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Bioaccumulation and critical body residue of PAHs in the amphipod, *Diporeia* spp.: additional evidence to support toxicity additivity for PAH mixtures

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are considered to act additively when exposed as congener mixtures. Additive internal concentrations at the site of toxic action is the basis for recent efforts to establish a sum PAH guideline for sediment-associated PAH toxicity. This study determined the toxicity of several PAH congeners on a body residue basis in *Diporeia* spp. These values were compared to the previously established LR_{50} value for a PAH mixture based on the molar sum of PAH congeners and demonstrated similar LR_{50} values for individual PAH. These results support the contention that the PAH act at the same molar concentration whether present as individual compounds or in mixture. Aqueous exposures were conducted for 28 d, and the water was exchanged daily to maintain the exposure concentration. The concentration in the exposures declined by an average of 22% between water exchanges across all compounds, and ranged from 11% to 32%. The toxicokinetics were determined using both time-weighted-average (TWA) and time-variable water concentrations and were not statistically different between the two source functions. Toxicity was determined for both mortality and immobility (failure to swim on prodding) and on both a TWA water concentration and a body residue basis. The LC_{50} values ranged from 1757 $\mu g\,l^{-1}$ for naphthalene after 10 d exposure to 79.1 $\mu g\,l^{-1}$ for pyrene after 28 d exposure, and the EC_{50} ranged from 1587 $\mu g\,l^{-1}$ for naphthalene after 10 d exposure to 38.2 $\mu g\,l^{-1}$ for pyrene after 28 d exposure. The LR_{50} values for all congeners at all lengths of exposure were essentially constant and averaged 7.5 ± 2.6 $\mu mol\,g^{-1}$, while the ER_{50} for immobility averaged 2.6 ± 0.6 $\mu mol\,g^{-1}$. The bioconcentration factor declined with increasing exposure concentration and was driven primarily by a lower uptake rate with increasing dose, while the elimination remained essentially constant for each compound.
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Keywords: Polycyclic aromatic hydrocarbons; *Diporeia* spp.; Lethal body residue; Toxicokinetics

1. Introduction

Organism exposures in the environment are generally to complex mixtures and not to single compounds. Even when polycyclic aromatic hydrocarbons (PAHs) are the dominant contaminant at a site, the mixture contains multiple compounds. Recently, DiToro et al. (2000)

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established a theoretical framework for the additivity of toxicity for non-polar narcotic compounds. This framework is an outgrowth from experimental evidence suggesting that PAHs act additively to produce a toxic response. PAH mixture studies with amphipods used LC_{50} values and the toxic unit (TU) approach to establish additivity (e.g., Swartz et al., 1995; Ozretich et al., 2000). The use of a sum PAH model (Swartz et al., 1995) with TU resulted in improved prediction of observed toxicity for PAH contaminated marine sediments (Swartz et al., 1995; Ozretich et al., 2000). However, using a similar sum PAH model for the freshwater amphipod *Hyalella azteca*, additive toxicity of PAH explained the response of *H. azteca* to PAH contaminated sediment, but there were complications from apparent limitations to bioavailability and the presence of unknown compounds (Lee et al., 2001). Further, recent work with *H. azteca* suggests that the toxicity of a mixture of PAH congeners may be synergistic (Verrhiest et al., 2001). This likely reflects the confusion that can result from interpreting interactions based on the use of external concentrations.

All of the above studies were based on using LC_{50} values to establish additivity. However, because of bioavailability issues, the resultant conclusions of additivity have been established by inference. To circumvent such bioavailability questions, the use of body residues as the reference media for the dose of contaminants promoting toxicity to aquatic organisms was introduced by McCarty (1986) and later expanded in McCarty and Mackay (1993). The premise is that body residues better reflect the dose at the receptor and are not subject to the complications of interpreting the bioavailability associated with the exposure. The concentration of non-polar organic contaminants required to produce acute mortality in invertebrates and fish is expected to be relatively constant, ranging between 2 and 8 mmol kg^{-1} (McCarty and Mackay, 1993). Limited direct evidence for toxicity additivity was established with the freshwater amphipod *Diporeia* spp. exposed to a PAH mixture (Landrum et al., 1991). In this case, the LR_{50} (lethal body residue concentration for 50% mortality) was established for the molar sum of PAH to be in the same range as that required for acute toxicity of individual non-polar compounds for 50% mortality (McCarty and Mackay, 1993). This result depends on the presence of a threshold and compounds acting additively at the site of toxic action. If this is true, then reducing the numbers of compounds in the mixture would mean that the body residue of each compound would increase but the molar sum would remain constant. Further, in the extreme case of an individual compound, the body residue for an individual compound would have to be at the same level as that observed for the total molar concentration of the mixture. In one study, the LR_{50} for the mixture (6.1 μmol g^{-1}) was similar to that for *Diporeia* spp. exposed to pyrene (5.8 μmol g^{-1}, Landrum et al., 1994). However, this finding requires additional support to insure that it did not happen by chance.

The objectives of this study were (1) establish the toxicity of representative PAH congeners to the amphipod *Diporeia* spp. on a water concentration and body residue basis, (2) determine the impact of the increasing concentrations and body residues on toxicokinetics parameters, and (3) compare the LR_{50} for individual PAH congeners with values already established for PAH mixtures using *Diporeia* spp. to add additional support for molar additivity.

2. Materials and methods

2.1. Compounds

The ^{14}C-PAH used in the study, naphthalele (8.1 mCi $mmol^{-1}$), fluorene (14.6 mCi $mmol^{-1}$), phenanthrene (13.1 mCi $mmol^{-1}$), and pyrene (32.3 mCi $mmol^{-1}$) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Unlabeled PAHs were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). ^{14}C-PAH were tested for purity prior to use by thin-layer chromatography (TLC) on pre-coated silica gel 60F-254EM glass plates (Alltech Associated, Deerfield, IL, USA) using hexane:benzene (8:2) solvent system in combination with liquid scintillation counting. All compounds were determined to be >98% pure. Stock solutions were generated by adding known amounts of unlabeled and labeled PAH to an acetone carrier solvent. New specific activities were determined based on isotopic dilution. The concentrations of spiked test solutions were determined by liquid scintillation counting (LSC) on a Tri-Carb Liquid Scintillation Counter (Model 2500 TR, Packard Instrument Co., Meridien, CT, USA). Samples were corrected for quench using the external standards ratio method after subtracting background. The calculated specific activity, μCi ^{14}C μmol^{-1} total PAH, for each compound and exposure concentration was used to calculate the concentration in all matrices.

2.2. Exposure media

Water used in all experiments was collected from the Huron River upstream from Dexter, MI, USA, at the Hudson Mills Metropark. Hardness (165 to 250 mg l^{-1} $CaCO_3$, $n = 15$), alkalinity (170–250 mg l^{-1} $CaCO_3$, $n = 15$), and pH (8.1–8.3, $n = 15$) were measured prior to use in each experiment. Test solutions were prepared by adding a known amount of ^{14}C and ^{12}C-PAH to acetone to create the working stocks. The appropriate amount of the stock was then added to 3 l of filtered water (0.2 μm Fin-L-Filter, Cole-Palmer Instrument Co., Vernon Hills,

IL, USA). The volume of carrier solvent never exceeded 60 $\mu l l^{-1}$. The control received 60 $\mu l l^{-1}$ of acetone only.

2.3. Organisms

Field collected *Diporeia* spp. that passed through a 2-mm mesh and were retained in a 1-mm mesh (juvenile organisms approximately 5–11 months old) were used in all experiments. The animals were collected at a 45-m deep station west of Muskegon, MI (43° 11.27′ N, 86° 18.90′ W) and transported to the Great Lakes Environmental Research Laboratory in lake water that was kept cool with ice (Landrum, 1989). The organisms were held in Lake Michigan sediment with approximately 10 cm Huron River (MI) water for less than 1 month prior to testing. Huron River water was used because it has water quality characteristics, pH, hardness, and alkalinity, that are essentially the same as Lake Michigan water (Kane Driscoll et al., 1997).

2.4. Toxicity and bioaccumulation experiments

Toxicity and bioaccumulation of pyrene, naphthalene, phenanthrene, and fluorene were examined in 28-d water-only exposures. Amphipods were exposed to a range of contaminant concentrations (Table 1) in 300 ml beakers filled with 250 ml of test solution. Each exposure beaker received 10 *Diporeia* spp. at day 0. *Diporeia* spp. were placed in beakers with no substrate or food. *Diporeia* spp. can survive long starvation periods (1–2 mo) with little decline in lipid content (Gauvin et al., 1989). Experiments with *Diporeia* spp. were conducted at 4 °C. Experiments with naphthalene, pyrene, and phenanthrene were performed in 1997, and those with fluorene were performed in 1999. Four beakers per treatment were used for monitoring survival. The beakers were examined daily to record the number of live amphipods and to remove dead animals. The number of *Diporeia* spp. that appeared to be immobilized was also recorded daily. Immobility is defined as the inability of an amphipod to actively swim upon contact stimulus while exhibiting some respiratory movements. Immobility was not quantified for the fluorene study. Upon experiment completion, (28 days) surviving amphipods were used for body residue determinations. For naphthalene, phenanthrene, and pyrene, extra replicate beakers, in addition to the 4 to be used for effects measurements, were prepared so that body residues in live organisms could be determined at intermediate time points. Amphipods were sampled from one set of two beakers per treatment at days 2 and 5 and from a different set of two beakers at days 10 and 17 (or 18).

Three fourths of the test-solution in each beaker was manually exchanged daily. Exposure water (5 ml) was sampled from three beakers for compound concentration at the beginning of the experiment (day 0) and from

Table 1
The best estimate from non-linear regression (± standard error) for uptake clearance rate (k_u) and elimination rate (k_e) calculated from the time-weighted average water concentration and BCF estimated from k_u/k_e for *Diporeia* spp. exposed to select PAH congeners

Naphthalene				Phenanthrene				Pyrene			
Conc. ($\mu g l^{-1}$)[a]	k_u ($ml\,g^{-1}\,h^{-1}$)	k_e (h^{-1})	BCF	Conc. ($\mu g l^{-1}$)[a]	k_u ($ml\,g^{-1}\,h^{-1}$)	k_e (h^{-1})	BCF	Conc. ($\mu g l^{-1}$)[a]	k_u ($ml\,g^{-1}\,h^{-1}$)	k_e (h^{-1})	BCF
453.2	3.68 ± 0.8	0.005 ± 0.001	736	57.1	62.5 ± 10.3	0.006 ± 0.001	10 261	34.0	109.0 ± 24.3	0.003 ± 0.001	36 329
676.0	4.14 ± 0.8	0.009 ± 0.002	460	104.6	79.4 ± 20.0	0.009 ± 0.003	8900	51.3	70.9 ± 12.2	0.002 ± 0.0008	30 671
1204.0	6.85 ± 2.4	0.023 ± 0.008	303	214.4	57.2 ± 9.7	0.005 ± 0.001	11 216	84.5	43.7 ± 11.3	0.003 ± 0.001	16 810
2201.1	2.9 ± 1.5	0.006 ± 0.006	490	383.0	51.9 ± 17.5	0.005 ± 0.004	10 080	130.7	37.0 ± 5.3	0.003 ± 0.0007	12 316
				637.8	44.1 ± 6.7	0.008 ± 0.002	5376				

[a] Time weighted average water concentration.

two beakers from each treatment daily thereafter, before and after the water exchange. Amphipods from exposure beakers were sampled in groups of two to five individuals at the end of the experiment and from extra beakers at intermediate time points. They were blotted dry and weighed. Water and amphipod samples were transferred to 20 ml scintillation vials containing 12 ml scintillation cocktail (3a70b, Research Products International, IL, USA), and [^{14}C] activity was quantified by LSC. Measured specific activities were used for converting radioactivity concentration in water or amphipod samples to the molar concentration of contaminants. Because compound concentrations were calculated using [^{14}C] activity as a surrogate, all concentrations are reported as parent compound equivalents. However, since *Diporeia* spp. has limited ability to biotransform PAHs, essentially all (>95%) of the ^{14}C-bearing molecules in the tissues are expected to be parent compound (Landrum, 1988).

The toxicokinetics were determined by fitting the measured concentrations in the organisms to the water either expressed as the time-weighted-average concentration or as a variable water concentration. When the time-weighted-average concentration was used, the organism concentration was fit with a two-compartment model:

$$\mathrm{Ca} = \frac{k_\mathrm{u}\mathrm{Cw}}{k_\mathrm{e}}(1 - \mathrm{e}^{-k_\mathrm{e}t}) \quad (1)$$

where Ca is the concentration in the organism ($\mu g\,g^{-1}$), k_u is the uptake coefficient ($ml\,g^{-1}\,h^{-1}$), Cw = the time-weighted-average water concentration, k_e = the elimination rate constant (h^{-1}), and t = time (h). For the time-variable water concentration, the differential form of the model was used, and the water data was set up as a step function with first order decay between the water exchange times. This yields a variable water concentration, e.g. Fig. 1. The data for the time-variable approach were fit using a fourth order Runga–Kutta approach for numerical integration. The estimates for parameterizing the numerical integrations were determined from the toxicokinetics using the time-weighted average water concentration.

2.5. Statistics

The model fits and statistics for the models were determined using the program Scientist® version 2.01 (MicroMath Scientific Software, Salt Lake City, UT). Toxicity data was calculated using TOXSTAT® (West EcoSystems Technology, Cheyenne, WY). Comparisons of LC_{50}, EC_{50}, LR_{50} and ER_{50} values were performed by comparing 95% confidence intervals. Significance was set at $p \leqslant 0.05$.

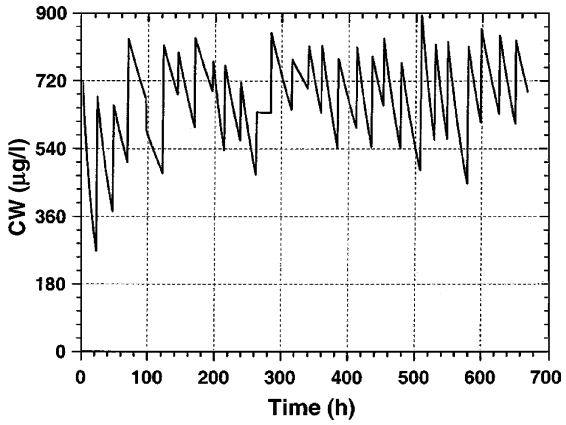

Fig. 1. Example (naphthalene 676 $\mu g\,l^{-1}$) of the time-variable water concentrations (CW) during static-renewal water exposures to PAH congeners.

3. Results

3.1. Toxicokinetics

Toxicokinetic data were only available for pyrene, naphthalene, and phenanthrene (Tables 1 and 2). The fits to the data using the time-weighted-average (TWA) concentrations were good to excellent, with coefficients of determination (COD) ranging from 0.74 to 0.93 except for the two highest doses for naphthalene where the data were much more variable (Table 1). For the highest naphthalene dose, the data were also limited due to organism mortality. In these two cases, the COD were low, 0.30 and 0.41, respectively. In general, the elimination coefficient did not vary with dose for each compound but did decline with increasing $\log K_{ow}$ (Table 4) of the selected compounds (Table 1). The uptake coefficient declined between the lowest and highest dose for pyrene and phenanthrene, but not for naphthalene, likely due to high among-replicate variability, particularly in the two highest concentrations. The resultant decline in uptake with a near constant elimination rate resulted in lower estimated bioconcentration factors (BCF) (Table 1) calculated as k_u/k_e with increasing concentrations, particularly for pyrene (Fig. 2). This trend was not as clear for either phenanthrene or naphthalene, although the calculated BCF was lower at the highest dose compared to the lowest dose (Table 1).

When the time-variable water concentration was employed, the toxicokinetic constants were essentially identical to those determined from those determined with the time-weighted-average model (Table 2) and the error estimates almost completely overlapped. However, the uptake curve based on the time-variable water concentration was not smooth and shows some short-term losses during the uptake as a result of the differences in

Table 2
The best estimate from non-linear regression (± standard error) for uptake clearance rate (k_u) and elimination rate (k_e) calculated from the time-variable water concentration for *Diporeia* spp. exposed to select PAH congeners

Naphthalene			Phenanthrene			Pyrene		
Conc. ($\mu g\,l^{-1}$)[a]	k_u ($ml\,g^{-1}\,h^{-1}$)	k_e (h^{-1})	Conc. ($\mu g\,l^{-1}$)[a]	k_u ($ml\,g^{-1}\,h^{-1}$)	k_e (h^{-1})	Conc. ($\mu g\,l^{-1}$)[a]	k_u ($ml\,g^{-1}\,h^{-1}$)	k_e (h^{-1})
453.2	3.68 ± 0.96	0.005 ± 0.002	57.1	62.5 ± 12.8	0.006 ± 0.002	34.0	109 ± 24	0.003 ± 0.001
676.0	4.13 ± 1.05	0.009 ± 0.003	104.6	79.4 ± 23.2	0.009 ± 0.003	51.3	70.9 ± 14	0.003 ± 0.001
1204.0	6.84 ± 4.22	0.022 ± 0.015	214.4	57.2 ± 11.3	0.005 ± 0.001	84.5	43.7 ± 11.9	0.002 ± 0.001
2201.1	NC	NC	383.0	51.9 ± 20.2	0.005 ± 0.0042	130.7	36.9 ± 5.6	0.003 ± 0.001
			637.8	44.1 ± 9.4	0.008 ± 0.003			

NC = Not calculated.
[a] Time weighted average water concentration.

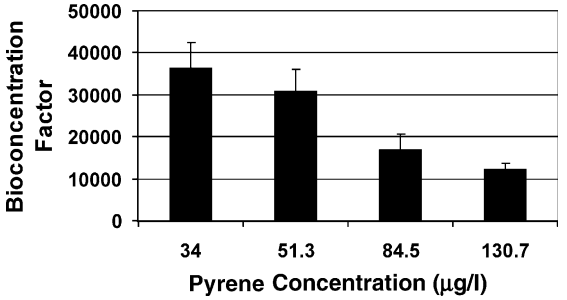

Fig. 2. Effect of the decreasing uptake rate coefficient on the bioconcentration factor for *Diporeia* spp. exposed to increasing concentrations of pyrene in water.

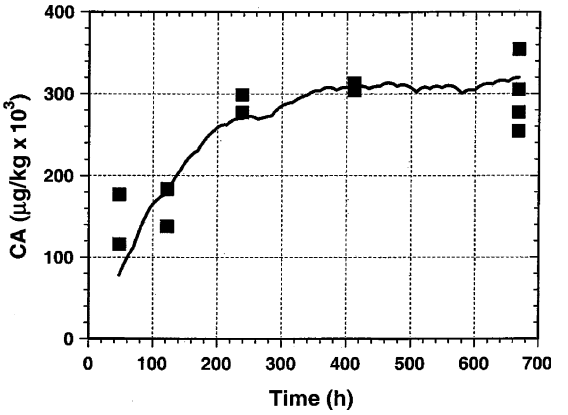

Fig. 3. Example of the time-variable accumulation (CA) of PAH (naphthalene 676 $\mu g\,l^{-1}$) by *Diporeia* spp.

water concentration and the time course of the water concentration (Fig. 3). Further, the COD (range 0.67–0.92 with the exception of the two high naphthalene doses that exhibited a COD of 0.27 and not determinable) were not as robust because the variation in the water concentration was incorporated into the model results. These fits to the data result in somewhat larger error estimated for the coefficients. The comparability between the toxicokinetic parameters calculated from the two models likely results because the water concentrations were renewed at much shorter intervals than the half-life for elimination. Thus, the overall trends are identical with those stated above. However, it was not possible to fit the highest dose for naphthalene to the time-variable water concentrations because of the paucity and variability of the data.

3.2. Toxicity

The PAH toxicity was evaluated using immobility and mortality as endpoints. The range of LC_{50} and EC_{50} values was more than a factor of 35, if the concentrations are converted to a molar basis, across compounds (Tables 3 and 4). Variation in the LC_{50} and EC_{50} values temporally and among compounds is consistent with the differences in the toxicokinetics and bioconcentration potential of the various compounds leading to the body burden required to produce effects (McCarty and Mackay, 1993). However, the LR_{50} and ER_{50} values were much less variable, with the standard deviation (SD) of the average across compounds less than 40% for the lethal endpoint and less than 25% for the immobility endpoint. This is consistent with the hypothesis that a constant threshold exists for 50% mortality, often called the critical body residue (CBR, McCarty and Mackay, 1993). An unexpected absence of time variability in the critical body residues was observed for both endpoints for all compounds. While time variability has been observed for other species, it has generally been over time courses beyond the point of toxicokinetic limits, i.e., after the organisms had reached steady state (Lee et al., 2002). Based on the kinetics data, the *Diporeia* in these toxicity studies were still generally under toxicokinetic control that is the concentrations in the organisms are increasing with exposure duration because the organisms are not at steady state. The estimated time to reach steady state (5 half-lives, determined from the k_e estimate) ranged from 322 to 1260 h. Naphthalene and phenanthrene both reached steady state by the end of

Table 3
Median effect concentration (EC_{50}) and median effect residue (ER_{50}) calculated for *Diporeia* spp. exposed to select PAH congeners

Compound	EC_{50} (95%CI) 2-d ($\mu g\,l^{-1}$)	ER_{50} (95%CI) 2-d ($\mu mol\,g^{-1}$)	EC_{50} (95%CI) 5-d ($\mu g\,l^{-1}$)	ER_{50} (95%CI) 5-d ($\mu mol\,g^{-1}$)	EC_{50} (95%CI) 10-d ($\mu g\,l^{-1}$)	ER_{50} (95%CI) 10-d ($\mu mol\,g^{-1}$)
Naphthalene	ID	ID	1587 (1378–1827)	2.7 (2.4–3.0)	1141 (991–1314)	2.7 (2.4–3.0)
Phenanthrene	295 (256–340)	2.9 (2.7–3.3)	74.3 (65.6–84.2)	2.3 (2.1–2.6)	38.2 (23.5–61.9)	1.7 (1.0–3.03)
Pyrene	ID	ID	ID	ID	NM	3.5 (3.0–4.1)

ID = Insufficient data.
NM = Would not model.

Table 4
Median lethal concentration (LC_{50}) and median lethal residue (LR_{50}) calculated for *Diporeia* spp. exposed to select PAH congeners

Compound	LC_{50} (95%CI) 10-d ($\mu g\,l^{-1}$)	LR_{50} (95%CI) 10-d ($\mu mol\,g^{-1}$)	LC_{50} (95%CI) 28-d ($\mu g\,l^{-1}$)	LR_{50} (95%CI) 28-d ($\mu mol\,g^{-1}$)	Log K_{ow}[a]
Naphthalene	1757 (1604–1924)	5.0 (4.4–5.6)	1266 (1087–1475)	5.8[b] (4.9–6.7)	3.35
Fluorene	ID	ID	542.7 (449.8–654.9)	12.3 (10.0–15.3)	4.18
Phenanthrene	168.4 (141.9–199.9)	8.4 (7.1–9.9)	95.2 (73.1–124.1)	7.6 (5.0–11.7)	4.57
Pyrene	ID	ID	79.1 (36.5–171.2)	6.1 (4.9–7.7)	5.18
PAH mixture[c]	NA	NA	NA	6.1 (3.7–21.3)	4.18–7.1[d]

NA = Not available.
ID = Insufficient data.
[a] Miller et al. (1985).
[b] Estimated from LC_{50} and BCF.
[c] Landrum et al. (1991).
[d] Range of log K_{ow} for individual components of the mixture.

the 28 d exposure period. Phenanthrene had reached steady state (525 h) just before the end of the exposure period. Thus, for these two compounds, the 28 d lethality endpoint was a steady-state value, while the immobility endpoints were found well before the organisms reached steady state. Even though naphthalene had reached steady state before 28 d, the LR_{50} values at 10 and 28 d were not different. A 28-d exposure corresponds to approximately 6% of the expected life span for *Diporeia* spp., and therefore represents an acute exposure period.

The immobility endpoint was observed early in the experiment (Table 3) and required significantly lower concentrations than those required to produce mortality (Table 4).

4. Discussion

4.1. Toxicokinetics

Constant exposure solution concentrations and adequate water quality can be accomplished either with a continuous flow-through system or through static renewal in long-term toxicity studies. Static renewal was used in this study to minimize the use of radiolabeled material and generation of radioactive waste while maintaining water quality. From Fig. 1, it is clear that the concentration varied between water exchanges. The variation was greatest during the first few days of exposure, which was likely due to the fact that sorption to the exposure vessel was greatest early on and became saturated after a few days. The remainder of the variation would have resulted from volatilization and accumulation by the organism. Food was not provided and therefore was not a source of sorption loss. After the first few days the variation in the water concentration was much lower. There was very little impact of the variation on the overall kinetics because the rate of exchange, approximately once every 24 h, was substantially shorter than one half-life, 77–346 h. Thus, the toxicokinetic parameters calculated from a time-weighted average water concentration and from a time-variable water concentration model were essentially identical and not statistically different as the error estimates overlapped. The comparison between the use of TWA and time-variable water concentrations for toxicokinetic determinations in static renewal systems has not previously been demonstrated. However, there has been the assumption that the TWA would produce an appropriate representation of exposure. This work clearly supports that assumption when the elimination half-life is shorter than the water exchange rate.

The PAH water concentration affected the toxicokinetics, primarily the uptake kinetics. At low concentrations, the uptake coefficients for pyrene and phenanthrene were similar to previously determined values determined at trace concentrations (Landrum,

1995). In general, the uptake coefficient declined with increasing PAH concentration, especially with pyrene. The effect of increasing contaminant concentration, which results in a lower uptake coefficient from water, was also observed for the zebra mussel exposed to pentachlorophenol (Fisher et al., 1999). The work with the zebra mussel only examined a single high concentration for comparison with the trace dose, at different temperatures, all of which demonstrated similar reductions in uptake with the increased concentration. It was necessary to use these lower uptake coefficients to properly predict the bioaccumulated residue for intoxication of zebra mussels by pentachlorophenol. The decline for the zebra mussel with this respiratory inhibitor was much greater than observed for *Diporeia* spp. with a non-polar narcotic such as PAH. Similar declines in the uptake rate coefficient were observed with DDT, DDD, and DDE for the amphipod *Hyalella azteca*, while such declines in uptake rate coefficients were observed for DDE but not DDT or DDD in *Diporeia* spp. (Lotufo et al., 2000). Of these compounds, only DDE acts like a non-polar narcotic based on the body residue required to produce mortality. In general, it appears that when contaminant concentrations increase to toxic levels, particularly for non-polar narcotics, the uptake coefficient typically declines. As animals become affected by the non-polar narcotics, their swimming activity is reduced to the point of paralysis, where respiration can still be observed in non-moving organisms. The decreased activity will decrease the volume of contaminated water encountering the surface of the organism and thus reducing contaminant uptake. The reduced BCF with increasing concentration has significant implications for assessing bioaccumulation and trophic transfer of contaminants. The BCF is generally thought to be constant essentially reflecting the thermodynamic properties governing the partitioning between water and the organism. However, if changes in organism behavior affects the BCF then performing assessments using a constant value may over estimate bioaccumulation and trophic transfer of contaminants.

Considering the immobilizing effect of PAHs, it is surprising that PAH elimination appeared not to be affected by PAH water concentration. The relative consistency in the elimination may result because the rate-limiting step is the rate of passive transfer across the membrane for elimination and not the encounter rate of water as is apparently the case for the uptake coefficient.

4.2. Toxicity

The concentration of PAHs in the water associated with mortality or immobility increased with increasing compound hydrophobicity. The toxicity expressed as the LC_{50} value ranged substantially, requiring substantially higher concentrations to produce either immobility or mortality with compounds that had lower $\log K_{ow}$ values. This is consistent with previous observations that LC_{50}s are typically higher for hydrophilic compounds compared to hydrophobic compounds. Elevated LC_{50}s are typically associated with low bioconcentration factors (BCFs) for compounds with lower $\log K_{ow}$ values. Thus, low $\log K_{ow}$ compounds were perceived to be less toxic. However, recent work has shown that compounds acting by non-polar narcosis all tend to require the same body residue for acute toxicity, and that the differences in the required water concentrations to produce the results are reflected in the differences in the BCF (McCarty and Mackay, 1993).

Further, the LC_{50} and EC_{50} values both exhibited time-dependent variation that was not observed in the body residue based values for mortality. This results from toxicokinetic control over the process prior to the organism achieving steady state. If the body residue required for acute mortality is constant, then while the organisms are not at steady state the toxicity based on the external concentration must be larger with shorter exposures to achieve the threshold for toxicity internally in accordance with Eq. (1).

Both immobility and mortality were associated with similar body residue effect concentrations for the PAH congeners investigated in this study, although the immobility endpoint required about half the body residue concentration required for mortality (Tables 3 and 4). For each endpoint, the body residue concentration required for 50% response was constant over the time frame for which it was measured. Since both responses are expected to come from the same mechanism of action, the difference in the required concentration reflects the additional level stress required for mortality to ensue. Organisms unable to swim should be considered ecologically dead, since they would likely succumb to predators and/or additional stresses in the field.

The temporally constant value for each endpoint is in contrast to some recent work that suggests that the internal body residue required to produce mortality exhibits a time dependence with decreasing concentrations with increasing exposure time (Chaisuksant et al., 1997; Yu et al., 1999; Lee et al., 2002). The constant body residue with increasing exposure observed in this study was probably due to the relatively short duration of the exposures compared to the life span of the organism and to the time required to reach steady state. *Diporeia* spp. had barely reached steady state by the end of the longest exposure duration. Thus, for the compounds under study, most of the toxicity test was under toxicokinetic control. Further, the duration of exposure represents only a small portion of the lifetime of the organism, as *Diporeia* spp. exhibit a life span of 1–3 years in the field (Lubner, 1979). The time-dependent relationship found by Yu et al. (1999) reflected the exposure relative to the lifetime of the organism and that found by Lee et al.

(2002) occurred after the organism had reached steady state, and the toxicity became dominated by toxicodynamics of the compound. Thus, it is not surprising that time-dependent toxicity based on body residue was not observed in this case. In addition, lethal body burdens determined in this study for different PAH congeners met the expected body burdens for acute non-polar narcosis i.e., 2–8 mmol kg^{-1} (McCarty and Mackay, 1993).

4.3. Support for additive mixture toxicity

Recent work has suggested that PAHs produce mortality by non-polar narcosis in aquatic organisms and act additively when in a mixture in the absence of phototransformation (Swartz et al., 1995; DiToro et al., 2000). Similarly, the toxicity of a mixture of PAH, including fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*b*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene and benzo[*ghi*]perylene, to *Diporeia* spp. appeared to meet the criteria for additivity based on the LR_{50} of 6.1 mmol kg^{-1} as the molar sum of PAH (Landrum et al., 1991). However, previous evidence that individual PAH congeners promote acute toxicity at the same 2–8 μmol kg^{-1} level was minimal with only one study exposing *Diporeia* spp. to pyrene (Landrum et al., 1994). In the present study of several additional PAH congeners, it is clear that all the congeners required a similar internal concentration for 50% mortality as that described above for acute mortality (2–8 mmol kg^{-1}), and the internal concentrations required for 50% mortality varied only by about a factor of 2, 5–12 mmol kg^{-1}. Thus, combining this information on several congeners of differing $\log K_{ow}$ with the observation that the internal body burden for 50% mortality was a similar concentration for a mixture of PAH based on molar additivity confirms the additivity of the PAH in *Diporeia* spp. Therefore, our study provides additional experimental confirmation for the theoretical work of DiToro et al. (2000) for an additivity model of non-polar narcosis.

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