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Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: role of physiological and environmental variables*

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The accumulation and elimination kinetics of selected polycyclic aromatic hydrocarbons and polychlorinated biphenyl congeners were determined for the amphipod, *Pontoporeia hoyi*, the major benthic invertebrate in the Great Lakes. Rates measured during the course of several field seasons, along with environmental and physiological variables, indicated that for compounds less water soluble than anthracene, the uptake rate constant (K_u) is inversely proportional to the mass of the organisms and directly proportional to experimental temperature. The role of temperature was again limited to the more water insoluble compounds. The depuration rate constant (K_d) was inversely proportional to the octanol-water partition coefficient, the mass of the organism, and the lipid content of the organisms. K_d was directly proportional to temperature, although the effect of temperature was again insignificant for the very water soluble biphenyl. The magnitude of the thermal effect was lower during the summer and early fall when the lipid content of the organisms was high. Both the uptake and elimination kinetics are dependent on a variety of physiological and environmental factors. The kinetic parameters must be normalized for the various factors before comparisons of kinetics in *P. hoyi* between collections at different times during the year and different sampling sites can be made.

Key words: Toxicokinetics; Amphipods; Polycyclic aromatic hydrocarbons; Temperature and toxicokinetics; Lipid content and toxicokinetics; Organism mass and toxicokinetics

INTRODUCTION

The Great Lakes are apparently recovering slowly from an onslaught of pollution that has accompanied the industrial, agricultural and urban development of the Great Lakes basin. While the levels of contaminants are declining in fish (Shear,

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1984; St. Amant et al., 1984; DeVault et al., 1986), problems remain. For instance, there are a host of chemicals associated with the sediments, benthos and food chain. Hundreds of compounds have been detected in lake trout from Lake Michigan (Hesselberg and Seelye, 1982). Reproductive failures of both lake trout (Mac et al., 1985) and chinook salmon (Giesy et al., 1986) have been linked to toxics and indicate the long-term nature of toxic contaminant problems in the Great Lakes. Most of the current toxic organic loads to the Great Lakes are thought to occur via the atmosphere and ground water sources. However, the role of sedimentary release of stored pollutants remains of considerable concern.

To understand the movement of toxic organics through the benthic portion of the Great Lakes ecosystem, I studied the toxicokinetics of organic xenobiotics in *Pontoporeia hoyi*, the most abundant, on a mass basis, Great Lakes benthic invertebrate. These invertebrates are a major food source for most of the fishes in the Great Lakes at some stage in their life cycle (Mosley and Howmiller, 1977). The benthic organisms live for 1 to 2 years dependent on depth (Marzolf, 1965) and field monitoring shows that the animals have high concentrations of chlorinated hydrocarbon pollutants (Evans et al., 1986). The toxicokinetic studies examined selected polycyclic aromatic hydrocarbons (PAH) and PCB congeners that represent significant pollutant classes in the Great Lakes (Nriagu and Simmons, 1984).

Current efforts to define the accumulation of organic xenobiotics to aquatic organisms are centered around the steady state bioconcentration concept (Kenega and Goring, 1980; Veith et al., 1980 and Hawker and Connell, 1986). Such approaches are not generally successful for predicting the concentrations of organic contaminants in the ecosystem. A kinetic approach should better account for the effects of changes in the environment and the organism with time for the accumulation and elimination of toxicants. Previous studies to define the toxicokinetics of selected compounds for a single species generally account for only a single major environmental or physiological variable that affects the toxicokinetics. Because seasonal changes in toxicokinetics (Dewaide, 1971; Smith, 1977; Landrum and Crosby, 1981) and toxicity (Zitko and Carson, 1977; Margrey et al., 1981) have been observed in marine organisms, I examined the effects of temperature, organism mass and organism lipid content on the accumulation and elimination kinetics in *P. hoyi* over several field seasons. Additionally, two populations of *P. hoyi* within the southern basin of Lake Michigan were examined for differences potentially related to differences due to species strain or the two exposure environments.

MATERIALS AND METHODS

Organism collection and culturing

Pontoporeia hoyi were collected in Lake Michigan approximately 3 miles southwest of Grand Haven, MI (Loran coordinates 49514.2, 32525.1) at a depth of 23 to 29 m by PONAR grab. The animals were screened from the sediment,

transported to the laboratory in cool lake water (transport time 4 h) and held in shallow aquaria containing 3 to 4 cm of Lake Michigan sediment and 10 cm of lake water at 4°C (Landrum, 1982a). *P. hoyi* were also collected from the Benton Harbor area (Loran coordinates 49939.8, 32926.8) at 54 m depth. (Note: there was a change in the nomenclature for these amphipods from *P. affinis* to *P. hoyi* about 1977, Segerstrale, 1977.)

Compound selection, purification and preparation

The compounds studied included ^{14}C -radiolabeled benzo(a)pyrene (BaP, 16.3 mCi mmol $^{-1}$, lot number 801023), phenanthrene (Phe, 14 mCi mmol $^{-1}$, lot number 840510), 2,5,2',5'-tetrachlorobiphenyl (TCB, 6.3 mCi mmol $^{-1}$, lot number 70809), and biphenyl (BI, 15.9 mCi mmol $^{-1}$, lot number 90916) purchased from Pathfinder Laboratories; anthracene (Anth, 3.3 mCi mmol $^{-1}$, lot number 770842) purchased from California Bionuclear and [^{14}C]benz(a)anthracene (BAA, 49 mCi mmole $^{-1}$, Batch 5), ^3H -radiolabeled BaP (23.8 Ci mmol $^{-1}$, Batch 75) and pyrene (PY, 34 Ci mmol $^{-1}$, custom synthesis) purchased from Amersham Corporation. The specific activity for PY was determined by a combination of liquid scintillation counting (LSC) and gas chromatography after purification of the crude product by thin layer chromatography (TLC) (Landrum et al., 1985). All compounds were dissolved in a methanol carrier. Compound radiopurity of greater than 98% was determined, prior to use, by TLC using hexane:benzene (8:2 v:v) and LSC. All solvents were of HPLC grade. All analytical procedures were performed under gold fluorescent light ($\lambda > 500$ nm) to minimize the photodegradation of the PAH.

Experimental

Wet weight, dry weight, ash free dry weight, and total lipid content were determined at each collection during the 1984 field year. Total lipid content was measured by gravimetric analysis for both the 1984 and 1985 field year (Gardner et al., 1985a). Wet weights were determined after blotting the animals on a tissue. Dry weights were determined by drying the animals overnight at 60°C and then placing them in a desiccator for 2 to 3 days to reach constant weight. Ash weights were determined after ashing the dry animals at 500°C for 1 h. The ash-free dry weight is the difference between the dry weight and the ash weight. All weights were measured on a Cahn model 4700 electrobalance.

Lake Michigan water, filtered through glass fiber filters (Gelman AE), labeled in bulk (5 l) was dispensed to replicate test chambers after 1–2 h equilibration with the compound. The methanol carrier (< 30 mg l $^{-1}$) added with the experimental pollutant should not affect the uptake rates (Landrum, 1982b). Pollutant concentrations for all studies were kept at or below the reported aqueous solubility except for BaP that was occasionally at or slightly above its reported water solubility (Table I). Water concentrations were based on measured radioactivity and the specific activity of the compound. Sorption to natural dissolved organic matter in the water column,

TABLE I

Variability and range of the xenobiotic water concentrations.

Compound	Relative SD of the mean exposure concentration	Concentration range ^a for all exposures ($\mu\text{g l}^{-1}$)	Aqueous ^b solubility ($\mu\text{g l}^{-1}$)
Biphenyl	8.5 \pm 3.4% (<i>n</i> = 11)	2.1-9.7	6920
Phenanthrene	10.8 \pm 5.0% (<i>n</i> = 36)	0.7-7.1	1000
Anthracene	7.7 \pm 2.6% (<i>n</i> = 20)	4.6-16.9	72
Pyrene	7.8 \pm 3.4% (<i>n</i> = 4)	0.002-0.011	132
Tetrachlorobiphenyl	11.8 \pm 5.7% (<i>n</i> = 11)	4.1-5.6	46
Benz(<i>a</i>)anthracene	12.6 \pm 4.1% (<i>n</i> = 6)	0.62-1.11	14
Benzo(<i>a</i>)pyrene	16.3 \pm 6.9% (<i>n</i> = 49)	0.002-2.6	0.5

^a Concentrations represent the total concentration not the freely dissolved and are the range of concentrations used over the entire course of the experiments; *n* equals one exposure.

^b Data taken from Kenega and Goring, 1980, and Mackay et al., 1980, 1983.

measured as dissolved organic carbon (DOC), was determined at 4 h, after addition of the animals by a reverse-phase separation technique (Landrum et al., 1984). The fraction of the compound freely dissolved, 'bioavailable', was calculated from a previously determined relationship between Log K_{rp} , the reverse-phase measured partition coefficient to DOC, and Log K_b , the partition coefficient to DOC determined kinetically with *P. hoyi* (Landrum et al., 1987).

Measurements of the uptake kinetics were performed in temperature-controlled flow-through chambers containing 0.2 l of water and 20-50 organisms. The experiments were run under low-level red light to minimize the response of the *P. hoyi* to light (Donner and Lindstrom, 1980). The water flowed into the test chambers at approximately 100 ml h⁻¹ to maintain a constant toxicant concentration. Animals (2-3 per replicate time point) were removed at 1, 2, 4 and 6 h, weighed, and frozen for later analysis. For studies above 4°C, the animals were acclimated gradually by elevating the aquarium temperature 1°C per day and holding the animals at the study temperature for 5-7 days prior to experimental use. To account for the time the animals were held in the laboratory during the thermal acclimation prior to experimental use, the toxicokinetics were also determined for animals collected at the same time and held at 4°C for the same duration. The kinetics for these 'holding time controls' were measured within 48 h of the thermally acclimated animals.

The *P. hoyi* are sediment dwelling organisms. The absence of a suitable substrate

TABLE II

The role of a substratum on the uptake rate constants.

Substrate	Date	Benzo(a)pyrene	Phenanthrene
		K_u (ml g ⁻¹ h ⁻¹)	K_u (ml g ⁻¹ h ⁻¹)
Sand	16 APR '85	68.6 ± 11.7	92.5 ± 19.5*
No sand	16 APR '85	71.4 ± 12.6	140.8 ± 12.2
Sand	8 MAY '85	171.4 ± 17.2	150.1 ± 10.9
No sand	8 MAY '85	176.2 ± 25.9	139.5 ± 16.9

* Significantly lower $P < 0.001$ compared to no sand treatment.

for burrowing may result in a stress that would alter the toxicokinetics, especially since uptake experiments are generally run in the absence of a substrate. This potential effect was examined twice by exposing the animals in the presence and absence of sand as a substratum. From preference studies run in this laboratory, sand is second most preferred substrate. Only the sandy sediment from Lake Michigan is more preferred (Landrum, unpubl. data). No consistent difference in the conditional uptake rate was observed (Table II). This is similar to observations for the amphipod *Hyalella azteca* (Landrum and Scavia, 1983).

To account for the extent of accumulation attributed to sorption to the organism plus that occurring from passive diffusion, accumulation was determined as above but using heat killed organisms. The compounds examined were BaP, Anth, and BI as representative of the range of physical chemical properties under study.

Elimination rate determinations involved transferring 10–20 labeled animals into 6-l glass aquaria containing uncontaminated water and Lake Michigan sediment. The animals were removed after approximately 2, 4, 7 and 14 days (exact times were used for calculations).

Biotransformation of the xenobiotics was examined in 20–60 animals at the end of the uptake studies. The organisms were blotted dry, weighed, and frozen at -20°C until analysis. Storage was for not more than one week. The animals were extracted in a Ten Brock tissue homogenizer with 2×20 ml ethylacetate:acetone (4:1 v:v) and 1×20 ml cyclohexane. The extracts were combined and filtered. The filtered residue was analysed for radioactivity as described below for the whole organism. The extracts were dried over anhydrous Na_2SO_4 and the volume reduced by rotary flash evaporation followed by evaporation under a stream of nitrogen to approximately 500 μl . The extracts were analyzed by TLC using hexane:benzene (8:2 v:v). Developed plates were divided into 4 or more sections corresponding to the R_f of the parent compound and 3 or more other sections including the origin. These sections were scraped from the plate and the activity determined. The total amount of metabolites was determined as the sum of all non-parent activity on the TLC plate and the activity of the filtered carcass residue.

To assess the concentrations of the xenobiotics in the water and animals, ^{14}C and ^3H activity were determined by placing the two to three animals per sample or 2 ml water directly into 12 ml of scintillation cocktail (Research Products International 3a70b). The animals were kept in the cocktail for at least 12 h prior to counting. The activity was assayed on a Packard 460C and/or LKB 1217 liquid scintillation spectrometer. Direct extraction by the cocktail gave results comparable with those obtained by predigesting the animals using the method of Mahin and Lofberg (1966). Samples were corrected for quench, using the external standards ratio method after subtracting background. All concentrations in animals and water were based solely on ^{14}C or ^3H activity, since previous studies had shown no biotransformation with *P. hoyi* and no degradation in the water over the length of time required to measure uptake (Landrum, 1982a, 1985a).

Calculation of kinetics parameters

The conditional uptake rate constants were calculated by fitting the data to a simple linear model:

$$C_a = K_w C_w t \quad (1)$$

where:

C_a = The concentration in the animal (ng g^{-1})

K_w = The conditional uptake rate constant ($\text{ml g}^{-1} \text{h}^{-1}$)

C_w = The total concentration of toxicant in the water measured from total activity (ng ml^{-1})

t = time (h).

To be valid, this simplified linear uptake model requires that two assumptions be met. First, the concentration in the water must be constant. The xenobiotic concentrations had a mean coefficient of variation of approximately 10% (Table I). Second, the elimination rate must be sufficiently slow and not result in significant loss over the time course of the uptake experiment. Even with the most rapidly eliminated compound at its highest elimination rate, the uptake lasted less than 20% of one elimination half life. The regression lines of C_a vs t for determining K_u had r^2 values of generally greater than 0.85 (Landrum, 1986) indicating that the above two assumptions had been met.

The water toxicant concentration must be corrected for the fraction of the xenobiotic bound to DOC, as this material is not apparently bioavailable (Landrum et al., 1985b, 1987). The methodology to measure the sorption to DOC had not been developed when some of the kinetics data was obtained. This is particularly true for Anth, TCB, and BI. To present consistent data for comparison, some estimation of the sorption to DOC had to be included. For all of the compounds where the reverse-phase sorption had been measured in Lake Michigan water at some point

during the course of the studies, a constant value of the sorption was used to correct all the uptake data for a particular compound (Landrum, 1986). The validity of this approach is based on the relatively small variation in sorption to DOC (coefficient of variation of < 10% for BaP; see results) and because the effect on the rate constant estimations for the less hydrophobic compounds should be lower than for compounds such as BaP. The only compound for which no correction was made was BI because a reliable estimate of the reverse-phase partition coefficient did not exist. Since BI is more water soluble than any of the other compounds, the potential effect should be minimal or at least less than that of Phe (only about 4%).

The depuration rate constants were calculated by fitting the data to a first order elimination model:

$$C_a = C_a^0 e^{-K_d t} \quad (2)$$

where:

K_d = the conditional depuration rate constant (h^{-1})

C_a^0 = the concentration of the toxicant in the organism at the start of the elimination experiment (ng g^{-1}).

All the data used to support the regressions and figures presented in this paper are available upon request from the author (Landrum, 1986). All regressions were performed using SAS version 5 (1985). All errors presented in the paper are standard deviations for means or standard errors from regression estimates.

RESULTS

Physiological and environmental parameters

The average mass of *P. hoyi*, generally increased from spring through late summer and then declined during the winter (Fig. 1). The data in Fig. 1 do not represent the total population distribution but rather the adult and juvenile population, used for toxicokinetic experiments, removed from the sediment with a 1-mm mesh screen. The sizes of the animals sampled varied most in the spring collections due to the presence of large fertile females and the smaller young-of-the-year. The average monthly dry/wet weight ratio was 0.269 ± 0.052 and the ash-free dry weight to wet weight ratio was 0.231 ± 0.046 . These are the average of the monthly means determined for eleven collections in 1984 containing a minimum of 24 animals per sample.

The lipid content for *P. hoyi* is higher than that for other Great Lakes benthos (Gardner et al., 1985b). The *P. hoyi* exhibit a seasonal trend in lipid content with a maximum occurring in late May to early June (Fig. 2). The lipid content was lower in 1985 than in 1984. The seasonal trend in the lipid content does not peak at the same time as the mass (Figs. 1 and 2). Therefore, the mass and lipid content of *P.*

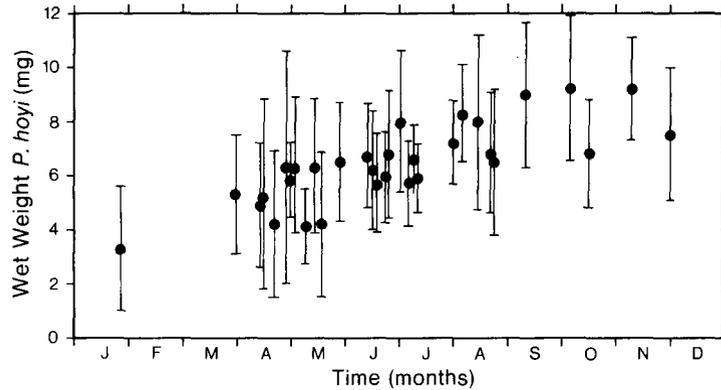


Fig. 1. Seasonal change in the wet weight of *P. hoyi* used for the toxicokinetic studies, collected at 23 to 29 m near Grand Haven, MI between April 1982 and December 1985.

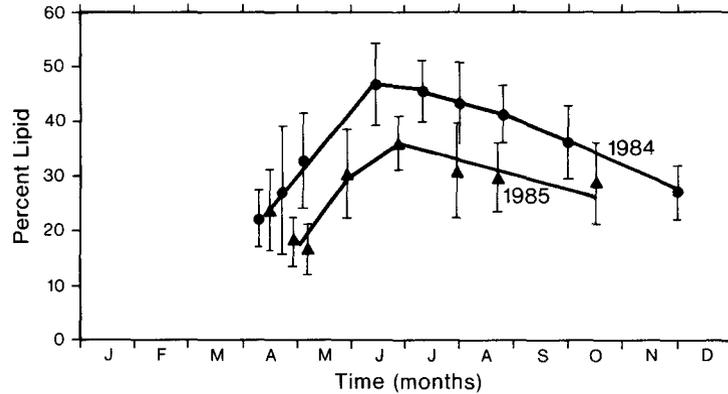


Fig. 2. Seasonal change in lipid content of *P. hoyi* on a percent dry weight basis for 1984 and 1985 field seasons.

hoyi were not well correlated. Previous work indicates a positive significant correlation between *P. hoyi* mass and lipid content but the relationship only accounts for about 8% of the data variability (Gardner et al., 1985b).

The bottom temperature at the site of collection, 23 to 29 m depth, reaches a maximum of approximately 16°C around middle to late August then slowly declines until reaching a minimum of 1–2°C in winter. The temperature is subject to frequent upwellings when the wind pattern is correct (Mortimer, 1971, Bell and Eadie, 1983). These upwelling events result in considerable variability in observed summer temperatures.

The DOC in the water column is fairly constant over the course of the season and averages approximately 2 to 3 mg l⁻¹. However, apparently high levels of DOC oc-

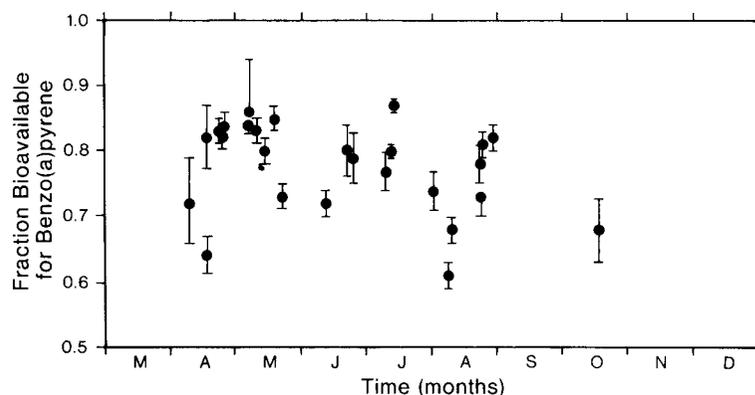


Fig. 3. Fraction of benzo(a)pyrene in the water column that is bioavailable as measured by the reverse-phase method and corrected for the bias between this chemical measure and the bioavailable fraction based on the relationship between sorption measured by reverse-phase separation and determined by kinetics (Landrum et al., 1987). Data from 1983–1985.

cur in the winter ($5\text{--}6\text{ mg l}^{-1}$) due to strong resuspension of bottom material with the subsequent humic and fulvic acid desorption from particulate matter (Eadie, pers. communic.). The sorption of toxic organics by DOC also shows some seasonality with the minimum sorption (maximum fraction freely dissolved, bioavailable) occurring in the spring (Fig. 3). This minimum in the sorption of BaP occurs at about the same time as the spring diatom bloom. DOC sorption of xenobiotics should be measured to account for changes in sorption resulting in bioavailability changes (Landrum et al., 1985b, 1987) because the sorption characteristics can differ seasonally and with different DOC sources (Landrum et al., 1984; Morehead et al., 1986). The annual average fraction freely dissolved BaP is 0.77 ± 0.07 ($n = 26$) for filtered Lake Michigan water.

Kinetics: uptake

The variability observed in the accumulation rates and respective rate constants was examined in relation to selected environmental and physiological factors.

The role of sorption to the carapace and the potential of passive diffusion into dead animals only account for a small fraction of the observed conditional uptake rate constants. For heat-killed animals, the uptake rate constants were generally less than 15% of the average annual values for the three PAH tested, BaP ($4.6 \pm 1.8\text{ ml g}^{-1}\text{ h}^{-1}$), Anth ($11.1 \pm 2.2\text{ ml g}^{-1}\text{ h}^{-1}$) and BI ($14.3 \pm 4.6\text{ ml g}^{-1}\text{ h}^{-1}$). The increase in the rate constants with increasing water solubility may reflect an increased aqueous diffusion rate for the more water soluble compounds. Thus, the reported uptake rate constants for these compounds are dependent on the organism functioning as a live entity.

The net accumulation of organic xenobiotics has been observed to be proportional to K_{ow} (Kenega and Goring, 1980; Veith et al., 1980; Hawker and Connell, 1986). Net accumulation is dependent on two factors, the rate of accumulation and the rate of elimination, and can be expressed as a two compartment model for accumulation from water. There was no relationship between the average annual conditional 4°C uptake rate constants and $\log K_{ow}$. However, the data are quite variable (Table III) even after correcting for sorption to DOC. In part, the variability can be explained by a relationship between the animal size (mass) and the uptake rate constant. For both BaP and TCB, the conditional uptake rate constant was inversely proportional to mass on a wet weight basis. BaP uptake yielded a strong relationship, $K_u = 266.2 (\pm 30.2) - (23.2 (\pm 5.1) \times \text{mass in mg})$ ($r^2 = 0.68$, $P < 0.02$, $n = 11$) while that for TCB was weaker, $K_u = 167.5 (\pm 16.2) - (4.3 (\pm 2.1) \times \text{mass in mg})$ ($r^2 = 0.38$, $P < 0.08$, $n = 8$) (Fig. 4a). The BaP data were only the data where the binding to DOC was measured and the laboratory holding time was 5 days or less. Data collected for PY and BAA was insufficient to determine trends between K_u and mass. The linear regression of K_u versus mass for the more water soluble compounds was not statistically significant. However, K_u tended to increase with increasing mass for Phe and BI while for the less water soluble Anth K_u tended to decrease with an increase in mass (Fig. 4b).

The preceding discussion demonstrates that relationships between K_u and physical chemical properties such as $\log K_{ow}$ cannot be clearly defined until the dependence of K_u on mass is first removed. Since the water soluble compounds do

TABLE III
Average annual 4°C uptake and depuration rate constants.

Compound	$\log K_{ow}^a$	Average K_u ($\text{ml g}^{-1} \text{h}^{-1}$)	Average K_d (h^{-1})
Anthracene	4.45	131.1 ± 40 ($n = 15$)	0.0033 ± 0.0019 ($n = 11$)
Benz(a)anthracene	5.9	138.6 ± 26.2 ($n = 6$)	0.0022 ± 0.0023 ($n = 2$)
Benzo(a)pyrene	6.5	116.8 ± 43 ($n = 35$)	0.0016 ± 0.0011 ($n = 31$)
Biphenyl	3.2	95.4 ± 28.2 ($n = 9$)	0.012 ± 0.0046 ($n = 9$)
Phenanthrene	4.16	129.0 ± 31 ($n = 27$)	0.0046 ± 0.0027 ($n = 21$)
Pyrene	5.2	199.2 ± 36.5 ($n = 4$)	0.0012 ± 0.0006 ($n = 3$)
Tetrachlorobiphenyl	4.4	134.9 ± 13.9 ($n = 9$)	ND ($n = 9$)

^a Data taken from Kenega and Goring, 1980, Mackay et al., 1980, and Mackay et al., 1983.

not show significant mass dependence, the average K_u for the experiments at 4°C should be representative of the respective mass independent rate constants. For TCB and BaP, the intercept at mass equals zero should represent the mass independent conditional rate constant for those compounds. Although there was not a large enough data set to examine the mass dependence of PY, its high water solubility suggests that it will be in the same category as the other relatively highly water soluble compounds; therefore, an average K_u was used. (The water solubility for PY is intermediate between that of Phe and Anth.) A plot of the average K_u 's for the water soluble compounds and the intercepts for TCB and BaP against $\text{Log } K_{ow}$ indicates that the higher $\text{Log } K_{ow}$ compounds are accumulated more rapidly ($K_u = -84.4(\pm 30.0) + 53.5(\pm 6.3) \text{ Log } K_{ow}$, $r^2 = 0.95$, $n = 6$, Fig. 5) and K_u is inversely related to the log of the aqueous solubility of the compounds

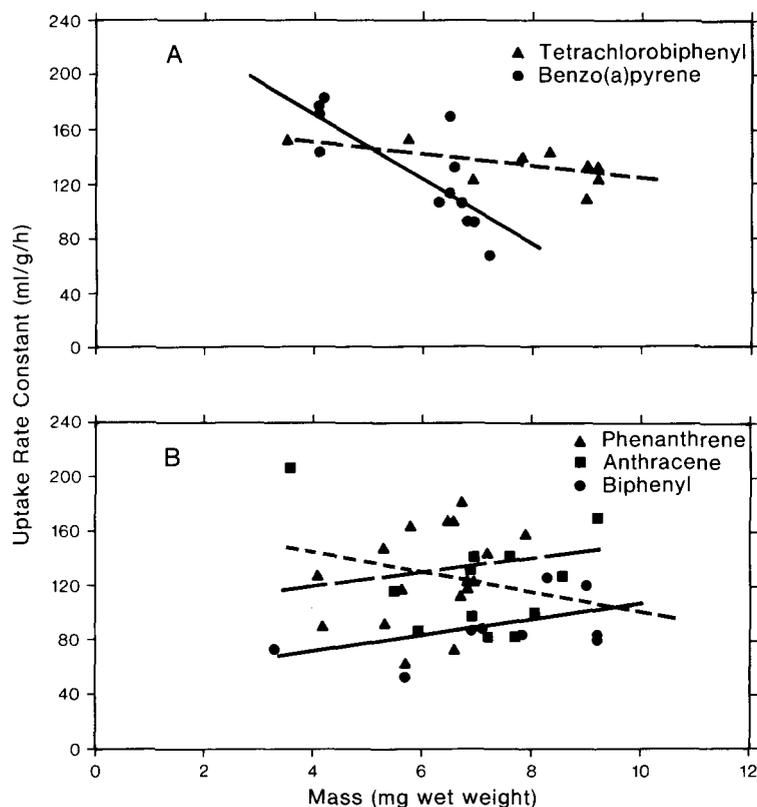


Fig. 4. (A) The relationship between K_u and organism mass for benzo(a)pyrene (solid line) and tetrachlorobiphenyl (dashed lined). (B) The scatter plots of K_u with mass for the more water soluble compounds: anthracene (dotted line), phenanthrene (dashed line) and biphenyl (solid line). The lines are the least squares fit through the data.

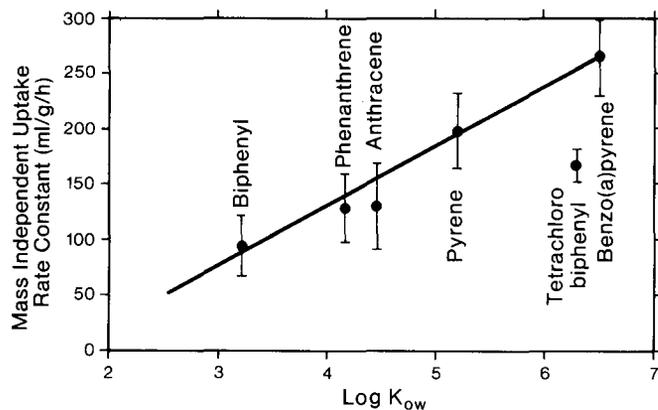


Fig. 5. After removing the effect of mass on K_u , the uptake rate constant was linearly proportional to the $\log K_{ow}$. Error bars represent one standard error from the regression for BaP and TCB or the standard deviations on the means for the individual compounds for the water soluble compounds.

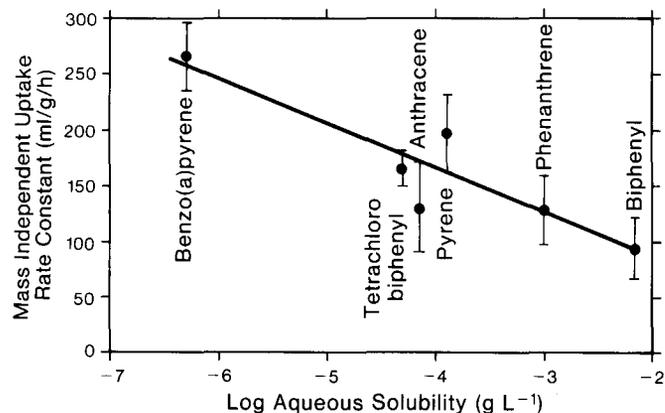


Fig. 6. The mass independent uptake rate constant was also inversely proportional to the log of the aqueous solubility. Error bars represent one standard error from the regression for BaP and TCB or the standard deviations on the means for the individual compounds for the water soluble compounds.

$(K_u = 7.7(\pm 38.0) - 39.5(\pm 9.2) \log \text{aqueous solubility (g l}^{-1}\text{)}, n = 6, r^2 = 0.82, \text{ Fig. 6})$. Correcting the aqueous solubilities for the melting point differences after Yalkowsky and Valvani (1980) did not provide significant improvement in the regression equation between K_u and \log aqueous solubility.

Because the compounds of interest are highly lipophilic and showed a significant relationship with $\log K_{ow}$, the potential role of lipid content on the conditional uptake rate constant was examined. Linear regressions of lipid content and the conditional uptake rate constants for BaP and Phe (1984 and 1985) were not significant.

The role of exposure temperature was examined three times for BaP and Phe and twice for Anth. The duration that animals are held in the laboratory can markedly influence the toxicokinetics of some marine organisms (Landrum and Crosby, 1981). Since the animals were held in the laboratory for a considerable period of time during temperature acclimation, it was necessary to control for this hold-time. There was no statistically significant correlation between duration of holding in the laboratory up to 38 days and either the uptake or depuration kinetics. Therefore, the effect of temperature on the conditional rate constants could be examined without having to consider the duration the animals were held in the laboratory. The conditional uptake rate constant for Anth increased with increasing temperature over the range of 4 to 15°C ($K_u = 81.5(\pm 29.5) + 10.9(\pm 3.3) \times T^\circ\text{C}$, $n = 7$, $r^2 = 0.68$, $P < 0.05$, Fig. 7A). For TCB the slope of the temperature curve was somewhat, but not statistically, greater than that for Anth ($K_u = 76.1(\pm 3.8) + 12.2(\pm 0.5) \times T^\circ\text{C}$, $r^2 = 0.998$, $n = 3$, Fig.B). The uptake of BaP (Fig. C) also increased with increasing temperature yielding an overall regression of $K_u = 53.1(\pm 31.2) + 10.6(\pm 4.2) \times T^\circ\text{C}$, $r^2 = 0.44$, $n = 10$. Phe did not show a significant relationship between temperature and the conditional uptake rate constant even though the uptake was performed simultaneously with those for BaP. There was, however, a trend for Phe in the same direction as that of Anth, TCB and BaP ($K_u = 122.4(\pm 19.2) + 3.52(\pm 2.9) \times T^\circ\text{C}$, $n = 12$, $r^2 = 0.11$, $P = 0.25$) while BI exhibited no thermal dependence. The slope of the temperature dependence for the more hydrophobic compounds appears to be relatively constant (average slope 10.9 ± 1.5). This average slope would yield an average Q_{10} of 3.1 ± 1.2 for Anth, BaP and TCB.

Kinetics: depuration

The elimination or depuration is the second part of the net accumulation equation and seems to be most responsible for the extent of accumulation of nonpolar materials. Elimination can be markedly altered by biotransformation. The biotransformation of the selected organics was not observable within the time frame of the experiments (this work, and Landrum, 1982b; Landrum et al., 1985a). Thus, the elimination of the nonpolar xenobiotics was as parent compound. As with the uptake rate constants, the depuration rate constants must be considered conditional constants due to various experimental conditions and physiological states of the organisms over the study period. The depuration rate constants (slopes for the regression of $\ln C_a$ vs t) for some of the PAH, particularly BaP were on occasion not significantly different from zero (approximately 20% of the BaP values and $< 10\%$ for all other compounds except TCB) because the elimination was slow and the biological variability was large (Landrum, 1986). However, the estimate of the rate constant derived from the data was used as the best estimate of the depuration for that experiment, regardless of significance, and was used for examination of the effect of various variables on the conditional constants. Non-significant estimates

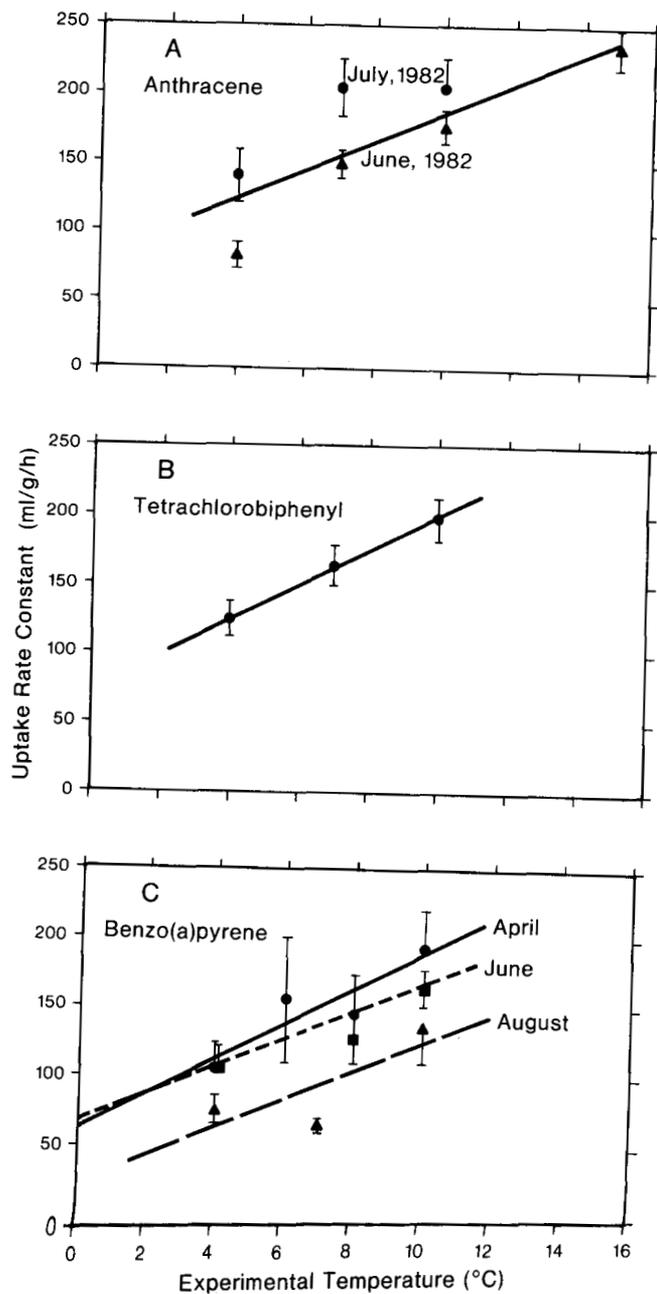


Fig. 7. The effect of temperature on the conditional uptake rate constants for anthracene (A), tetrachlorobiphenyl (B), and benzo(a)pyrene (C) ($K_u = 63.3 + 12.5 \times T^{\circ}\text{C}$, $r^2 = 0.81$ April collection; $K_u = 67.9 + 8.8 \times T^{\circ}\text{C}$, $r^2 = 0.90$ June collection and $K_u = 23.9 + 9.95 \times T^{\circ}\text{C}$, $r^2 = 0.61$ August collection).

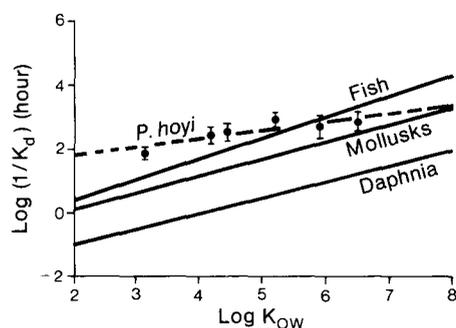


Fig. 8. Depuration was most appropriately described by $\log(K_d^{-1}) = 0.25 \log K_{ow} + 1.36$ when the temperature was held at 4°C. The slope of this line is about half that found by Hawker and Connell (1986) for fish, daphnia and mollusks.

were used because (1) the only alternative would have been the use of zero; (2) the use of zero would have been even less appropriate as an estimate since some loss was observed and (3) the use of non-significant best estimates is a statistically justifiable approach (ASTM, 1983). The conditional depuration rate constants at 4°C for the various PAH (Table III) were related to K_{ow} by $\log(K_d^{-1}) = 0.25(\pm 0.03) \log K_{ow} + 1.36(\pm 0.14)$, $r^2 = 0.53$, $n = 72$ (Fig.8). This relationship breaks down when TCB is included. Over the course of eleven studies at temperatures as high as 10°C, elimination of TCB was not detected during eleven days of depuration. Based on an assumed loss of 10% in 10 days that could be easily detected even with the biological variability, the elimination rate that would result would yield a half life for TCB of 65 days. Therefore, the half life for TCB is at least 4 times longer than that for BaP.

The depuration rate constants were also inversely proportional to organism mass for each compound (Table IV). A regression of the slopes for each compound of the depuration rate versus mass was inversely proportional to $\log K_{ow}$. This implies

TABLE IV

The effect of organism mass on the depuration rate constant.

Compound	Relationship of K_d with mass				
	Slope	Intercept	r^2	P	n
Biphenyl	0.0011 ± 0.0008	-0.021 ± 0.006	0.22	<0.2	8
Anthracene	0.00092 ± 0.00003	-0.0098 ± 0.0022	0.52	<0.2	9
Phenanthrene	0.0013 ± 0.0005	-0.012 ± 0.0032	0.28	<0.03	16
Pyrene	0.00071 ± 0.00002	-0.0059 ± 0.0002	0.99	<0.03	3
Benz(a)anthracene		Insufficient data			
Benzo(a)pyrene	0.00031 ± 0.00011	-0.0036 ± 0.0007	0.23	<0.02	24

Relationship has the form $K_d = \text{Slope} \times \text{mass} + \text{intercept}$, ± standard error.

that the diffusion out of the organism becomes slower as the hydrophobicity increases, as expected. Combining mass and K_{ow} in a multiple linear regression yields a relationship for the elimination of any PAH from *P. hoyi* at 4°C: $\log(K_d^{-1}) = 0.26(\pm 0.03) \log K_{ow} + 0.05(\pm 0.02) \times (\text{Mass in mg}) + 0.99(\pm 0.22)$, $r^2 = 0.55$, $n = 73$, $P < 0.0001$, that accounts for 55% of the variability in K_d .

The depuration rate constant at 4°C was also inversely related to the *P. hoyi* lipid content for BaP, $K_d = -5.0(\pm 2.0) \times 10^{-5} \times (\% \text{lipid}) + 0.003(\pm 0.001)$ ($r^2 = 0.34$, $P < 0.05$, $n = 12$, Fig. 9) and for Phe, $K_d = -2.2(\pm 0.5) \times 10^{-4} \times (\% \text{lipid}) + 0.012(\pm 0.001)$ ($r^2 = 0.71$, $n = 10$, $P < 0.0025$, Fig. 9). If the lipid content is added as a factor to account for K_d variability the multiple linear regression with mass is not improved for BaP; in fact, the regression does not have as good an F statistic as the individual regressions for K_d vs mass or lipid content alone. For Phe, the inclusion of lipid content with mass in a multiple linear regression significantly improves the relationship over either variable alone ($K_d = -2.6(\pm 0.5) \times 10^{-4} \times (\% \text{lipid}) + 4.9(\pm 4.0) \times 10^{-4} \times (\text{mass in mg}) + 0.0103(\pm 0.0002)$, $r^2 = 0.75$, $n = 10$, $P < 0.005$). The improvement in the relationship for Phe, for this small subset of the data, occurs because the lipid content controls most of the variability ($K_d = -2.2(\pm 0.5) \times 10^{-4} \times (\% \text{lipid}) + 0.0119(\pm 0.001)$, $r^2 = 0.71$, $n = 10$, $P < 0.003$ and $K_d = -5.9(\pm 5.6) \times 10^{-4} \times (\text{Mass in mg}) + 0.0082(\pm 0.0036)$, $r^2 = 0.11$, $P < 0.35$, $n = 10$). For both BaP and Phe, the data set available for examination of the relationship of lipid content, mass and depuration was smaller than was available for mass and K_d only.

Lipids are extremely important in the storage of organic xenobiotics. As shown, the depuration rate constants are inversely proportional to organism lipid content measured prior to initiation of depuration. In four experiments, two with Grand

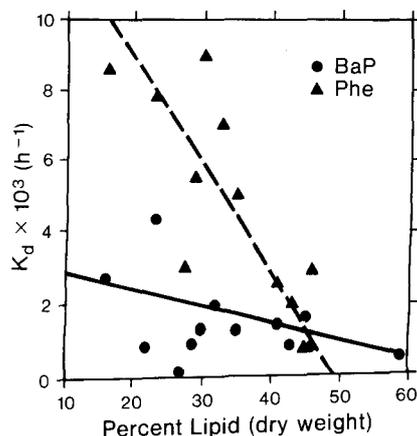


Fig. 9. Depuration was inversely proportional to the percent lipid on a dry weight basis for benzo(a)pyrene (solid line) and phenanthrene (dashed line).

TABLE V
Elimination rate constants for lipid and xenobiotics.

Date/compound	Grand Haven	Benton Harbor
	K_d (h^{-1})	K_d (h^{-1})
<i>August 22, 1985</i>		
Lipid	-0.0008 ± 0.0007	-0.002 ± 0.0008
Benzo(a)pyrene	-0.0009 ± 0.0003	-0.0013 ± 0.0004
Phenanthrene	-0.0055 ± 0.0007	-0.009 ± 0.0005
<i>October 16, 1985</i>		
Lipid	ND	-0.0014 ± 0.0009
Benzo(a)pyrene	-0.0023 ± 0.0006	-0.0013 ± 0.0005
Phenanthrene	-0.0044 ± 0.0005	-0.005 ± 0.0007

ND = None detected.

Represents the standard error of the slope of the regression line for first order elimination.

Haven-collected organisms and two with Benton Harbor-collected organisms, the time course for lipid loss was examined simultaneously with the elimination of BaP and Phe (Table V). The depuration rate constants for BaP tracked very closely the loss rate constant for lipid, except for one set of Grand Haven-collected organisms where BaP was eliminated faster than lipid was lost. Phe elimination generally increased with the increasing loss of lipid. However, the Phe depuration rate constants were approximately 4 to 5 times greater than that for BaP or lipid loss.

The thermal dependence of the depuration rate constant did not appear constant over the season (Table VI). As with the uptake rate constants, the depuration rate constants were not affected by the time held in the laboratory. Both BaP and Phe

TABLE VI
Thermal dependence of the depuration rate constant.

Compound	Date	Relationship of K_d with temperature				
		Slope ($\times 10^3$)	Intercept ($\times 10^3$)	r^2	P	n
Phenanthrene	April '85	2.57 ± 0.5	-7.85 ± 3.8	0.96	<0.15	3
	July '85	0.25 ± 0.25	1.6 ± 0.2	0.48	<0.5	3
	August '85	0.42 ± 0.28	0.4 ± 0.2	0.99	<0.05	3
Benzo(a)pyrene	April '85	0.67 ± 0.3	-2.2 ± 2.0	0.69	<0.17	4
	July '85	No change detected with temperature				
	August '85	0.10 ± 0.02	0.47 ± 0.1	0.96	<0.12	3
Biphenyl	June 82	1.5 ± 0.5	4.8 ± 3.5	0.91	<0.2	3
Anthracene	June '82	1.5 ± 0.2	4.4 ± 2.0	0.88	<0.006	6

The relationship is $K_d = \text{Slope} \times \text{Temperature} + \text{intercept}$.

were run simultaneously as dual labeled experiments and both show the same change in the relative effect of temperature between the spring and summer experiments. The slope of the temperature curve drops during the summer by about one order of magnitude. In general, the change in K_d in the spring would be about $0.0016 \pm 0.0008 \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$ for all the compounds represented in Table III and $0.0003 \pm 0.00016 \text{ h}^{-1} \text{ } ^\circ\text{C}^{-1}$ in the summer and fall as represented by BaP and Phe. The impact of the thermal effect on the kinetics rate constants should be small since there is little change in the environmental temperature in the spring when the laboratory depuration rate constants are most sensitive to thermal effects and when the environmental temperatures change in the late spring to summer the rate constants are apparently less sensitive to change in temperature.

Geographical comparisons

On three occasions, animals were collected at both Grand Haven and at Benton Harbor on the same day. The Benton Harbor site is in a high sedimentation area that has high concentrations of contaminants in the sediments (Eadie et al., 1982). The site is also about twice as deep as the Grand Haven site. In all cases, the animals collected from the Benton Harbor area were larger than those collected on the same day in Grand Haven. Size differences were significant for the August and October collections (Table VII). Correspondingly, the conditional uptake rate constants were lower for the Benton Harbor animals. However, after correcting for the mass dependency of the uptake rate constant, only one set, the May collection, had significant differences ($P < 0.05$) between the two collection sites for BaP and Phe, with the Grand Haven animals exhibiting the greater uptake. After accounting for differences in organism size, no statistical differences in the depuration rate constants were found between animals collected at Grand Haven and those collected in Benton Harbor.

DISCUSSION

In shallow water, *P. hoyi* have a one year life cycle (Marzolf, 1954). The *P. hoyi* release their young in the winter and early spring. The animals increase in size through the summer and reach an apparent maximum in the late summer or early fall. The size of the individuals then declines as the young are released and the spent females die off. The size increase does not seem to correlate with the accumulation of lipids which occurs mostly in the spring through early summer. The lipid content reduction between the two field seasons could have been due to changes in the quality or quantity of food. The peak in lipid content corresponds or lags slightly the peak in spring diatom production (Fahnensteil and Scavia, 1987). These changes in size, composition and reproductive state have a major impact on the accumulation and elimination of organic xenobiotics (Table VIII). As a result, these physiological

TABLE VII

Comparisons between Benton Harbor- and Grand Haven-collected animals.

Variable/date		Benton Harbor	Grand Haven
Mass (mg/animal)			
6 May '85		5.6 ± 1.8	4.1 ± 1.4
22 Aug '85		9.2 ± 1.4	6.5 ± 2.7*
16 Oct '85		8.8 ± 1.8	6.8 ± 2.0*
K_u (ml g ⁻¹ h ⁻¹)			
6 May '85	(BaP)	85.2 ± 15.7	143.8 ± 20.2*
	(Phe)	94.0 ± 13.1	129.1 ± 11.1*
22 August '85	(BaP)	75.2 ± 15.7	112.3 ± 24.8
	(Phe)	124.2 ± 16.8	167.5 ± 28.6
16 Oct '86	(BaP)	84.1 ± 8.9	103.8 ± 9.4
	(Phe)	89.0 ± 7.6	120.3 ± 27.8
K_d (h ⁻¹)			
6 May '85	(BaP)	0.0032 ± 0.0006	0.0027 ± 0.0004
	(Phe)	0.0085 ± 0.0004	0.0086 ± 0.0006
22 Aug '85	(BaP)	0.0013 ± 0.0004	0.0023 ± 0.0006
	(Phe)	0.005 ± 0.0007	0.0044 ± 0.0005
16 Oct '85	(BaP)	0.0013 ± 0.0004	0.0009 ± 0.0003
	(Phe)	0.009 ± 0.0005	0.0055 ± 0.0007

* $P < 0.05$.

variables must be accounted for to make comparisons between measurements of toxicokinetics from collection to collection and from collections made at different sites.

The presence of dissolved organic carbon affects the accumulation of organic xenobiotics by aquatic organisms, especially the more water insoluble compounds (Landrum et al., 1985, 1987). The water concentration must be corrected for the amount of contaminant bound to DOC prior to calculating the conditional uptake rate constants and making comparisons on any basis. Failure to make a correction for xenobiotic binding to DOC would cause significant under-estimates of the conditional uptake rate constants for compounds with Log K_{ow} greater than about 4.5 to 5. It was interesting to find a seasonal pattern for the binding of BaP to organic carbon from lake water. The BaP was least bound in the spring when the DOC in the water column was likely dominated by products released from diatoms.

The conditional uptake rate constant was also dependent on the mass of the organism for those compounds that were relatively water insoluble, BaP and TCB. Mass dependency was not significant for the more water soluble compounds. The

data for Phe were often accumulated in the same animals as those for BaP by performing dual labeled experiments, [^3H]BaP and [^{14}C]Phe. Thus, the animals were exposed to a mixture of two compounds, one of which exhibited a reduction in accumulation with an increase in animal mass, while the other compound did not exhibit this effect. There was no apparent difference in the individual rate constants whether measured as single compound exposures or in combination at the levels employed. Thus, the individual compounds yielded results that were self consistent but varied between compounds. The differences in the role of mass may occur from differences in the kinetics of transport across the membrane responsible for entrance into the organism and/or the kinetics of distribution to the final storage site.

The rate of accumulation of the compounds under study was proportional to the log K_{ow} once the mass dependency was accounted for. Diffusion rate and membrane permeability are major factors that influence the transfer kinetics as the hydrophobicity of the compounds change. Where the rate controlling step in the uptake rate is controlled by membrane permeation an increased accumulation will occur with increased lipophilicity (K_{ow}) (Gobas et al., 1986); thus, from the data, K_u should be under membrane permeation control. However, the accumulation kinetics are complex and apparently change rate-determining steps at lower K_{ow} 's based on the cutoff of the mass dependency at lower K_{ow} .

The lipid content of the animals did not affect the uptake rate constant. Since in-

TABLE VIII
Factors affecting the toxicokinetics.

Compound	Rate constant	Log K_{ow}	Log S^a	Organism	% Lipid mass	Temp.
BaP	K_u	+	-	-	0	+
	K_d	-	ND	-	-	+
BAA	K_u	+	-	ID	ND	ND
	K_d	-	ND	ID	ND	ND
TCB	K_u	+	-	+	ND	+
	K_d	ID	ND	ID	ND	ID
Anth	K_u	+	-	0	ND	+
	K_d	-	ND	-	ND	+
Py	K_u	+	-	ID	ND	ND
	K_d	-	ND	ID	ND	+
Phe	K_u	+	-	0	0	0
	K_d	-	ND	-	-	+
Bi	K_u	+	-	0	ND	ND
	K_d	-	ND	-	ND	ND

+ = Positive interaction, - = negative interaction, 0 = significant relationship but the slope was not different from zero, ID = indeterminate (no statistically determinable relationship), ND = not determined (not examined).

^a Log S equals Log aqueous solubility.

creases in lipid, above some baseline level, are undoubtedly storage lipids, they will act as final storage sites but are removed from the site of uptake. Therefore, the lipid content should not influence the uptake rate.

Temperature effects on K_u , as with mass, were dependent on the relative aqueous solubility of the compound. BzP, TCB and Anth uptake were clearly dependent on the experimental temperature. The effect of temperature on K_u appeared to be limited to compounds whose log aqueous solubility is less than that of Phe, -3.0 ($\log g\ l^{-1}$). Neither Phe or BI exhibited any significant temperature dependency for accumulation. This further supports a hypothesis that the uptake kinetics may be composed of multiple components contributing to the rate of accumulation.

Overall, the accumulation of organic xenobiotics from water was dependent on the size of the organism, the temperature, and the log K_{ow} or aqueous solubility of the compound. Further, the binding of the organic xenobiotics to DOC in natural waters must be accounted for, as well as the sorption to particles to assess uptake correctly.

The elimination of the organic xenobiotics occurs as a partitioning phenomenon particularly since no kidney-like function appears to exist. Even though biotransformation of xenobiotics is known to occur among amphipods (Landrum and Scavia, 1983; Reichert et al., 1985), there was no measurable biotransformation for *P. hoyi*; thus, the losses observed were loss of parent compound. The high lipid content of the *P. hoyi* may account, in part, for the absence of measurable biotransformation. Because compounds primarily stored in lipid may not have sufficient contact with biotransformation sites at sufficiently high concentrations to produce detectable levels of metabolites. The high lipid content accounts in large part for the extremely slow elimination rates. This high lipid content in conjunction with the absent or extremely slow biotransformation capability may well be responsible for the absence of elimination of TCB by *P. hoyi*. The presence of a peak in lipid content is curious since most benthic organisms have rather stable lipid contents over the season because they have a fairly stable food supply (Gardner et al., 1985b). This spring peak suggests that *P. hoyi* does not have a stable food supply and is a selective feeder. The selectivity of feeding in this organism has also been observed as an absence of continuous feeding (Quigley, 1988). This discontinuous feeding response may account, in part, for the observed reduction in elimination with the increase in organism size since larger animals were less likely to feed (Quigley, 1988). The movement of food through the intestine of lower animals is apparently important for elimination of foreign compounds (Leversee et al., 1982; Abedi and Brown, 1961; Landrum and Scavia, 1983; Frank et al., 1986). This is especially true since the *P. hoyi* excrete their fecal material with a peritrophic membrane (Quigley, 1988) that was extremely important for elimination of DDT and DDE by mosquito larvae (Abedi and Brown, 1961). Therefore, reduced feeding should result in reduced formation of peritrophic membrane with subsequent reduced elimination of organic xenobiotics.

The relationship between $\log(K_d^{-1})$ and $\log K_{ow}$ yielded a slope that was approximately half that for the relationship developed between elimination rate constants and K_{ow} for fish, daphnia, and mollusks (Hawker and Connell, 1986). The intercept for *P. hoyi* is approximately two orders of magnitude higher than those for the other animals but with the lower slope the regression lines intersect at a $\log K_{ow}$ of about 5.5. This difference apparently reflects the differences in the intrinsic ability of different species to eliminate the compounds. The mechanism for this difference reflects the complex interaction between lipid content, surface to volume ratio and specific metabolic activity of the organisms.

The relationship between the inverse of the $\log(K_d^{-1})$ with $\log K_{ow}$ and organism mass suggests that the elimination rate for these hydrophobic molecules is diffusion layer limited because of the dependence on both K_{ow} and organism size (Gobas et al., 1986). Again, this is only for the PAH and will not account for the TCB data. This low elimination of TCB and the reasons for it require further research.

The redistribution of the compound from storage sites may play a role in xenobiotic elimination. The rate of lipid loss was the same as that for BaP elimination suggesting that BaP becomes mobilized as lipid is consumed. While Phe loss was proportional to loss of lipid, more than one mechanism is suggested for mobilizing Phe because the depuration rate constant was 4 to 5 times larger than that for BaP or lipid loss. The TCB was an anomaly in that there was no measurable elimination in 11 days. This very slow elimination implies that the organism will not reach steady state for TCB for at least a year and will likely not reach steady state at all. Therefore, it is expected that *P. hoyi* will accumulate TCB and probably other PCB congeners for its entire life with little loss. PAH, in particular BaP, elimination rates seemed to be directly related to the loss of lipid from the organism. Such lipid losses were still not sufficient to result in measurable losses of TCB. Further, PAH of similar and greater K_{ow} are eliminated much more rapidly and the elimination cannot be attributed to biotransformation; the storage site for the TCB may be different than that for the PAH. Alternatively, the kinetics of dissociation from the storage site may be much slower for PCB than that of PAH. Since lipid is the likely storage site for TCB as well as the PAH, partitioning theory needs to be updated to incorporate structural characteristics in the description of sorption to account for the differences observed in elimination.

All the compounds examined, except TCB, had increased elimination rate constants with increased temperature. The influence of temperature on the elimination was apparently seasonal and related in part to seasonal lipid levels. In spring, the elimination rate constant increased to a greater extent with temperature elevation than was observed with the higher lipid content summer organisms.

Organisms were collected from the Grand Haven area and the Benton Harbor area on the same day and assayed for the accumulation and elimination of BaP and Phe simultaneously. These two sites were chosen because the concentrations of PAH and other pollutants in the sediments are much higher in the Benton Harbor

area than in the Grand Haven area (Eadie et al., 1982; Cahill and Shimp, 1984). Since many of the persistent organic pollutants, especially PAH and PCB, induce the mixed function oxidase system in mammals and fish, which would in turn affect elimination, we hypothesized that the higher exposure would result in some induction and therefore measurable differences in the toxicokinetics. Differences in uptake by the organisms between the two sites were, in all but one case, accounted for by the differences in the mass of the organisms. The absence of significant site differences lies, in part, in the large confidence intervals resulting from biological and experimental variability. In all cases, the animals collected at Benton Harbor were larger than those collected on the same day from Grand Haven. The size differences between the two sites may result from any of several environmentally related causes such as increased nutrient availability at the Benton Harbor site (Eadie et al., 1983.), stimulation of growth by low level toxic stress, as has been observed in the laboratory (Kielty et al., 1985), and/or other significant environmental factors. Because of the higher level of MFO inducing compounds at the Benton Harbor site, we hoped to detect biotransformation, but none was observed. Further, enhanced biotransformation would likely result in enhanced elimination since the metabolites are more polar. The absence of increased depuration, even after accounting for differences in the mass of the organisms, also suggests a low biotransformation capacity and little or no MFO induction.

The bioconcentration factors, derived from the above kinetics data, are large relative to the bioconcentration observed for other organisms of similar size (Landrum and Scavia, 1983; Laversee et al., 1982; Gerould et al., 1983). For Anth the maximum BCF would be approximately 36 000 when determined as a ratio of K_u/K_d at 4°C while that for *Hyaella azteca* was approximately 1000 at 20°C (Landrum and Scavia, 1982). Although *P. hoyi* can survive at 20°C, the laboratory LT_{50} was observed to be 7°C (Smith, 1972). However, if the kinetics data were extrapolated to 20°C, using the maximum thermal variance observed, the expected BCF would be about 10 000, approximately the same as the K_{ow} . This suggests that *P. hoyi* eliminate Anth about an order or magnitude slower than *Hyaella* at an assumed similar temperature since the K_{us} are about the same. However, *Hyaella* do biotransform Anth (Landrum and Scavia, 1983) which may account for some of the elimination rate differences between the two species. Because *P. hoyi* accumulates relatively large quantities of organic pollutants without biotransforming them, they should be an excellent source of these compounds for higher levels in the food chain.

Overall, the accumulation and loss of compounds at trace levels seems independent of whether the exposure occurs as a single compound or as a part of mixture. Both physiological and environmental variables, such as lipid content, temperature and organism mass, influence the kinetics and their impact appears to disappear when the $\log K_{ow}$ becomes sufficiently small or aqueous solubility increases. While the physical-chemical properties, such as $\log K_{ow}$, explain a substantial portion of the variability, they are not always adequate to permit predication across classes of

compounds. For instance, TCB shows substantially lower elimination than could be explained from log K_{ow} predictions based on PAH. This implies that our understanding of partitioning theory and the factors affecting the movement into and out of organisms needs improvement. Finally, kinetics processes are complex and have more than one potential step or mechanism for determination of the rate process and the role of physical-chemical properties that influence these steps for transport into or out of organisms require more study.

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