*Environmental Toxicology and Chemistry*, Vol. 10, pp. 35–46, 1991
Printed in the USA. Pergamon Press plc

0730-7268/91 $3.00 + .00

# TOXICOKINETICS AND TOXICITY OF A MIXTURE OF SEDIMENT-ASSOCIATED POLYCYCLIC AROMATIC HYDROCARBONS TO THE AMPHIPOD *DIPOREIA* SP.

PETER F. LANDRUM,* BRIAN J. EADIE and WARREN R. FAUST
Great Lakes Environmental Research Laboratory, NOAA, 2205 Commonwealth Blvd., Ann Arbor, Michigan 48105

(*Received* 3 *January* 1990; *Accepted* 26 *March* 1990)

**Abstract**—Amphipods, *Diporeia* sp., were exposed to a reference sediment dosed with two radiolabeled polycyclic aromatic hydrocarbons (PAHs) and sediments dosed with a mixture of PAHs at four concentrations: 21.4, 41.0, 119.6, and 327.0 nmol $g^{-1}$ dry sediment, as the molar sum of the PAH congeners. *Diporeia* sp. were sampled for mortality and toxicokinetics for up to 26 d. Significant sediment avoidance was observed at the highest dose out to 6 d of exposure. The toxicity for the mixture was 38 ± 3% after 19 d of exposure at the highest dose, 327 nmol $g^{-1}$ dry sediment as the molar sum of the PAHs. The measured organism concentration required to produce the mortality at day 19 was 2.9 $\mu$mol $g^{-1}$ as the sum of the bioaccumulated PAHs. The uptake clearance (g dry sediment $g^{-1}$ organism $h^{-1}$) from sediments for the radiotracers increased with dose to an apparent plateau. Uptake clearance is the conditional constant relating the contaminant flux into the organism to the contaminant concentration in the referenced environmental compartment, in this case the sediment. This enhanced bioavailability with dose occurred even in the absence of overt effects and in the absence of changes in the measured partition coefficients for phenanthrene (273 ± 98) and pyrene (540 ± 212), between the freely dissolved radiotracers in interstitial water and the sediment particles. These changes in bioavailability with changes in PAH concentration suggest that predictions of bioaccumulation of PAH congeners from sediments under different field concentration conditions will not be possible with standard partitioning relationships.

**Keywords**—Polycyclic aromatic hydrocarbons *Pontoporeia* *Diporeia* sp. Toxicokinetics Sediments

## INTRODUCTION

Contaminated sediments represent the legacy of past and current discharges of contaminants to our freshwater and marine aquatic environments. The extent of the problem is not yet completely defined, but highly contaminated sites have been reported for both marine [1] and freshwater [2,3] systems. These contaminated sediments represent a large potential source and in some cases the only apparent source of contaminants to the food chain. Field studies have provided evidence that sediment-associated contaminants are affecting portions of the ecosystem such as the benthic community structure in portions of the Great Lakes [4]. In spite of this evidence, questions remain: What amount of sediment-associated contaminants are biologically available? What levels of sediment-associated contaminants produce intoxication? What are the major factors that modify the toxicity and bioavailability of sediment-associated contaminants?

To address some of the above questions, the amphipod *Diporeia* sp.—the major Great Lakes benthic invertebrate on a mass basis [5]—was exposed to a range of laboratory-dosed sediments containing a mixture of polycyclic aromatic hydrocarbons (PAHs). (Note: The amphipod used for these studies has previously been named *Pontoporeia hoyi*; the change to *Diporeia* sp. is recent [6]). High levels of PAHs have been reported in the Great Lakes [7] and have been correlated with a high tumor incidence in bottom-dwelling fish [8,9]. *Diporeia* do not metabolize PAHs [10,11] and high concentrations have been measured for *Diporeia* collected from lakes Michigan and Erie [12,13], thus this amphipod may constitute a major vector for the transport of PAHs to fish. Exposure of *Diporeia* to PAHs dosed into sediments either as single compounds or in pairs at low concentrations (0.001–2 nmol $g^{-1}$) indicated that the uptake was

*To whom correspondence may be addressed.
GLERL Contribution No. 697.

apparently independent of the presence of other PAH or native contaminants and was inversely proportional to the log of the octanol–water partition coefficient ($K_{ow}$) [14]. The objective of this study was to extend our understanding of the bioavailability of sediment-associated PAHs to benthos by (a) examining the uptake clearance of multiple PAH congeners exposed in a mixture, (b) examining the role of PAH sediment concentration on the uptake clearance, and (c) determining the toxicity of a mixture of PAHs by examining mortality and sediment avoidance. (Note: The uptake clearance is the conditional constant relating the contaminant flux into the organism to the contaminant concentration in the referenced environmental compartment, in this case the sediment.)

## MATERIALS AND METHODS

### *Collection of environmental samples*

*Diporeia* were collected from Lake Michigan off Grand Haven, Michigan, at a depth of 24 m with a PONAR grab sampler. *Diporeia* collected from this site have very low background concentrations of PAH [13]. The *Diporeia* were gently removed from the sediment with a screen, placed in clean lake water, and kept cold with ice during transport to the laboratory. *Diporeia* were kept in aquaria containing about 2 to 3 cm of sediment and 7 to 10 cm of lake water at 4°C [10].

Water used throughout the work was Lake Michigan surface water (collected about 1 m below the surface) and stored at 4°C. Lake Michigan sediment was obtained by PONAR grab approximately 5 mi off Grand Haven, Michigan, at a 45-m depth. This sediment has somewhat higher background concentrations of PAH than the site from which the *Diporeia* were collected; however, the organic carbon level is much higher, and thus the sediment would have a more consistent food supply for the longer exposure [13]. The sediment was sieved at 1 mm to remove animals and large debris, and kept at 4°C. The sediment was characterized by wet sieving a subsample to determine the amount of material, on a dry weight basis, that passed an 88 $\mu$m sieve. The organic carbon content of both the whole sediment and the sieved fractions was determined by a wet oxidation method on an Oceanography International carbon analyzer [15].

### *Sediment dosing*

The sediment was dosed with two radioactive tracers, [$^{14}$C]phenanthrene (PHE, universally labeled, specific activity 14 mCi mmol$^{-1}$) and [$^{3}$H]-pyrene (PY, specific activity 34 Ci mmol$^{-1}$) and an additional nine nonlabeled PAHs: anthracene, fluorene, fluoranthene, chrysene, benzo[*a*]pyrene, benzo[*e*]pyrene, perylene, benzo[*b*]fluoranthene, and benzo[*ghi*]perylene.

A PAH solution was prepared by dissolving 30 mg of each of the nonlabeled compounds in 50 ml acetone. The appropriate amount of this mixture was added to make a dosing solution of 25 ml in an acetone carrier. The dosing solution was then added to approximately 3 L of a 1:4 sediment to water slurry (w:v) as follows: The 1 mm sieved sediment was thoroughly mixed. An amount of wet sediment corresponding to 600 g dry weight was placed in a 4-L beaker, and 2500 ml lake water was added. The following solutions were added dropwise while the mixture was stirred vigorously at room temperature: 42:0 $\mu$l methanol containing [$^{14}$C]PHE (2.76 $\mu$Ci/600 g dry sediment); 880 $\mu$l methanol containing [$^{3}$H]PY (13.5 $\mu$Ci/600 g dry sediment); and 25 ml acetone containing the appropriate amount of the above PAH solution for each contaminant level. The amount of acetone and methanol carriers added to each sediment was held constant. The sediment containing only the radiolabeled compounds was also dosed with the same amount of carrier solvent as those containing the nonlabeled PAHs. The mixture was stirred at room temperature for 4 h and kept at 4°C thereafter. The small amount of water-soluble carrier should have only a minimal effect on the PAHs partitioning to the sediments [16].

The nonlabeled compounds were tested at four different dose levels (Table 1) with the radiotracers added at the same concentration for each dose level: 0.13 ± 0.02 nmol g$^{-1}$ for PHE and 0.25 ± 0.01 pmol g$^{-1}$ for PY. The reference sediment concentration contained the same amount of radiotracers as did all the other dose levels, but contained only the natural background concentrations of the nonlabeled PAHs (Table 1).

### *Experimental procedure*

Two days after the sediment was dosed, the overlying water, containing most of the acetone and methanol carriers, was decanted. The sediment was stirred for homogeneity, and 45 g of wet sediment was transferred to 400-ml beakers. The amount of acetone (<1.6 $\mu$l ml$^{-1}$) and methanol (<0.01 $\mu$l ml$^{-1}$) remaining was not expected to affect the toxicokinetics, based on previous studies investigating the role of cosolvents on uptake kinetics [17]. The estimate of the amount of carrier assumes that 80% of the water was decanted, that there was no loss due to volatilization, and that the

Table 1. Concentration of PAHs (nmol $g^{-1}$) in exposure sediment

| PAH | log $K_{ow}$[a] | Dose level 0[b] | 1 | 2 | 3 | 4 | % Recovery[c] |
|---|---|---|---|---|---|---|---|
| Fluorene | 4.18 | ND | 2.1 | 5.7 | 16 | 39.3 | 28.7 |
| Phenanthrene | 4.57 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 61 |
| Anthracene | 4.54 | ND | 2.4 | 5.6 | 15.4 | 47.7 | 31.6 |
| Fluoranthene | 5.2 | ND | 2.2 | 4.8 | 13.9 | 39.2 | 31.8 |
| Pyrene[d] | 5.2 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 38 |
| Chrysene | 5.79 | ND | 2.0 | 4.6 | 12 | 34.2 | 33.6 |
| Benzo[*b*]fluoranthene | 5.98[e] | 0.11 | 2.0 | 3.5 | 10.9 | 29.5 | 32.4 |
| Benzo[*e*]pyrene | 5.98[e] | 0.32 | 2.3 | 3.9 | 12.7 | 31.3 | 37.4 |
| Benzo[*a*]pyrene | 5.98 | 0.18 | 2.1 | 3.8 | 11.4 | 32.1 | 34.8 |
| Perylene | 6.5 | 0.15 | 4.4 | 6.2 | 18.1 | 50.8 | 45 |
| Benzo[*ghi*]perylene | 7.1 | ND | 1.8 | 2.8 | 9.1 | 22.8 | 30.8 |
| Total PAHs | | 0.89 | 21.4 | 41.0 | 119.6 | 327 | |

ND = not detected.
[a]From Miller et al. [32].
[b]Reference Sediment only radiotracers added to sediment.
[c]Mean recoveries of PAHs for all dose levels based on expected nominal concentrations dosed to the sediments.
[d]Concentration in pmol $g^{-1}$.
[e]Log $K_{ow}$ estimated from a regression of molecular weight vs. log $K_{ow}$.

carriers dispersed into the fresh overlying water. The following samples were taken in triplicate from each dose level at the beginning, middle, and end of the sediment distribution to the exposure beakers: 2 g sediment for analysis of nonlabeled PAHs by gas chromatography (GC); 2 g sediment for wet and dry weight determination; and 5 g sediment desiccated with sodium sulfate, extracted with ethyl acetate, and counted for radioisotope activity. Lake water (250 ml) was then carefully added to each beaker with minimal sediment disturbance. On the following day (3 d after dosing the sediment), an initial water sample was taken and 20 test organisms were added to each beaker.

Groups of 20 *Diporeia* were exposed to each sediment dose in triplicate for each sample point: 3, 6, 12, 19, and 26 d. Thus, the total number of beakers was 15 per dose level. Two additional beakers at each concentration, containing no organisms, were sampled on day 15 for measurement of the radiotracer concentration in sediment interstitial water.

The distribution of the radiolabeled compounds in the overlying water was determined from an initial water sample of 25 ml, taken in duplicate from each dose level: 2 ml was counted for total activity, 20 ml was filtered to determine particle-bound contaminant (with a back-up filter to correct for sorption), and 2 ml of filtrate was counted. Fifteen milliliters of filtrate was run through a C-18 Sep Pak (Waters Associates). The Sep-Pak was subsequently eluted with 3 ml of methanol, which was counted to determine the amount of freely dissolved compound in the water column [18,19].

Three beakers from each dose level were sampled at random at each time point (3, 6, 12, 19, 26 d) to obtain the following:

- Overlying water—Total, free, and bound radioisotope activity, as described above, and oxygen concentration [20] were measured.
- Test organisms—The number of *Diporeia* in overlying water was recorded to check for sediment-avoidance behavior. The number of surviving *Diporeia* was recorded for toxicity determinations. The radioisotope concentration of two individuals (wet weight basis) was measured for toxicokinetics. The individual lipid content of two organisms was determined by microgravimetric analysis [21]. The remaining live organisms were combined, weighed, and extracted by homogenizing in a tissue grinder with methylene chloride for determination of their PAH content by gas chromatography.
- Sediment—A subsample was taken for wet and dry weight. A second subsample was taken for later extraction and PAH determination via gas chromatography. A third subsample was desic-

cated with anhydrous sodium sulfate and extracted with ethyl acetate, and the radioisotope activity was determined.

- Pore water—On day 15, the overlying water was removed by aspiration from beakers containing no organisms. The sediment was transferred to centrifuge tubes and centrifuged at approximately 1,000 *g* for 10 min. The total, free, and bound concentration of the two radioisotopes was determined for the resulting pore water as described above for the overlying water.

*Partition coefficients*

The partition coefficients were determined as the ratio of the PAH congener concentration on the sediment particles divided by the "freely dissolved" PAH congener concentration in the interstitial water. The freely dissolved concentration could be measured only for the radiolabeled compounds. The freely dissolved concentration was determined in a manner similar to a previous study that examined the distribution of compounds between suspended particles, dissolved organic carbon, and freely dissolved fractions [22]. The freely dissolved concentration is determined as the sum of twice the compound that sorbed to the back-up filter, which accounts for filter sorption when removing the suspended particles from the interstitial water, divided by the total volume of interstitial water filtered plus the difference in the concentrations of the filtrate and the concentration of bound compound determined after passing the filtrate through a Sep Pak [18]. The freely dissolved concentration is an operationally defined value and an estimate of the truly dissolved compound.

*Analyses*

Approximately 2 g (dry) of sediments was soxhlet-extracted for 4 h in methanol; 200 ml methylene chloride was then added and extraction continued overnight. The extracted specimens, sediments, and *Diporeia* were washed twice with an equal amount of hexane-extracted distilled water. The washed extract was rotary-evaporated to a few milliliters, and 50 ml of pentane was added. The sample was further concentrated to approximately 1 ml on the rotary evaporator. The extract, now in pentane, was introduced to an activated silica gel column (12 × 1 cm), and the saturated hydrocarbons were eluted with 12 ml of pentane. The PAHs were eluted with 12 ml of pentane:methylene chloride (5:1). The PAH sample was rotary evaporated to approximately 1 ml, and approximately 2 ml of hexane was added. The resultant mixture was then evaporated to 1 ml under a gentle stream of nitrogen for gas chromatographic analysis. Extraction yields were calculated by using the [$^{14}$C]- and [$^{3}$H]-labeled PAHs. (Extraction recoveries for the various PAHs were fluorene, 94%; anthracene, 100%; fluoranthene, 101%; chrysene, 103%; benzo[*b*]fluoranthene, 102%; benzo[*e*]pyrene, 92%; benzo[*a*]pyrene, 94%; perylene, 96%; and benzo[*ghi*]perylene, 116%; based on a spiked sample.)

Gas chromatographic analysis was performed on an HP 5880 gas chromatograph equipped with automated dual injection ports (280°C) and two HNU Model PI-52 photoionization detectors (10.2 eV lamps at 280°C). The capillary columns were 25-m BP-1 and BP-5. The samples were run from 120 to 270°C at 4°C/min. Carrier ($H_2$) flow was approximately 1 ml/min. Compound identification required that retention times on both columns be within 0.5% of standard runs for the same day. A program was developed for this instrument that used both peak area and height to calculate final concentrations.

Radioactivity was measured with an LKB 1217 liquid scintillation counter using RPI scintillation cocktail 3a70B. The data were corrected for quench using the external standards ratio method after correcting for background.

*Statistics*

Student's *t* test was used when comparing means or slopes of regression lines. Differences between means and slopes were considered significant when $p < 0.05$. Probit analyses for biological dose response were calculated using the probit routine in SAS® [23].

## RESULTS

*Sediment characteristics*

The bulk dry sediments had an organic carbon content of 1.0 ± 0.2%. The ash-free dry weight was 1.9 ± 0.2% of the dry weight. Slightly greater than half of the sediment mass (56.4%), on a dry-weight basis, passed through an 88-μm screen, while 43.6% was retained by the 88-μm screen. The organic carbon content of the fractions was 0.26 ± 0.06% for >88 μm and 2.1 ± 0.3% for <88 μm fraction.

The homogeneity of the mixed sediments was assessed by analyzing the radiotracers in samples taken at the beginning, middle, and end of the sediment distribution to the exposure beakers. The coefficients of variation were low: 4.7 ± 2.1% (mean ± 1 SD) for the PHE and 4.8 ± 2.6% for the

PY at the various dose levels. The low coefficients of variation suggest that the sediment was homogeneously dosed on a bulk sediment basis.

Based on the amount of compound dosed to the sediment slurry, only 61 ± 8% of the PHE and 38 ± 1.8% of the PY sorbed onto the sediment. The amount sorbed for the nonlabeled PAHs was similar to that of the PY, based on the amount dosed to the sediments (Table 1). The modest sorption of PHE to the sediment is similar to that in previous studies [14] and is likely due in part to the decanting of the overlying water and the loss of some of the fine material and PHE associated with it. The reason for the lower amount of PY sorbed to the sediment is not clear, because previous studies that dosed PY into sediments resulted in much higher sorption [14]. The amount of carrier required to produce the dosed sediments may have been sufficient to increase the solubility of the PAHs, resulting in greater amounts of PAHs decanted with the overlying water. Reductions in the partitioning of organic contaminants to soils have been found when the amount of cosolvent was at concentrations of a few percent or greater [16]. Why there should have been a difference between PHE and all the other PAH congeners in the extent of partitioning to the sediment is not clear. (Note: The uptake kinetic and LC50 values were calculated from the measured sediment values. Thus, the failure of the PAHs to sorb to the sediments as expected would not affect our results or conclusions.)

The measured radiotracer concentrations in the sediments were generally not statistically different between the initial measurement at day 0 and day 26 except at the highest dose, where PY concentration decreased approximately 12% and PHE decreased approximately 20%. Such differences were not observed for the concentrations of the nonlabeled compounds. This may have been due in part to different extraction procedures for the two types of samples. The coefficient of variation for the sediment analyses was 23 ± 7% across the range of compounds; the coefficient of variation for the animals was similar among compounds and among dose levels: 14.9 ± 10.4% except for the lowest dose, level 1, where the coefficient of variation was 28.4 ± 24%.

The partition coefficients, ratio of the concentration on the sediment particles divided by the freely dissolved concentration in the interstitial water for the radiolabeled PAH congeners, were measured at each dose level on day 15 of the exposure, and were nearly constant across dose levels. However, the partition coefficient for PHE at higher concentrations of total PAHs declined while PY increased at the highest total PAH concentration (Table 2). The partition coefficient between sediment interstitial water and the sediment particles for benzo[*a*]pyrene determined under similar conditions was 378 ± 18 [24]. Thus, the partition coefficients between sediment interstitial water and sediment particles were similar among the PAHs for 15-d adsorption and do not exhibit a regular relationship with the hydrophobicity, as represented by the compounds' octanol-water partition coefficient ($K_{ow}$).

Table 2. Partitioning between the interstitial water and the sediment particles for phenanthrene and pyrene on day 15 of exposure

| PAH concentration (nmol $g^{-1}$) | $K_p$ (PHE) | $K_p$ (PY) |
|---|---|---|
| 0.88 (reference sediment) | 317 | 420 |
| 21.3 | 374 | 454 |
| 41.1 | 338 | 421 |
| 119.5 | 162 | 487 |
| 327 | 175 | 916 |

The concentration of oxygen averaged 8.4 ± 1.2 $\mu g\ ml^{-1}$ at day 3 of exposure and 8.02 ± 0.88 $\mu g\ ml^{-1}$ at day 19. The amount of radiotracer in the overlying water was only about twice background, and more than 95% of both PY and PHE were associated with particles and dissolved organic matter. Although even a small amount of dissolved material might greatly influence the accumulation of a contaminant based on the previously determined water uptake clearances [11], previous studies with dosed sediments have shown that overlying water is unimportant when the contaminant is sorbed to sediment for laboratory exposures [14].

### *Bioaccumulation*

The measured concentration of PAHs in *Diporeia* generally increased with increasing sediment dose and exposure time (Table 3). Perylene and the benzo[*ghi*]perylene concentrations were very inconsistent in the organisms. Their apparent absence in several cases and the inconsistent concentrations, when the concentration of the other nonpolar compounds such as benzo[*a*]pyrene (BaP) were very consistent, suggested that the peaks detected by gas chromatography may contain co-eluting interferences. These two compounds are eliminated from further discussion, as

it is not clear whether they were biologically available from sediments.

In addition to the general increase in total organism PAH concentrations with increasing length of exposure, the relative concentrations of the more lipophilic components—compounds with the larger log $K_{ow}$'s (Table 1)—increased in comparison to the less lipophilic components (Table 3). This change in relative concentrations of PAH congeners occurs because (a) the more water-soluble/less lipophilic contaminants become less biologically available more rapidly than the less water-soluble/more lipophilic compounds [14], and (b) the more water-soluble congeners are more rapidly eliminated by the *Diporeia* than the less water-soluble compounds [11]. The change in bioavailability occurs in spite of the essentially constant chemically measured concentrations in the sediments.

The bioavailability of the PAHs from sediment was determined as the uptake clearance (previously defined) of the PAH by *Diporeia* from sediment. The uptake clearance was calculated using the following general model:

$$dC_a/dt = K_s * C_s * e^{-\lambda t} - K_d * C_a \quad (1)$$

where $K_s$ is the uptake clearance (g dry sediment $g^{-1}$ organism $h^{-1}$), $C_s$ is the concentration in the sediment (nmol $g^{-1}$ dry sediment), $\lambda$ is the rate constant ($h^{-1}$) for reduction in the bioavailable concentration of the specific PAH congener [14], $K_d$ is the depuration rate constant ($h^{-1}$), and $C_a$ is the concentration in the animal (nmol $g^{-1}$). For some of the congeners, the $K_d$ value had not previously been determined and was estimated from a regression between $K_d$ and log $K_{ow}$ [11]. The data for compounds with a log $K_{ow} < 5$ were fit to the integrated form of Equation 1 using NLIN [24] and the average depuration rate constant [11] supplied. For compounds with a log $K_{ow} > 5$ the model could be simplified to a one- or two-compartment donor-dependent model with $\lambda$ equal to zero [14].

The $K_s$ values for the radiotracers, PHE and PY, at the reference concentration (Table 4) were similar to those previously determined [14]. As the concentration of the total PAHs added to the sediment increased, the $K_s$ values increased to an apparent plateau for both PHE and PY (Fig. 1). The $K_s$ values for the highest dose were somewhat lower (but not statistically lower) than those for the next lower dose. Similar changes in the $K_s$ values

Table 3. The concentrations of PAHs in *Pontoporeia hoyi* (nmol $g^{-1}$)

| Dose level | Time (d) | Flu | Ant | Fla | Chry | BbFl | BeP | BaP | Per | BghiPer |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 8.9 | 7.3 | 6.7 | 3.7 | 1.3 | 2.5 | 3.0 | 3.1 | 2.1 |
| 1 | 6 | 12 | 12 | 11 | ND | ND | 6.4 | 8.2 | 1.8 | 5.9 |
| 1 | 12 | 11 | 14 | 14 | 6.0 | ND | 4.2 | 7.9 | 0.6 | 3.1 |
| 1 | 19 | 7.3 | 9.4 | 15 | 8.0 | 7.2 | 3.9 | 8.3 | ND | 3.9 |
| 1 | 26 | 7.9 | 11 | 25 | 8.9 | 8.5 | 7.4 | 4.8 | 10 | 5.7 |
| 2 | 3 | 49 | 42 | 35 | 10 | ND | 10 | 5.9 | ND | 8.4 |
| 2 | 6 | 57 | 67 | 62 | 20 | 21 | 20 | 20 | ND | 10 |
| 2 | 12 | 44 | 61 | 81 | 30 | 20 | 22 | 18 | ND | 6.3 |
| 2 | 19 | 40 | 48 | 70 | 29 | 22 | 23 | 13 | ND | ND |
| 2 | 26 | 28 | 45 | 74 | 40 | 25 | 28 | 19 | ND | 25 |
| 3 | 3 | 116 | 115 | 119 | 19 | 16 | 14 | 10 | 22 | 5.5 |
| 3 | 6 | 149 | 163 | 175 | 35 | 44 | 42 | 32 | 92 | ND |
| 3 | 12 | 119 | 177 | 213 | 59 | 74 | 64 | 58 | 101 | 15 |
| 3 | 19 | 99 | 205 | 361 | 81 | 72 | 77 | 53 | ND | ND |
| 3 | 26 | 68 | 153 | 327 | 93 | 84 | 102 | 82 | 101 | ND |
| 4 | 3 | 614 | 383 | 628 | 62 | 107 | 101 | 94 | 66 | 47 |
| 4 | 6 | 577 | 426 | 707 | 77 | 126 | 92 | 100 | ND | 51 |
| 4 | 12 | 607 | 474 | 891 | 105 | 184 | 141 | 152 | 93 | 128 |
| 4 | 19 | 446 | 459 | 1,300 | 116 | 255 | 205 | 204 | ND | ND |
| 4 | 26[a] | 71 | 92 | 158 | 33 | 12 | 41 | 42 | 5.7 | 8.7 |

ND = not detected. Fluorene (Flu), Anthracene (Ant), Fluoranthene (Fla), Chrysene (Chry), Benzo[*b*]fluoranthene (BbFl), Benzo[*e*]pyrene (BeP), Benzo[*a*]pyrene (BaP), Perylene (Per), and Benzo[*ghi*]perylene (BghiPer).
[a]The low values for level 4 on day 26 reflect a recovery problem for this sample.

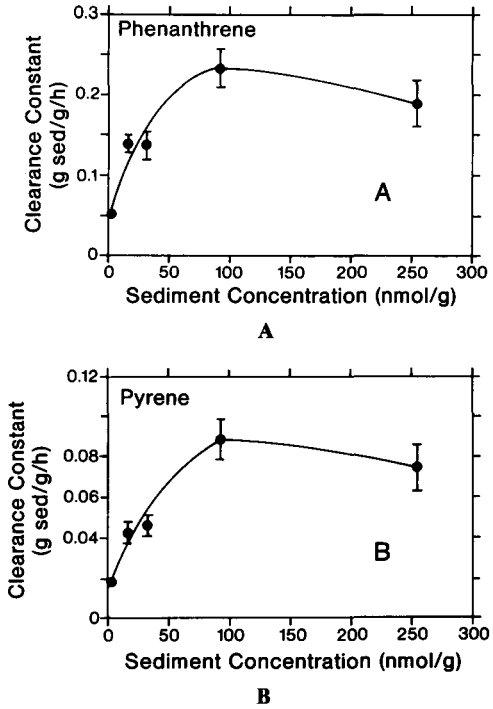


Fig. 1. Uptake clearance of phenanthrene (**A**) and pyrene (**B**) by *Diporeia* as a function of the total concentration of PAHs in the sediment.

with PAH sediment concentrations were observed for the nonlabeled compounds. Within a fixed sediment concentration, the log $K_s$ values were inversely proportional to the log $K_{ow}$ values (Fig. 2). The slopes of the regression lines for the various dose levels were not significantly different. Regression coefficients for the higher concentrations were lower than those previously determined at low PAH concentrations and generally reflect the added variability of the gas chromatographic analysis compared to radiotracer analysis (Fig. 2).

*Toxicity*

The PAH toxicity was detected in two ways: sediment avoidance and mortality. Sediment avoidance was observed only early in the experiment, with 17 ± 8% avoidance observed at 72 h and 17 ± 10% avoidance observed at 144 h for the highest sediment dose, 327 nmol $g^{-1}$ dry sediment as the sum of molar concentrations. After 144 h, the number of *Diporeia* found above the sediment surface ranged from 0 to 4 with most of the samples in the 0 to 2 range (Table 5). Although there were consistently more animals avoiding the sediment at the highest dose, the mean was not statistically different from the reference sediment, level 0, which was generally zero avoidance during the later portions of the experiment (Table 5).

Table 4. Uptake clearance ($K_s$) values for PAH congeners at several dose levels

| | $K_s$ (g dry sediment $g^{-1}$ $h^{-1}$) Dose level | | | | |
|---|---|---|---|---|---|
| PAH congener | 0[a] | 1 | 2 | 3 | 4 |
| Fluorene | ND | 0.077 (0.014)[b] | 0.152 (0.028) | 0.137 (0.017) | 0.27 (0.06) |
| Phenanthrene | 0.051 (0.004) | 0.139 (0.011) | 0.137 (0.017) | 0.233 (0.023) | 0.189 (0.029) |
| Anthracene | ND | 0.057 (0.009) | 0.139 (0.019) | 0.112 (0.013) | 0.12 (0.023) |
| Fluoranthene | ND | 0.036 (0.012) | 0.165 (0.01) | 0.112 (0.03) | 0.205 (0.07) |
| Pyrene | 0.019 (0.002) | 0.045 (0.006) | 0.048 (0.006) | 0.098 (0.014) | 0.079 (0.014) |
| Chrysene | ND | 0.023 (0.007) | 0.037 (0.001) | 0.023 (0.0005) | 0.03 (0.005) |
| Benzo[*b*]fluoranthene | ND | 0.0113 (0.002) | 0.117 (0.089) | 0.0322 (0.003) | 0.042 (0.011) |
| Benzo[*e*]pyrene | ND | 0.0021 (0.0018) | 0.0069 (0.002) | 0.0094 (0.002) | 0.0093 (0.002) |
| Benzo[*a*]pyrene | ND | ND | 0.0051 (0.002) | 0.0093 (0.002) | 0.0094 (0.001) |

[a]Reference concentration—only radiotracers added to the sediment.
[b]Numbers in parentheses represent the standard error of the regression fit for the data to the appropriate kinetic model.

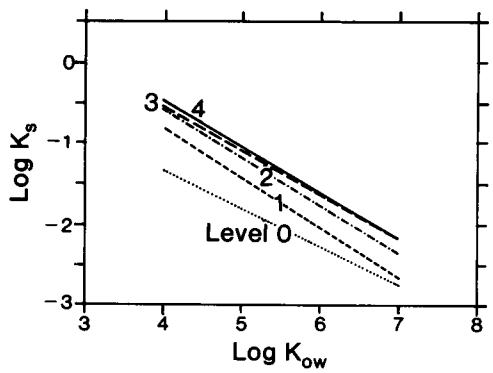


Fig. 2. Log of the uptake clearance at the various dose levels as a function of log $K_{ow}$. The level zero was taken from Landrum [14] (log $K_s = -0.48 \pm 0.04$ log $K_{ow} + 0.59 \pm 0.21$; $r^2 = 0.90$, $n = 19$). The range for the slopes at the higher doses was −0.58 to −0.68, and the intercepts ranged from 1.77 to 2.39 with $r^2$ values ranging from 0.70 to 0.77.

The mortality estimates were determined in several ways, based on concentration in the sediments, the time required to produce mortality at the highest dose, and the concentration of PAHs in the organisms measured at day 26. Whereas the mortality at the highest dose, 327 nmol $g^{-1}$ dry sediment, averaged 38 and 28%, respectively, on days 19 and 26, it is useful to estimate an LC50 for comparative purposes (Table 5). The LC50 at day 26 was estimated to be 601 (389–1,739, 95% C.I.) nmol $g^{-1}$ dry sediment as the molar sum of the added PAH.

The cumulative concentration in *Diporeia* required to elicit 38 ± 3% mortality at day 19 was 2.9 nmol PAH $g^{-1}$ organism measured as the sum of the molar concentrations of the individual PAH (Table 3). A 26-d LC50 can be estimated from the measured PAH concentrations in *Diporeia* by probit analysis if the concentration at day 19 is used for the highest PAH dose, because calculated concentrations from the gas chromatographic analysis of the day 26 sample were anomalously low, presumably due to a low recovery for this sample. Substituting the concentrations on day 19 for those on day 26 for the highest dose may not be too unreasonable, because the concentrations in the organisms did not change much between 19 and 26 d at the next lower concentration that exhibited similar accumulation kinetics to the highest concentration. Again, the concentrations of perylene or benzo[*ghi*]perylene in *Diporeia* were not included for reasons described above. With these restrictions, the estimated 26-d LD50—the cumulative concentration in the organisms for 50% mortality—was 6.1 (3.7–21.3, 95% C.I.) μmol $g^{-1}$ organism. This estimate is useful for comparative purposes, but must be considered no more than an estimate because the total toxicity did not exceed

Table 5. Toxic response of *Diporeia* sp. to PAHs in sediments as sediment avoidance and mortality

| Exposure (days)[a] | Sediment avoidance Dose level | | | | | Number surviving of 20 Dose level | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| 3-A | 0 | 0 | 5 | 0 | 5 | 20 | 18 | 18 | 20 | 18 |
| 3-B | 1 | 0 | 1 | 2 | 2 | 20 | 19 | 19 | 19 | 20 |
| 3-C | 1 | 0 | 0 | 1 | 3 | 20 | 20 | 18 | 19 | 19 |
| 6-A | 0 | 1 | 0 | 2 | 5 | 17 | 20 | 20 | 20 | 38[b] |
| 6-B | 0 | 0 | 1 | 3 | 1 | 19 | 19 | 19 | 20 | 19 |
| 6-C | 1 | 0 | 0 | 1 | 4 | 17 | 19 | 18 | 19 | 19 |
| 12-A | 0 | 1 | 1 | 4 | 0 | 18 | 19 | 20 | 18 | 20 |
| 12-B | 0 | 0 | 2 | 0 | 1 | 17 | 19 | 18 | 18 | 17 |
| 12-C | 2 | 0 | 0 | 1 | 2 | 18 | 20 | 20 | 19 | 17 |
| 19-A | 0 | 0 | 1 | 0 | 1 | 17 | 19 | 17 | 17 | 12 |
| 19-B | 0 | 0 | 0 | 0 | 0 | 19 | 17 | 19 | 19 | 13 |
| 19-C | 0 | 0 | 0 | 0 | 2 | 17 | 19 | 20 | 20 | 12 |
| 26-A | 0 | 0 | 2 | 0 | 3 | 18 | 18 | 20 | 15 | 13 |
| 26-B | 0 | 0 | 0 | 0 | 0 | 19 | 18 | 20 | 19 | 14 |
| 26-C | 0 | 0 | 0 | 0 | 2 | 21 | 18 | 20 | 19 | 16 |

[a]The letters A–C associated with the exposure times indicate the various replicates taken.
[b]40 animals (2 × 20) were inadvertently added to this replicate.

50% mortality and the concentration in the organisms for the highest dose had to be estimated due to analytical problems.

## DISCUSSION

Homogeneous doses were necessary to ensure that exposures were uniform. The small coefficients of variation for the initial PHE and PY concentrations suggest that the doses were homogeneous on a bulk sediment basis at the beginning of the experiment. The absence of apparent concentration changes between day 0 and day 26 for most dose levels suggests that the exposures, as measured chemically, remained fairly constant over the course of the studies. Further, the excellent concentration comparisons between the beginning and the end of the experiment are further support that the sediment was homogeneously dosed on a bulk sediment basis.

Calculation of a bioaccumulation factor (BAF) (concentration in the organism divided by the concentration in the sediment) after 26-d exposure results in a range generally higher than that found in the field [12] for the same PAH congeners, except for BaP (Table 6). The PHE and PY at level zero were lower by approximately a factor of three than the values for the remainder of the dataset and generally were closer to those values found under field conditions. This may reflect the generally lower concentrations in the sediments from the field collections of *Diporeia* compared to the experimental conditions, parts per billion in the field samples versus parts per million in the sediments with nonlabeled PAHs, or the fact that these are mean values for organisms collected from a wide range of sediment types [12]. Differences between BAFs calculated from field results and BAFs calculated from sediments containing the nonlabeled PAHs might also occur, in part, because steady state had not necessarily been achieved, particularly for the field results in which changing environmental and physiological conditions altered the toxicokinetics [11]. The similarity of the PY and PHE, in the absence of nonlabeled PAHs, to the field data suggests that the kinetics in the field are similar to the kinetics measured in the laboratory at concentrations similar to those in the environment. This finding suggests that predictions at high concentrations cannot be made from kinetics measured at low concentrations.

Table 6. Bioaccumulation factors for *Diporeia* exposed to PAH-contaminated sediments

| Compound | This study at 26 d[a] | Field[b] |
|---|---|---|
| Fluoranthene | 11 – 23 | 1.5 |
| Chrysene | 4.4 – 7.7 | 1.2 |
| Benzo[*a*]pyrene | 2.3 – 6.9 | 2.3 |
| Phenanthrene | | |
| No added PAH[c] | 3.4 ± 0.3 | 3.3 |
| Added PAH[d] | 7.0 – 26.9 | |
| Pyrene | | |
| No added PAH[c] | 5.1 ± 0.8 | 2.5 |
| Added PAH[d] | 13.1 – 28.6 | |

[a]Omits the highest dose for all the PAH congeners measured by gas chromatography due to analytical difficulties.
[b]From Eadie et al. [13].
[c]Sediments dosed only with the radiotracers, only one sediment with three replicates.
[d]Sediments dosed with the radiotracers and various levels of nonlabeled PAHs.

In previous studies performed at lower concentrations and in simpler mixtures, the uptake clearance appeared to be independent of the concentration of other contaminants [14]. Thus, the increase in the uptake clearances with increasing PAH concentrations was unexpected. The increase occurred even in the absence of any apparent toxic effects for the mixture as measured by either mortality or sediment avoidance, e.g., the difference in the kinetics between level 0 and level 1 (Table 4). The reason for this increase is not clear but may be due to sublethal effects even at the lowest dose of the nonlabeled mixture, increasing the overall activity of the organism, including increased respiration, ingestion, and movement through the sediment. A similar change in uptake clearances of chlorinated hydrocarbons was observed with *Diporeia*. However, for the chlorinated hydrocarbon mixture, acute mortality was observed [22]; thus, the changes in the uptake clearances were attributed to the changes in the physiological state of the organisms.

At each specific PAH concentration, the uptake clearances were inversely proportional to the log $K_{ow}$. Because the slopes of the regression lines were not significantly different (Fig. 2), the mechanisms for accumulation of these contaminants from sediment at the different concentrations are assumed to be via the same routes. Therefore, changes in the observed uptake clearances between doses may result from changes in the fraction of the total dose that is bioavailable. This apparent change in bioavailability occurs despite an apparently constant partition coefficient between the interstitial water and sediment particles.

A recent mechanistic simulation model suggests

that one of the important variables affecting the accumulation of sediment-sorbed contaminants, other than ingestion of contaminated matter and desorption of contaminants from sediment particles, is the volume of interstitial water that benthic organisms contact [25]. Because desorption is very slow, organisms rapidly deplete the interstitial water in their immediate vicinity of dissolved contaminant; subsequently, the rate of uptake from the interstitial water becomes dependent on the rate of desorption from sediment particles. However, if the organism moves to new patches of interstitial water that are in equilibrium with the sediment particles, uptake can be enhanced because the dissolved compound is readily available. As a result, increasing the movement of organisms through the sediment should increase the rate of accumulation. This behavioral process would yield increased uptake clearances without requiring any change in the partitioning between sediments and interstitial water. Further, as the organisms become intoxicated, movement would be expected to decline, resulting in lower uptake clearances as is observed for the highest dose.

A second explanation for the enhanced bioavailability with increasing PAH dose may be the binding of contaminants to different sites on the sediment particles. Multiple binding sites of different affinities for the PAH may exist. If a set of higher affinity, less bioavailable, sites is available but limited in number, then, at lower concentrations, a larger fraction of the PAHs would be less available. This explanation could account in part for the changes in uptake clearances with changes in concentration, but such a speculative explanation would seem to require a change in the measured partition coefficient to lower values at higher concentrations. The partition coefficient for PHE did seem to decline slightly at the higher concentrations, but that for PY did not; nor was the relative decline of the same magnitude as the relative increased uptake clearance. Thus, this speculative explanation requires more supporting data before it can be seriously considered.

It is not possible from this dataset to define the mechanism for the apparent enhanced bioavailability. However, the hypothesis that the organisms' physiology is changing is more plausible than that the binding sites are different, particularly as the partition coefficients do not change markedly among the different sediment doses. Improving our understanding of this issue will require improved data on the ingestion and assimilation efficiency of particulate-associated contaminants, the desorption rates from sediment particles, and the behavior of organisms in the sediment matrix.

The observed avoidance of the sediments, although small at the doses tested, was much greater than that observed for exposure to a chlorinated hydrocarbon mixture that produced a much greater level of mortality [22]. Perhaps the organisms can sense the PAHs but not the chlorinated hydrocarbons. Whereas more organisms were generally found above the sediment at the highest concentration, even though the number was not statistically significant compared to the reference sediment at the later parts of the experiment, the decline in the number of *Diporeia* found above the sediment with increased exposure suggests that *Diporeia* cannot completely avoid contact with undesirable sediments if no alternative sediment is available.

The oxygen concentrations in the exposure beakers were relatively high and remained at 61 to 65% of saturation. Thus, the observed mortality was not a result of low oxygen in the exposure chambers. The toxicity of the PAH mixture required relatively high sediment concentrations, suggesting that the PAH bioavailability was relatively low. The most likely mechanism for toxicity was nonpolar narcosis, supported by the high organism PAH concentration required to produce mortality (2.9 nmol $g^{-1}$ for 38% mortality at day 19). Because the organisms were continuously exposed, the dose to *Diporeia* accumulated over the entire exposure period. Therefore, the use of the concentration in the organism at the end of the exposure as the total dose to the organism will be an upper limit of the dose required to produce the toxic effect.

The cumulative concentration *estimated* to achieve 50% mortality was similar to that suggested by McCarty [26], as required for an acute toxic response by the nonpolar narcotic action of organic compounds in fathead minnows. McCarty's data are all single-compound values; the *Diporeia* concentrations are the sum of the molar concentrations in the organisms. Although comparisons of concentrations required to produce mortality may vary due to species sensitivity, the similarities between the levels help support the concept that the observed toxic effect results from nonpolar narcosis. It is interesting to note that the lipid content for fathead minnows, the organism used for McCarty's work, is in the range of 7 to 15% of dry weight [27–30], only about a factor of 2 lower than the range for *Diporeia* [21]. Thus, storage of the

contaminants in lipid pools should not be radically different, and should result in similar concentrations available at the receptor to produce effects.

The comparison between the single-compound concentration required to produce 50% mortality and that of the sum of the molar concentrations required to produce mortality for the mixture suggests that, for toxicity measured as 26-d mortality, the PAHs are approximately additive with no overt evidence of synergism or antagonism. The suggestion of additivity can be further supported by the measured PY LD50 at 31-d exposure for *Diporeia* of 5.8 $\mu$mol $g^{-1}$ organism (4.3–36.8, 95% C.I.; P. Landrum, unpublished data). Thus, the same molar concentrations for a single congener and the mixture were required to produce mortality with approximately the same duration of exposure.

## CONCLUSION

High sediment concentrations of total PAHs in the range of 100 $\mu$g $g^{-1}$ dry sediment are required to produce mortality in 26 d. Such levels occur in rivers and embayments where PAH discharges have occurred [31] but are not generally found in the open water sediments of the Great Lakes—the habitat of *Diporeia* (sp) [6,31]. Further, the amount of accumulated PAHs in *Diporeia* for mortality suggests that the PAHs are acting as a nonpolar narcotic. The PAH action appears to be additive, but this will require further testing for confirmation. The rate of PAH accumulation is dependent on the concentration of PAHs in the sediment and is not predictable through measured partitioning between interstitial water and sediment particles. The BAFs estimated from 26-d exposures were generally greater than those found in the field samples, except for the exposures in which only radiotracers were added to sediments. The elevated BAF values may result in part from excitatory effects of the PAHs on the organisms enhancing the accumulation. The partition coefficients measured between the sediment particles and the interstitial water did not change markedly among the doses, further suggesting that changes in organism behavior may be responsible for the observed changes in bioavailability. These behavioral changes may be from increased ingestion or contact with larger than expected quantities of interstitial water. However, additional research will be required to elucidate the mechanism, whether physical or biological, responsible for the increased bioavailability with increasing PAH concentration.

## REFERENCES


1. **National Oceanic and Atmospheric Administration.** 1988. A summary of selected data on chemical contaminants in sediments collected during 1984, 1985, 1986, and 1987. Progress Report. NOAA Technical Memorandum NOS OMA 44. National Status and Trends Program for Marine Environmental Quality, Rockville, MD.
2. **International Joint Commission.** 1987. Guidance on characterization of toxic substances problems in areas of concern in the Great Lakes Basin. Report to the Great Lakes Water Quality Board. Windsor, Ontario, Canada.
3. **International Joint Commission.** 1987. Guidance on assessment and remediation of contaminated sediment problems in the Great Lakes. Report to the Great Lakes Water Quality Board. Windsor, Ontario, Canada.
4. **Nalepa, T.F.** and **P.F. Landrum.** 1988. Benthic invertebrates and contaminant levels in the Great Lakes: Effects, fates and role in cycling. In M. S. Evans, ed., *Toxic Contaminants and Ecosystem Health: A Great Lakes Perspective.* John Wiley & Sons, New York, NY, pp. 77–102.
5. **Marzolf, G.R.** 1965. Substrate relations of the burrowing amphipod, *Pontoporeia affinis*, in Lake Michigan. *Ecology* **46**:579–592.
6. **Bousfield, E.L.** 1989. Revised morphological relationships within the amphipod genera *Pontoporeia* and *Gamaracanthus* and the "glacial relict" significance of their post glacial distributions. *Can. J. Fish. Aquat. Sci.* **46**:1714–1725.
7. **Eadie, B.J.** 1984. Distribution of polycyclic aromatic hydrocarbons in the Great Lakes. In J.O. Nriagu and M.S. Simmons, eds., *Toxic Contaminants in the Great Lakes.* John Wiley & Sons, New York, NY, pp. 195–212.
8. **Baumann, P.C., W.D. Smith** and **M. Ribick.** 1982. Hepatic tumor rates and polynuclear aromatic hydrocarbon levels in two populations of brown bullhead (*Ictalurus nebulosus*). In M. Cooke, A.J. Dennis and G.L. Fisher, eds., *Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry.* Sixth International Symposium. Battelle Press, Columbus, OH, pp. 93–102.
9. **Couch, J.A.** and **J.C. Harshbarger.** 1985. Effects of carcinogenic agents on aquatic animals: An environmental and experimental overview. *Environ. Carcinog. Rev.* **3**:63–105.
10. **Landrum, P.F.** 1982. Uptake, depuration and biotransformation of anthracene by the scud, *Pontoporeia hoyi. Chemosphere* **11**:1049–1057.
11. **Landrum, P.F.** 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: Role of physiological and environmental variables. *Aquat. Toxicol.* **12**:245–271.
12. **Eadie, B.J., W.R. Faust, P.F. Landrum** and **N.R. Morehead.** 1985. Factors affecting bioconcentration of PAH by the dominant benthic organisms of the Great Lakes. In M.W. Cooke and A.J. Dennis, eds., *Polynuclear Aromatic Hydrocarbons: Mechanisms, Methods and Metabolism.* Eighth International Symposium. Battelle Press, Columbus, OH, pp. 363–377.

13. **Eadie, B.J., P.F. Landrum** and **W. Faust.** 1982. Polycyclic aromatic hydrocarbons in sediments, pore water and the amphipod *Pontoporeia hoyi* from Lake Michigan. *Chemosphere* **11**:847–858.
14. **Landrum, P.F.** 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod, *Pontoporeia hoyi. Environ. Sci. Technol.* **23**:588–595.
15. **Menzel, D.W.** and **R.F. Vaccaro.** 1964. The measurement of dissolved and particulate carbon in sea water. *Limnol. Oceanogr.* **9**:138–142.
16. **Nkedi-Kizza, P., P.S.C. Rao,** and **A.G. Hornsby.** 1985. Influence of organic cosolvents on sorption of hydrophobic organic chemicals by soils. *Environ. Sci. Technol.* **19**:975–979.
17. **Landrum, P.F.** 1983. The effect of co-contaminants on the bioavailability of polycyclic aromatic hydrocarbons to *Pontoporeia hoyi.* In M.W. Cooke and A.J. Dennis, eds., *Polynuclear Aromatic Hydrocarbons: Formation Metabolism and Measurement.* Seventh International Symposium. Battelle Press, Columbus, OH, pp. 731–743.
18. **Landrum, P.F., S.R. Nihart, B.J. Eadie** and **W.S. Gardner.** 1984. Reverse-phase separation for determining pollutant binding to Aldrich humic acid and dissolved organic carbon of natural waters. *Environ. Sci. Technol.* **18**:187–192.
19. **Landrum, P.F., S.R. Nihart, B.J. Eadie** and **L.R. Herche.** 1987. Reduction in bioavailability of organic contaminants to the amphipod, *Pontoporeia hoyi,* by dissolved organic matter of sediment interstitial waters. *Environ. Toxicol. Chem.* **6**:11–20.
20. **Grasshoff, K., M. Ehrhardt** and **K. Kremling.** 1983. *Methods of Seawater Analysis,* 2nd ed. Verlag Chemie, Weinheim, FRG, pp. 61–72.
21. **Gardner, W.S., W.A. Frez, E.A. Chichocki** and **C.C. Parish.** Micromethod for lipid analysis in aquatic invertebrates. *Limnol. Oceanogr.* **30**:1099–1105.
22. **Eadie, B.J., N.R. Morehead** and **P.F. Landrum.** 1990. Three-phase partitioning of hydrophobic organic compounds in Great Lakes waters. *Chemosphere* **20**:161–178.
23. **SAS Institute.** 1985. *SAS® User's Guide: Statistics, Version 5 Edition.* Cary, NC.
24. **Landrum, P.F., W.R. Faust** and **B.J. Eadie.** 1989. Bioavailability and toxicity of a mixture of sediment-associated chlorinated hydrocarbons to the amphipod *Pontoporeia hoyi.* In. U.M. Cowgill and L.R. Williams, eds., *Aquatic Toxicology and Hazard Assessment: 12th Volume.* STP 1027. American Society for Testing and Materials, Philadelphia, PA, pp. 315–329.
25. **Landrum, P.F.** and **J.R. Robbins.** 1990. Bioavailability of sediment associated contaminants to benthic invertebrates. In R. Baudo, J. Giesy and H. Muntao, eds., *Sediments: Chemistry and Toxicity of In-Place Pollutants.* Lewis Publishers, Chelsea, MI, Chapter 8, pp. 237–263.
26. **McCarty, L.S.** 1986. The relationship between aquatic toxicity QSARS and bioconcentration for some organic chemicals. *Environ. Toxicol. Chem.* **5**:1071–1080.
27. **Goran, D.,** and **P. Kosian.** 1983. Influence of diet and body lipids on the bioconcentration of endrin from water in the fathead minnow (*Pimephales promelas*). EPA 600/3-83-077. U.S. Environmental Protection Agency, Duluth, MN.
28. **Mac, M.J.** 1984. Flow-through bioassay for measuring bioaccumulation of toxic substances from sediment. EPA 905/3-84-007. Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, IL.
29. **Mac, M.J.** and **W.A. Wilford.** 1986. Bioaccumulation of PCBs and mercury from Toronto and Toledo harbor sediments. In *Evaluation of Sediment Bioassessment Techniques,* Report to the Great Lakes Water Quality Board. International Joint Commission, Windsor, Ontario, Canada, pp. 81–90.
30. **Mac, M.J., C.C. Edsall** and **R.J. Hesselberg.** 1985. Accumulation of PCBs and Hg by fish and earthworms during field and laboratory exposures to Green Bay sediments. Administrative Report No. 85-4, USFWS-GLFL/AR-85-4. Great Lakes Fishery Laboratory, U.S. Fish and Wildlife Service, Ann Arbor, MI.
31. **Rathke, D.E.** and **G. McRae,** eds. 1989. 1987 Report on Great Lakes water quality. Appendix B—Great Lakes surveillance, Vol. 1. International Joint Commission, Great Lakes Regional Office, Windsor, Ontario, Canada.
32. **Miller, M.M., S.P. Wasik, G. Huang, W. Shiu** and **D. Mackay.** 1985. Relationships between octanol-water partition coefficient and aqueous solubility. *Environ. Sci. Technol.* **19**:522–529.