, v:v) and was quantified by liquid scintillation counting (LSC). Samples were counted on a LKB 1217 liquid scintillation counter. The data were corrected for quench using the external standards ratio method after subtracting for background. Compound radiopurity was > 98% for all compounds. Analytical procedures were performed under gold fluorescent light (λ > 500 nm) to minimize the PAH photodegradation. All compounds were dissolved in an acetone carrier. The acetone concentration in the exposure water ranged between 0.005 and 0.01 mL L^{-1}.

Organisms

Crayfish: Crayfish, *Pacifastacus leniusculus,* were obtained through Carolina Biological Supply (Burlington, NC, USA). Upon arrival, crayfish were placed in a shallow 5 gallon aquarium with a sand substrate and submerged pieces of PVC pipe for refuge. The crayfish were fed daily ~ 500 mg of ground hamburger per organism. Any uneaten food was removed after 1 h and discarded. The aquarium was aerated, and the water was renewed by means of a flow-thru system with a flow rate of 500 mL h^{-1}. Twenty-four hours prior to the start of the tests, feeding was discontinued.

Zebra Mussels: Adult zebra mussels were collected from Lake St. Clair (42°20'00'' N and 82°47'30'' W) at a depth of 5 m using an epibenthic sled. The collected mussels were cleaned with lake water, placed in a cooler, covered with wet paper towels, and transported to the laboratory. At the laboratory, mussels were transferred to an aerated aquarium and maintained at the water temperature measured at the site of collection, which ranged from 8 to 15°C. Mussels in culture were fed a daily diet of *Chlamydomonus* spp. The *Chlamydomonus* was prepared in 2000 mL flasks in stock solution using Guillard WC culture medium (Guillard and Lorenzen 1972). This algal culture was allowed to grow for 7 d at 15°C with a photoperiod of 16 h light/8 h dark. The algae were then added to the mussel cultures at a rate of 200 mL of algal solution per day.

The culture water was renewed once a week by changing half the water of the holding tanks with Lake Michigan water at the culture temperature. The culture water was also monitored twice a week for ammonia concentration (Aquarium Pharmaceuticals, INC, Chalfont, PA). Throughout the course of the mussel culturing, the ammonia concentration never exceeded 2 mg L^{-1}. Furthermore, no mussels were used after being held in culture for more than 3 weeks.

Lipid Analysis

Mussels for these studies were 2.5–3.0 cm in length. The lipid content of 10 individual zebra mussels was determined for each collection of organisms to monitor health. The lipids were measured using a microgravimetric procedure with chloroform/methanol extraction (Gardner *et al*. 1985). Since the crayfish tissues were large, sub-samples of each tissue were taken for lipid analysis.

Crayfish Water-Only Uptake Static Exposures

Crayfish, 3–4 cm in length (1–2 g) were exposed under static conditions to aqueous solutions containing two radiolabeled compounds as ^{3}H and ^{14}C labeled pairs in filtered lake water. Filtered lake water (12 L), adjusted to the experimental temperature, was dosed in bulk with radiolabeled compounds in the following combinations: ^{3}H-chrysene/^{14}C-HCBP and ^{3}H-pyrene/^{14}C-BaP. The water was allowed to equilibrate for 1 h after dosing. For each set of compounds, crayfish were individually housed in 1 L beakers containing 700 mL of exposure solution at 20°C under yellow fluorescent lights, with six crayfish per exposure and an exposure duration of 6 h. Oxygen was determined at the beginning and end of the exposure by using an Orion O_2 electrode. Water samples (2 mL) and one crayfish were removed every hour to determine contaminant concentrations. Each crayfish was dissected into the following parts: hepatopancreas, abdominal muscle, thorax muscle, carapace, and a combination of stomach, brain, and adrenal glands (viscera). The stomach, brain, and adrenal glands were added together because of their small size and the difficulty in separating them cleanly. Tissue samples were removed, weighed, and placed in 12 mL of scintillation cocktail (RPI 3a70B). Each tissue was then sonicated for 1 min. using a Tekmar 375-watt ultrasonic processor (Tekmar Co., Cincinnati, OH USA). The carapace was placed in scintillation cocktail for extraction by the cocktail without sonication for 24 h and then removed before counting. Sorption to the beaker was also determined by measuring the radioactivity in an acetone rinse of each beaker. At the end of the exposures, the total mass balance ranged from 85 to 93%. The ^{14}C and ^{3}H tracers were counted simultaneously on an LKB 1217 liquid scintillation counter using dual-label counting. The data were corrected for quench using external-standards ratio method after correcting for background.

Crayfish Water-Only Uptake Flow-Thru Exposures

For comparison with the static exposures, crayfish were exposed under flow-thru conditions for 6 h at a flow rate of 200 mL h^{-1} in 250 mL exposure chambers. Two crayfish were added to each of the three exposure chambers. Since there were a sufficient number of chambers (6 in total), duplicate experiments were performed. The crayfish were not fed during any portion of the uptake study. Water samples (2 mL) and one crayfish were removed every hour to determine contaminant concentrations. Procedures for determining crayfish concentrations were the same as described above.

Crayfish Elimination Studies

Crayfish for the elimination studies were exposed at the same time as each of the static or flow-thru experiments were being conducted. The pre-exposure from the static experiment consisted of exposing five crayfish to four liters of exposure solution. The pre-exposure for the flow-thru experiment involved exposing four crayfish in one flow-thru chamber. For both, static and flow-thru experiments, the following protocol was identical. The contaminated crayfish were transferred to 4 L of uncontaminated aerated water under the same conditions as those used for uptake studies. The rates of elimination were determined by sampling over a 10 d period post-exposure (24, 144, 192, 288, and 360 h for crayfish from the static exposure and 24, 96, 216, and 360 h for crayfish from the flow-thru exposure). During this time, the water was continuously exchanged by using a flow-thru system exchanging at least 100% of the water daily. The crayfish were fed as described for the culture conditions. At each of the time points, one crayfish was removed at each time point, dissected and the tissue residues determined as described above.

Kinetics

Accumulation for the static exposures was modeled through a mass balance model (Equation 1, Landrum *et al*. 1992). Using the initial rates assumptions, the model assumed that during the uptake phase, elimination was not significant, and the total mass of contaminants remained constant throughout the exposure and could be found either in the water or the organism. The mass balance indicated that the amount sorbed to the beaker was 0.8 ± 0.2% for PY (mean ± SD, n=6 in all cases), 8.1 ± 3.2% for Ch, 9.5 ± 1.6% for BaP, and 18.1 ± 8.2% for HCBP. The amount in the system was corrected for the amount bound to the beaker for HCBP only, because past experience suggested that losses of less than 10% have little impact on kinetic calculations (Landrum *et al.* 2003). The initial rate assumption was supported by the relatively slow elimination (half-life = 53-693 h) compared to the duration of exposure (6 h).

$$Q_a = A(1 - e^{-K_1 t}) \tag{1}$$

where

Q_a = amount of contaminant in tissue (μg)
A = total amount of contaminant in a treatment beaker at t=o (μg)
t = time (h)

Solving for k_1

$$k_1 = \frac{(-\ln(1 - Q_a / A)}{t} \tag{2}$$

k_1 is a conditional rate constant, which is a system dependent value, and must be converted to a system independent clearance (k_u) by the following equation (Landrum 1983)

$$k_u = k_1 \text{ (volume of water (mL) /wet mass of tissue (g))} \tag{3}$$

The uptake clearance, k_u, describes the amount of water scavenged of contaminant per amount of tissue per time (mL g^{-1} wet tissue h^{-1}).

The elimination rate coefficient, k_e, was determined using a first-order elimination model.

$$C_a = C_a^o \bullet e^{-k_e t} \tag{4}$$

where:

k_e = fraction of material eliminated with time (h^{-1})
C_a = amount of contaminant in the tissue (μg g^{-1}wet tissue)

log Bioconcentration Factors (*BCF*, mL g^{-1}) were calculated from uptake and elimination rate constants (Bailer *et al.* 2000):

$$\log(\hat{BCF}) = f(\hat{k}_u - \hat{k}_e) = \log(\hat{k}_u) - \log(\hat{k}_e) \tag{5}$$

The hat (^) above the *BCF*, k_u, and k_e is a notion that indicates that we are referencing an estimator or estimate and not a known parameter.

The standard errors (*SE*) of the *BCF* were estimated by applying the delta method to the *BCF* estimates, which yielded an appropriate *SE* associated with the estimated *BCF*.

$$SE(\hat{BCF}) \approx \sqrt{\frac{\sigma_u^2}{k_e^2} - \frac{2k_u\sigma_{ue}}{k_e^3} + \frac{k_u^2\sigma_e^2}{k_e^4}} \tag{6}$$

where the variance associated with the k_u estimator is σ_u^2, the variance associated with the k_e estimator is σ^2_e, and the covariance between these two estimators is denoted σ_{ue}.

For the flow-thru studies, a constant infusion (2 compartment) model is used because the water concentration did not change over the time course of the experiment. The variability in the water concentrations for pyrene and chrysene was 2-4%.

$$C_a = \frac{k_u}{k_e} C_w (1 - e^{-k_e t}) \tag{7}$$

where

k_u = uptake rate coefficient (mL g^{-1} h^{-1})
k_e = the elimination rate coefficient calculated by equation 4
C_a = concentration in the crayfish (µg g^{-1})
t = time (h)
C_w = concentration in the water (µg ml^{-1})

In all kinetic studies, C_a is determined as the sum of the amount of compound in each tissue divided by the total wet weight.

Absorption Efficiencies:
Zebra mussels were added to an exposure solution containing either radiolabeled ^{3}H-chrysene/^{14}C-HCBP or ^{3}H-pyrene/^{14}C-TCBP at water concentrations of 10,000 dpm mL^{-1} for the ^{3}H compounds and 1000 dpm mL^{-1} for the ^{14}C labeled compounds. The mussels were removed after 3 h, and the soft tissues were dissected. To obtain an estimate for the amount of activity in each piece of zebra mussel tissue fed to the crayfish, six zebra mussels exposed to treated water were dissected and the radioactivity determined by the same procedure as for crayfish tissues. The average amount of contaminant per zebra mussel (pyrene 0.17 µg ± 0.003, chrysene 0.52 µg ± 0.21, TCBP 25.8 µg ± 0.5, HCBP 2.91 µg ± 1.2) was used to set the amount ingested by the crayfish. Tissue from one mussel was then fed to one crayfish, which was housed in a 600 mL beaker covered with black paper to prevent startling the crayfish. Any crayfish that did not ingest the tissue within 10 minutes was removed from the experiment. Only crayfish that ingested the entire mussel tissue, were sampled for contaminant distribution. The crayfish were allowed to digest the tissue for 1 hr prior to sampling. After exposure, the crayfish were removed from the water and sacrificed by dipping them in liquid nitrogen. The same organs as described above were removed, weighed, placed in scintillation cocktail, and analyzed after 24 h by LSC.

Absorption Efficiencies were calculated by:

$$\% AE = ((Amt_i - Amt_e) / Amt_i)* 100 \quad (8)$$

where:

Amt_i = the amount ingested by the crayfish (dpm) based on the average amounts determined above
Amt_e = the amount eliminated in the feces (dpm)

Statistics
Student's t-tests were performed with SYSTAT® version 10.0 (SPSS, Chicago, IL, USA) to determine statistical differences between means. Differences were considered significant when $p<0.05$. The regression package in Scientist® (Micromath Inc, St. Louis, MO, USA 1995), was used for non-linear regressions.

RESULTS

Uptake Rate Coefficients
Uptake rate coefficients for the whole crayfish, from static water exposures, were not significantly different between all compounds, but uptake rate coefficients did decrease linearly with increasing octonal-water partition coefficient (K_{ow}), k_u = -4.32 (1.45 *SE*) (log K_{ow}) + 54.03 (8.52 *SE*), r^2=0.65, n=24 (Table 1a). In the static exposures, the uptake coefficient for pyrene for all tissues except gills was larger than for the gill tissue with other compounds. The gill tissues exhibited relatively large uptake coefficients for all compounds, which is reasonable as they represent the major route of accumulation for these materials (Table 2-5). Although not significant, gill tissue also exhibited a general increase in the uptake coefficient with increasing log K_{ow} except that the k_u for HCBP was somewhat lower than that for BaP.

Uptake rate coefficients for the whole crayfish, from flow-thru water exposures, were not significantly different between all compounds with the exception of pyrene (Table 1b). The k_u for pyrene was statistically larger than for the remaining three compounds. Uptake rate coefficients for the various tissues from flow-thru water exposures were significantly different between compounds (Tables 2-5) and were similar between replicated flow-thru tests.

Uptake rate coefficients between the two different experimental designs were statistically different for all compounds tested; specifically the k_u values were generally larger for the static experiments except for pyrene. The k_u from the various tissues for the two different experimental designs varied, and no particular experimental design proved to generate higher or lower k_u values with respect to the other design.

One dynamic feature from both experimental designs is that the k_u for the hepatopancreus, thorax, viscera, and abdominal muscle changed by more than a factor of 10 from the low log K_{ow} compound pyrene to the highest log K_{ow} compound HCBP (Table 2-5). While for the gills, a maximum change in the k_u was only a factor of two. This likely reflects the rapid flushing rate for the gills and the absence of restrictive barriers to accumulation. All other tissues depend both

on circulation rate, which governs redistribution within the crayfish, and gill transfer rate, both of which serve as kinetic barriers.

Comparing the uptake rates of *Pacifastacus leniusculus* with other species of similar size, the zebra mussel *Dreissena polymorpha* has a very high clearance rates relative to the crayfish (pyrene 350 mL g^{-1} h^{-1} , BaP 760 mL g^{-1} h^{-1} and, HCBP 900 mL g^{-1} h^{-1}, Bruner *et al.* 1993). This reflects the high pumping volumes of the mussels, which is required because they are filter feeders. However, the uptake clearance of the crayfish is more similar to that for small bluegill sunfish 67 ml g^{-1} h^{-1} for anthracene and 62 ml g^{-1} h^{-1} for BaP (Spacie *et al*. 1983). The bluegill and the crayfish are more similar physiologically as they do not use filtration as a feeding mechanism.

Elimination Rate Coefficients

The elimination rate coefficients for the whole crayfish from static water-only exposures decreased linearly with respect to the log K_{ow} and k_e values are significantly different between each compound (Table 1a). The k_e values for static water-only exposures for the whole crayfish were substantially different from the k_e values for the individual tissues (Table 6-9). For pyrene and chrysene, the k_e values for the tissues were generally similar to the whole body values, while for BaP and HCBP, the tissue values were generally larger than that determined for whole organisms. Further, for HCBP and BaP, all the tissues exhibited significantly larger k_e values than the whole body k_e. The lower k_e values for the whole animal verses specific tissues may reflect distribution limitations from the tissue to the gill.

There are also statistical differences in k_e values between the static and flow-thru water exposures for chrysene and BaP in whole crayfish. The k_e values were generally larger for the flow-thru studies perhaps representing better maintenance of an activity gradient between the organism and the water (Table 6-9). For pyrene, flow-thru conditions yielded the higher k_e values in the abdominal mussel, gills, hepatopancreas, and thorax muscle but not in the viscera. In the case of chrysene, BaP, and HCBP, all k_e values were higher in the flow-thru conditions. There are also statistical differences between replicate tests for both the static and flow-thru exposures for pyrene, chrysene, and BaP (Table 6-9). As with the static experiments, the individual tissues exhibited larger k_e values than the whole organism.

As with the uptake rates, the elimination rate constants can be compared between aquatic organisms of similar size. The elimination rate constant for *P. leniusculus* are smaller than those for *D. polymorpha* where k_e was pyrene 0.021 h^{-1}, BaP 0.009 h^{-1}, HCBP 0.005 h^{-1} despite the fact that the lipid content of the two species was similar (Bruner *et al.* 1994). The faster rates for elimination in the zebra mussels also reflect the high filtration rates. As with the uptake rates, the elimination rates for small bluegill sunfish are more similar but are still faster than for the crayfish, 0.040 h^{-1} for anthrancene and 0.010 h^{-1} for BaP. Some of the difference in this case may be differences in the biostransformation capacity of the fish. While biotransformation was not determined in the crayfish, bluegill were able to readily biotransform the PAH congeners.

Bioconcentration Factors

In general there was no trend in *BCF* verses log K_{ow} for whole crayfish exposed either in static or flow-thru experiments (Tables 1a and 1b) In general, the calculated *BCF* was greater from the static tests except for pyrene. As with the whole organism, there were no trends in the log

BCF from static water exposures for the individual tissues (Table 10). In contrast, the log *BCF* for the individual tissues from flow-thru exposures, with the exception of pyrene, increased with increasing log K_{ow} (Table 11). This may result from the pyrene k_u in the hepatopancreas being a factor of 10 greater than the other three compounds in the same tissue.

Absorption Efficiencies

Crayfish absorption efficiencies were high and increased linearly with respect to the contaminant's log K_{ow} (Table 12). Absorption efficiencies ranged from 91.2 to 96.1%. These efficiencies are similar to those of zebra mussels, which ranged from 91.5 to 97.6%, when exposed to radiolabeled algae with the same contaminants (Gossiaux *et al*. 1998). The high absorption efficiencies for accumulation from food likely reflects the high digestibility of the food for both the crayfish and the zebra mussel.

Lipid Analysis

The percent lipid within the crayfish ranged from 1.55% in the abdominal mussel to 15.57% in the hepatopancreas on a dry weight basis (Table 13). Lipid normalized concentrations in crayfish for both aqueous and feeding exposures were larger for pyrene in all tissues than all other compounds (Figures 3 and 4). However, the concentration of pyrene remained roughly the same for the viscera whether the crayfish were exposed to the contaminants in aqueous exposures or feeding exposures.

Contaminant Distributions

From the water-only exposures, the fraction of contaminant in tissue increased linearly with increasing log K_{ow} for the carapace and gills and decreased for the rest of the tissues, likely reflecting distribution limitations relative to the log K_{ow} across the four compounds (Figure 1). The fraction of pyrene, which has the smallest log K_{ow}, was greatest in the hepatopancreas and lowest in the gill tissue. While the fraction of HCBP with the largest log K_{ow}, was greatest on the carapace and smallest in the viscera (Figure 1).

Fractional contaminant distributions after the ingestion of pre-exposed zebra mussels, exhibited a linear increase for the hepatopancreas and decreases for most of the other tissues relative to the log K_{ow} (Figure 2). While HCBP was found at smallest percent accumulation in the hepatopancreas following water-only exposures, it had the greatest percent accumulation when exposure occurred via ingestion (Figures 1 and 2). Similarly, while HCBP exhibited the largest fraction on the carapace following the aqueous exposures, it had the smallest concentrations following the ingestion exposures (Figures 1 and 2). The decline in the importance of the carapace is clear in that the carapace would serve as a site of sorption for HCBP with aqueous exposure. For the exposure via feeding, the hepatopancreas is apparently kinetically closer to the site of uptake. In mammals, the bloods flow carries materials from the GI tract through the portal vein almost directly to the liver. Apparently, a similar flow occurs in the crayfish based on the greater accumulation in the hepatopancreas when exposed via feeding compared to exposure via an aqueous solution.

SUMMARY

In summary, whole crayfish had higher uptake rates for pyrene in both static and flow-thru exposures, while HCBP yielded uptake rates that were the lowest for both experimental designs. Upon examination of tissue uptake rates, it was discovered that the uptake rates varied between

tissues and compounds. However, from static exposures, k_u values for the gill tissues generally increased with increasing log K_{ow}, while k_u values for the hepatopancreas decreased with increasing log K_{ow}. Elimination rate constants for whole crayfish from static-exposures decreased with increasing log K_{ow} and were lower than k_e values for individual tissues exposed to the same contaminants. Elimination rate constants for tissue from flow-thru exposures were statistically greater than those from static exposures. The k_e values from flow-thru exposures were also larger than k_e values from whole crayfish flow-thru exposures. Absorption efficiencies were high (>91%) and increased linearly with respect to the contaminants log K_{ow}. Fractional contaminant distribution from aqueous exposures was greatest for the contaminant with the lowest log K_{ow}, pyrene, in the abdomen, hepatopancreaus, thorax, and viscera tissues, while the contaminant with the great log K_{ow}, HCBP was greatest in the gills and carapace. When these fractions were lipid-normalized, pyrene was in the greatest concentration for all tissues. Fractional contaminant distributions for crayfish after consumption of zebra mussel tissues revealed variable distribution for each tissue. However, HCBP was found in the greatest percent in the hepatopancreaus, while pyrene was greatest in carapace and viscera. When contaminant concentrations were lipid-normalized, pyrene again was in the greatest concentration for all tissues.

REFERENCES

Bailer, A. J., S.E. Walker, and K.J. Venis. Estimating and testing bioconcentration factors. *Environmental Toxicology and Chemistry* 19:2338-2340 (2000).

Bruner, K.A., S.W. Fisher, and P.F. Landrum. The Role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling: I. The effect of body size and lipid content on bioconcentration of PCBs and PAHs. *Journal of Great Lakes Research* 20:725-734 (1994).

Bruner, K.A., S.W. Fisher, and P.F. Landrum. The Role of the zebra mussel, *Dreissena polymorpha,* in contaminant cycling: II. Zebra mussel contaminant accumulation from algae and suspended particles, and transfer to the benthic invertebrate, *Gammarus fasciatus*. *Journal of Great Lakes Research* 20:735-750 (1994).

Fisher, S.W., D.C. Gossiaux, K.A. Bruner, and P.F. Landrum. Investigations of the toxicokentics of hydrophobic contaminants in the zebra mussel (*Dreissena polymorpha)*. In *Zebra Mussels: Biology, Impacts, and Controls*. T.F. Nalepa and D.W. Schloesser (eds.), pp 465-490. Lewis Publishers, Chelsea, MI (1993).

Gardner, W.S., W.A. Frez, and E.A. Cichocki. Micromethod for lipids in aquatic invertebrates. *Limnology and Oceanography* 30:1099-1105 (1985).

Gossiaux, D.C., P.F. Landrum, and S.W. Fisher. Effect of temperature on the accumulation kinetics of PAHs and PCBs in the zebra mussel, *Dreissena polymorpha. Journal of Great Lakes Research* 22:379-388 (1996).

Gossiaux, D.C., P.F. Landrum, and S.W. Fisher. The assimilation of contaminants from suspended sediment and algae by the zebra mussel, *Dreissena polymorpha*. *Chemosphere* 35:3181-3197 (1998).

Guillard, R.R.L,. and C.J. Lorenzen. Yellow-green algae with chlorophyllide *c*. *Journal of Phyology* 8:10-24 (1972).

Landrum, P.F. The effect of co-contaminants on the bioavailability of polycyclic aromatic hydrocarbon to *Pontoporeia hoyi*. In *Polynuclear Aromatic Hydrocarbons: Formation, Metabolism, and Measurement*. M.Cooke and D.J. Dennis, (eds.) pp. 731-743. Battelle Press, Columbus, OH (1983).

Landrum, P.F., W.A. Frez, and M.S. Simmons. The effect of food consumption on the toxicokinetics of benzo(a)pyrene and 2,2',4,4',5,5'-hexachlorobiphenyl in *Mysis relicta*. *Chemosphere* 25:397-415 (1992).

Landrum, P.F., G.R. Lotufo, D.C. Gossiaux, M.L. Gedeon, and J-H Lee. Bioaccumulation and critical body residue of PAHs in the amphipod, *Diporeia* spp.: Additional evidence to support toxicity additivity for PAH mixtures. *Chemosphere* 51:481-489 (2003).

Love, J., and J.F. Savio. 1993. Crayfish (*Orconectes virilis*) predation on zebra mussels (*Dreissena polymorpha*). *Journal of Freshwater Ecology* 8:253-259 (1993).

Nalepa, T.F. Estimates of macroinvertebrate biomass in Lake Michigan. *Journal of Great Lakes Research* 15:437-443 (1989).

Table 1a. Uptake, elimination rate coefficients and log bioconcentration factors (*BCF*) for whole body analysis from static aqueous exposures. Numbers in parentheses are standard deviations.

Compound	log K_{ow}	k_u (mL g^{-1} h^{-1})	k_e (h^{-1})	log *BCF*
Pyrene	5.2	33.1 (4.3)	0.013 (0.001)	3.41 (0.09)
Chrysene	5.8	31.4 (2.7)	0.008 (0.002)	3.60 (0.25)
Benzo(a)pyrene	5.9	25.9 (3.9)	0.003 (0.0004)	3.94 (0.16)
Hexachlorobiphenyl	6.7	23.8 (2.6)	0.001 (0.0005)	3.30 (0.50)

Table 1b. Uptake, elimination rate coefficients and log bioconcentration factors (*BCF*) for whole body analysis from flow-thru aqueous exposures. Numbers in parentheses are standard deviations.

Compound	log K_{ow}	k_u (mL g^{-1} h^{-1})	k_e (h^{-1})	log *BCF*
Pyrene	5.2	60.5 (10.7)	0.016 (0.007)	3.58 (0.47)
Chrysene	5.8	12.0 (1.7)	0.033 (0.001)	2.56 (0.15)
Benzo(a)pyrene	5.9	13.9 (1.4)	0.019 (0.001)	2.86 (0.11)
Hexachlorobiphenyl	6.7	11.7 (0.6)	0.001 (0.0002)	4.07 (0.21)

Table 2. Uptake rate coefficients (k_u ml g^{-1} h^{-1}) for pyrene from aqueous exposures. Numbers in parenthesis are standard deviations.

Exp Type	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Static	20.9 (1.8)	37.7 (18.3)	357.9 (140.1)	88.3 (50.3)	32.6 (10.1)
Flow-thru	18.8 (4.7)	21.2 (5.1)	573.1 (125.3)	66.6 (5.9)	24.9 (3.1)
Flow-thru	18.9 (1.7)	23.5 (4.4)	335.2 (20.1)	77.8 (22.5)	17.3 (1.9)

Table 3. Uptake rate coefficients (k_u ml g^{-1} h^{-1}) for chrysene from aqueous exposures. Numbers in parenthesis are standard deviations.

Exp Type	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Static	0.8 (0.7)	26.2 (20.1)	69.3 (25.9)	12.6 (10.4)	5.2 (2.1)
Flow-thru	3.7 (0.4)	14.4 (3.3)	52.3 (5.4)	4.5 (0.3)	4.4 (0.4)
Flow-thru	5.4 (0.2)	36.2 (7.4)	63.1 (12.6)	11.1 (1.6)	6.8 (0.4)

Table 4. Uptake rate coefficients (k_u ml g^{-1} h^{-1}) for BaP from aqueous exposures. Numbers in parenthesis are standard deviations.

Exp Type	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Static	3.6 (1.0)	63.8 (42.5)	37.8 (18.8)	15.9 (11.9)	5.2 (2.6)
Flow-thru	4.4 (0.4)	11.3 (1.6)	84.2 (19.1)	13.2 (1.2)	5.6 (0.4)
Flow-thru	1.1 (1.1)	41.1 (4.4)	57.8 (22.9)	5.0 (1.0)	7.1 (6.2)

Table 5. Uptake rate coefficients (k_u ml g^{-1} h^{-1}) for HCBP from aqueous exposures. Numbers in parenthesis are standard deviations.

Exp Type	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Static	6.8 (5.2)	60.9 (34.5)	58.5 (48.1)	2.3 (2.1)	7.5 (5.7)
Flow-thru	2.0 (0.3)	34.4 (7.2)	48.2 (3.8)	3.4 (0.4)	3.1 (0.2)
Flow-thru	2.0 (0.6)	69.4 (12.2)	41.3 (24.9)	3.8 (1.5)	3.8 (1.7)

Table 6. Elimination rate coefficients (k_e h^{-1}) for pyrene after aqueous exposure. Numbers in parenthesis are standard deviations.

Exp Type	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Static	0.0123 (0.0012)	0.0390 (0.0002)	0.0125 (0.0024)	0.0148 (0.0024)	0.0098 (0.0016)
Static	0.0191 (0.0029)	0.0212 (0.0033)	0.0164 (0.0018)	0.0158 (0.0008)	0.0155 (0.0019)
Flow-thru	0.0192 (0.0043)	0.0167 (0.0064)	0.0232 (0.0042)	0.0131 (0.0022)	0.0198 (0.0054)
Flow-thru	0.0692 (0.0017)	0.0654 (0.0265)	0.0183 (0.0112)	0.0086 (0.0017)	0.0117 (0.0031)

Table 7 Elimination rate coefficients (k_e h^{-1}) for chrysene after aqueous exposure. Numbers in parenthesis are standard deviations.

Exp Type	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Static	0.012 (0.002)	0.010 (0.003)	0.007 (0.001)	0.012 (0.001)	0.012 (0.008)
Flow-thru	0.077 (0.002)	0.105 (0.016)	0.032 (0.002)	0.042 (0.005)	0.025 (0.002)
Flow-thru	0.067 (0.003)	0.114(0.012)	0.039(0.002)	0.063(0.010)	0.011 (0.003)

Table 8. Elimination rate coefficients (k_e h^{-1}) for BaP after aqueous exposure. Numbers in parenthesis are standard deviations.

Exp Type	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Static	0.0089 (0.0015)	0.0074 (0.0013)	0.0093 (0.0012)	0.0087 (0.0012)	0.0078 (0.0014)
Static	0.0149 (0.0029)	0.0066 (0.0016)	0.0049 (0.0013)	0.0109 (0.0019)	0.012 (0.0021)
Flow-thru	0.0442 (0.0044)	0.0444 (0.0113)	0.0238 (0.0030)	0.0103 (0.0012)	0.0452 (0.0077)
Flow-thru	0.0826 (0.0041)	0.0651 (0.0082)	0.0253 (0.0025)	0.0188 (0.0032)	0.0223 (0.0021)

Table 9. Elimination rate coefficients (k_e h^{-1}) for HCBP after aqueous exposure. Numbers in parenthesis are standard deviations.

Exp Type	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Static	0.0032 (0.0026)	0.0061 (0.0044)	0.0004 (0.0002)	0.0021 (0.0005)	0.0027 (0.0002)
Flow-thru	0.0118 (0.0034)	0.0529 (0.0122)	0.0009 (0.0001)	0.0052 (0.0005)	0.0037 (0.0001)

Table 10. Average bioconcentration factors *BCF* (k_u/k_e) from static aqueous exposures.

	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Pyrene	1396	5722	25164	5779	2714
Chrysene	66	3500	9760	1033	448
BaP	323	9144	5889	1644	549
HCBP	219	9393	52750	1667	703

Table 11. Average bioconcentration factors *BCF* (k_u/k_e) from flow-thru aqueous exposures.

	Abdominal Muscle	Gills	Hepatopancreas	Viscera	Thorax Muscle
Pyrene	626	814	21663	3139	1369
Chrysene	64	227	1626	142	397
BaP	57	443	2911	774	221
HCBP	169	980	49722	1385	933

Table 12. Crayfish absorption efficiencies from ingestion of zebra mussel tissue.

Compound	% Absorption Efficiency	Standard Deviation
Pyrene	91.21	6.13
Chrysene	92.78	6.86
Tetrachlorobiphenyl	95.95	1.10
Hexachlorobiphenyl	96.05	4.17

n=10

Table 13. Percent lipid in crayfish.

Tissue	% Lipid	S.E.
Abdominal Muscle	1.55	0.43
Gills	11.85	2.85
Hepatopancreas	15.57	3.95
Thorax Muscle	3.40	0.58
Viscera	4.34	0.89

n=4

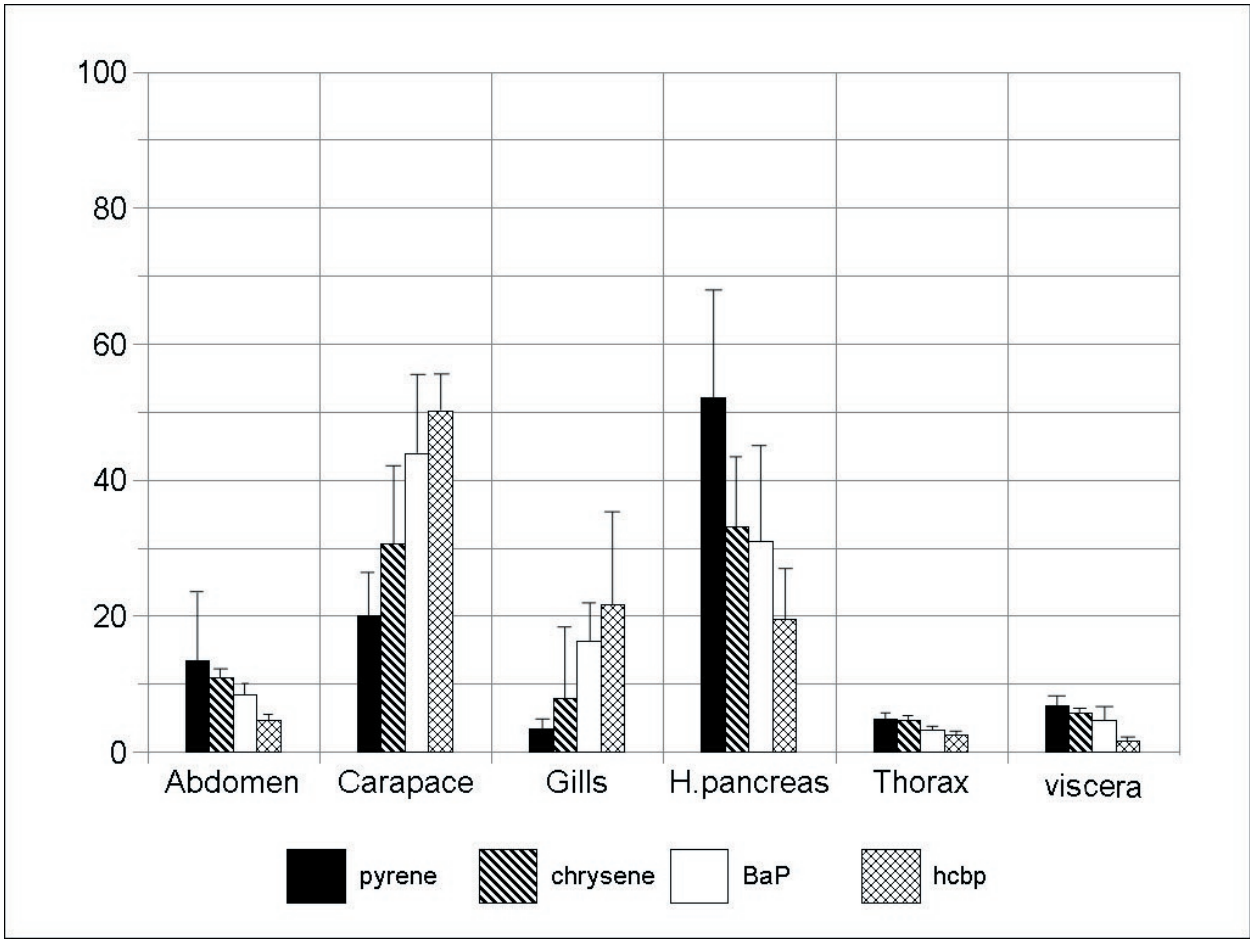

Figure 1. Percent of total contaminants (with stand errors bars, n=6) in crayfish tissues after aqueous exposures.

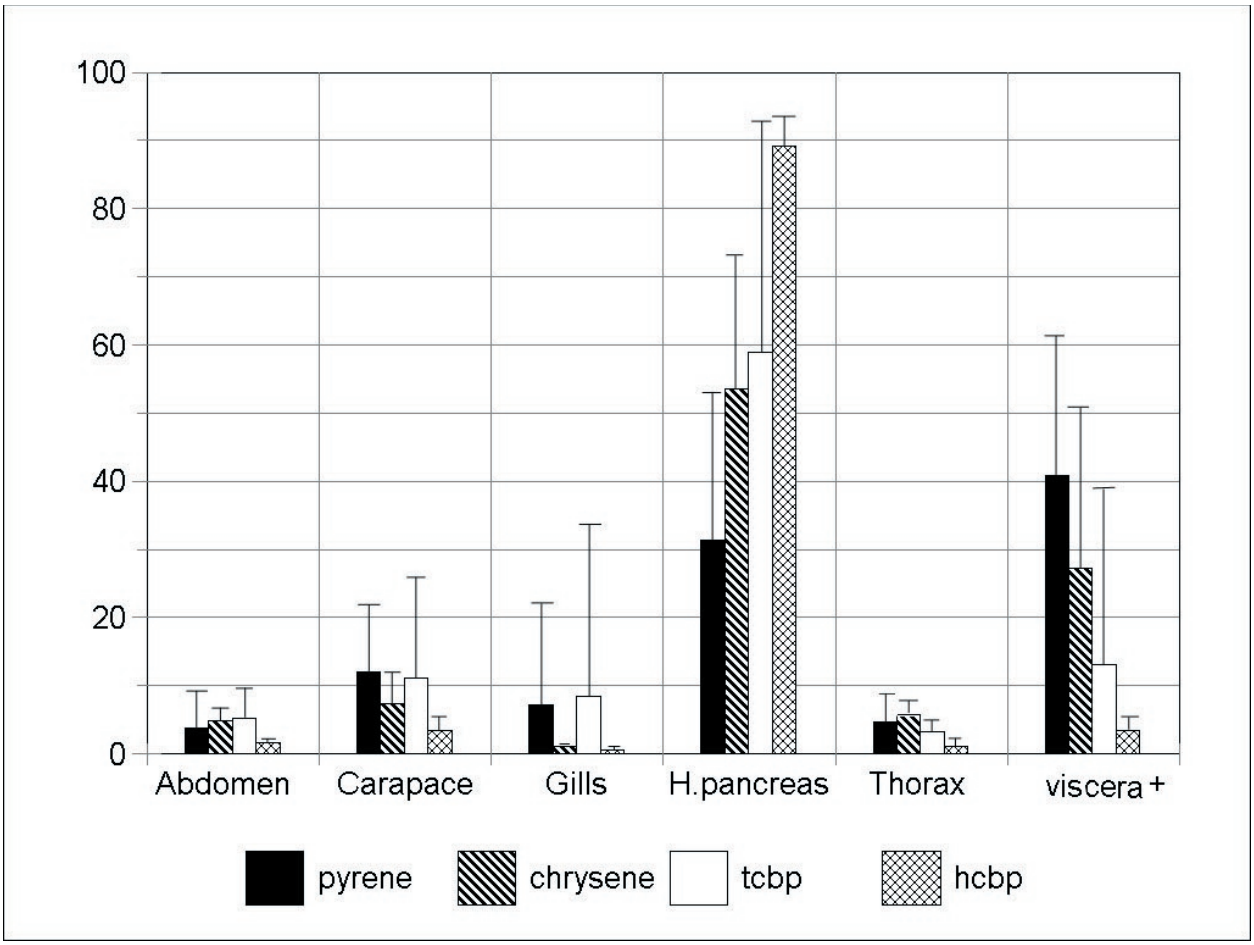

Figure 2. Percent of total contaminants (with stand errors bars, n=6) in crayfish tissues after consumption of contaminated zebra mussel tissue.

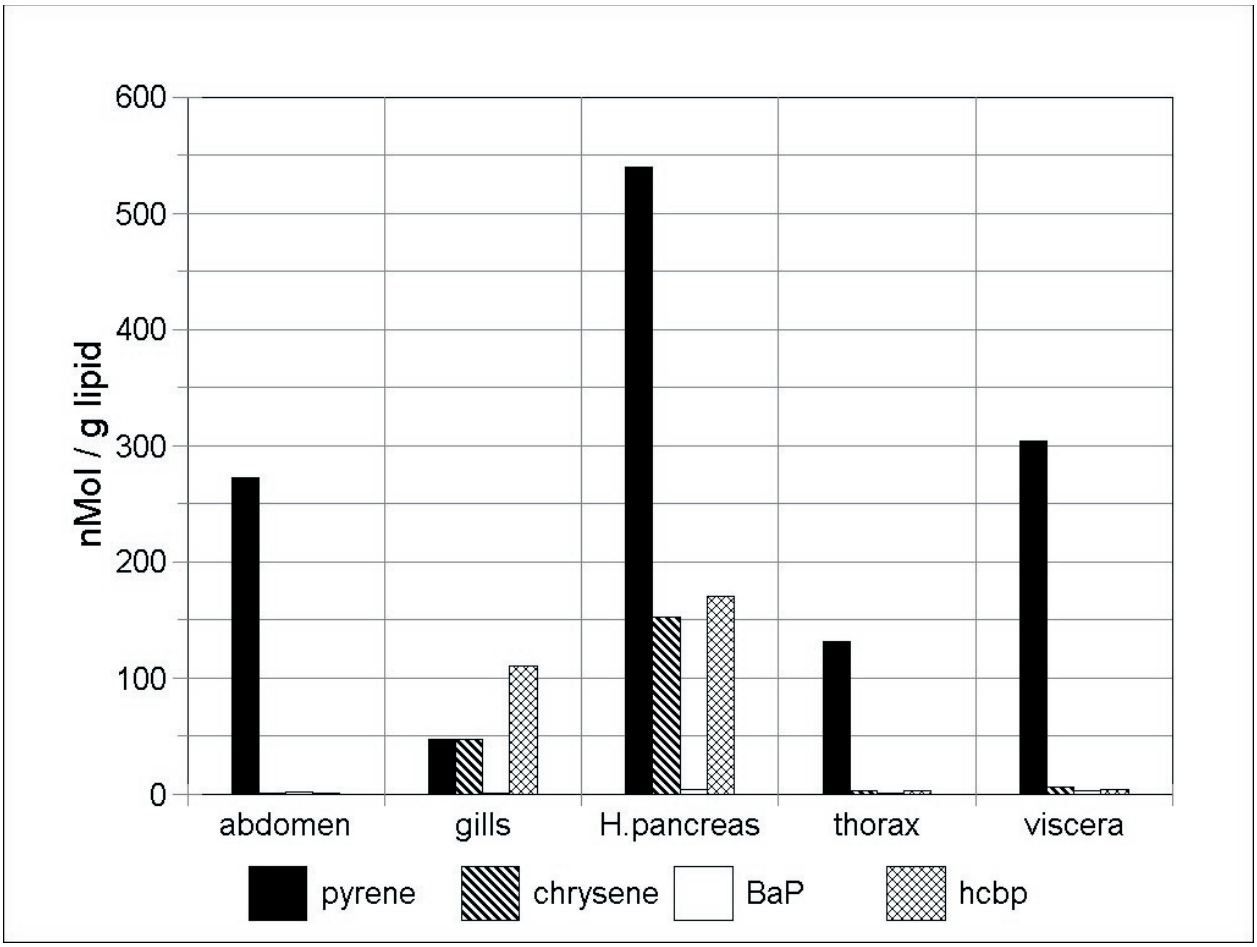

Figure 3. Lipid normalized contaminant concentrations in crayfish tissues after aqueous exposures.

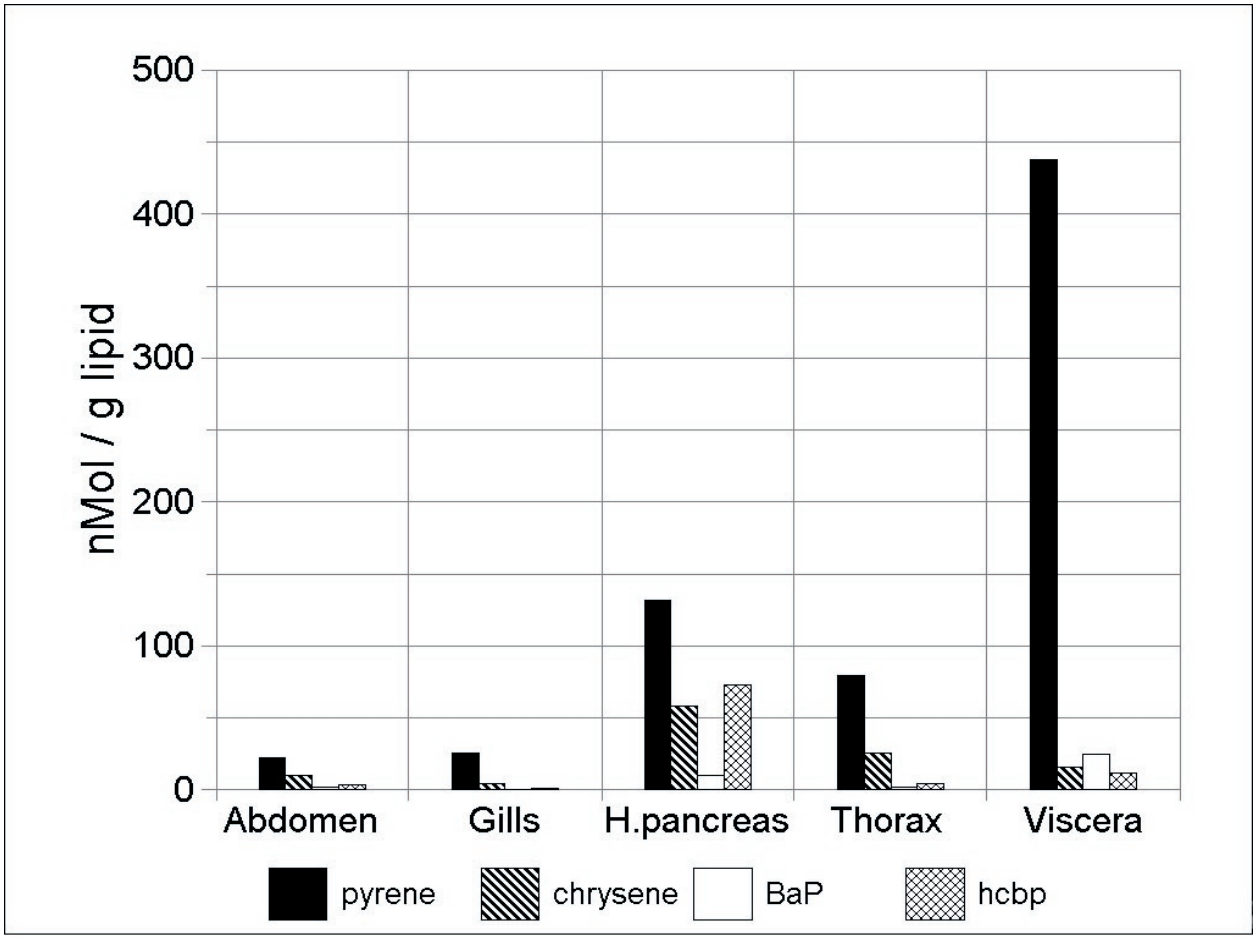

Figure 4. Lipid normalized contaminant concentrations in crayfish tissues after consumption of contaminated zebra mussel tissue.