SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 19, No. 4, pp. 1028–1035, 2000

Printed in the USA
0730-7268/00 $9.00 + .00

CRITICAL BODY RESIDUES IN THE MARINE AMPHIPOD *AMPELISCA ABDITA*: SEDIMENT EXPOSURES WITH NONIONIC ORGANIC CONTAMINANTS

AMANDA A. FAY,† BRUCE J. BROWNAWELL,‡ ADRIA A. ELSKUS,§ and ANNE E. MCELROY*‡
†The IT Group, 9300 Lee Highway, Fairfax, Virginia 22031, USA
‡Marine Sciences Research Center, State University of New York at Stony Brook, Stony Brook, New York 11794-5000, USA
§T.H. Morgan School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506, USA

(*Received* 23 *March* 1999; *Accepted* 13 *August* 1999)

Abstract—Body residues associated with acute toxicity were determined in the marine amphipod *Ampelisca abdita* exposed to spiked sediments. Nonylphenol and 2,2′,4,4′-tetrachlorobiphenyl critical body residues (CBRs, body residue of contaminant at 50% mortality) were 1.1 μmol/g wet tissue and 0.57 μmol/g wet tissue, respectively, values near the low end of the CBR range expected for compounds acting via narcosis. The polycyclic aromatic hydrocarbons tested, benzo[*a*]pyrene (B*a*P) and benz[*a*]anthracene (B*a*A), were not acutely toxic at exposure concentrations of up to 43 and 1,280 μg/g dry sediment for B*a*A and B*a*P respectively, and body burdens up to 1.2 μmol/g wet tissue (for B*a*P). Neither polycyclic aromatic hydrocarbon (PAH) was significantly metabolized by *A. abdita*. The microextraction technique employed here allowed residue analysis of samples containing as few as three amphipods (0.33 mg dry wt). The CBR approach avoids confounding factors such as variations in bioavailability and uptake kinetics and could be employed to assess the relative contribution of specific contaminants or contaminant classes in mixtures to effects observed in toxicity tests with *Ampelisca* and other organisms.

Keywords—Sediment toxicity test Nonylphenol Benzo[*a*]pyrene Polychlorinated biphenyl Metabolism

INTRODUCTION

Contaminated marine sediments are a major concern in many urban harbors and coastal areas. Because sediments serve as repositories for hydrophobic organic contaminants, they can become significant sources of these contaminants to aquatic life. Laboratory bioaccumulation and toxicity tests with benthic organisms are routinely used to assess potential risks of sediment-associated contaminants [1]. One species commonly used in whole-sediment toxicity tests is the marine amphipod *Ampelisca abdita*. This suspension and deposit feeder is often a dominant member of the benthic community, present at densities as high as 110,000/m^2, and can be an important food source to benthivorous fish [2,3]. Tests with *A. abdita* are often used to determine whether dredged marine sediments must be disposed of as hazardous waste [4]. Therefore, improved understanding of the factors that control toxicity in this organism not only has biological and chemical significance but also economic importance.

One method for examining the relationship between the accumulation of a toxicant and its effects on an organism is the critical body residue approach (CBR) [5]. Based on the premise that a toxicant must reach a threshold concentration within the organism before an adverse response is elicited, actual body residues of compounds are measured in relation to specific toxic effects observed [6,7]. The CBR approach has certain advantages over standard comparisons between toxicity and contaminant concentration in the exposure medium [7]. By taking advantage of a more direct dose-response relationship, the CBR approach does not rely on external dose as a surrogate for internal dose at the site of toxic action [8]. In addition, the acute CBR (or lethal body burden) obtained should be less dependent on experimental conditions such as exposure time and concentration than the more commonly used LC50 approach [9]. Associating biological effects with contaminant residues in the body of the organism should be more consistent and meaningful than associating toxicity with contaminant concentrations in the water or sediment [10] because bioavailability and multiple uptake paths are implicitly considered [11].

The CBR theory and approach has been evaluated most thoroughly for compounds acting via narcosis. Narcosis is a general, nonspecific, reversible mode of toxic action produced in most organisms whereby the presence of hydrophobic organic chemicals in membranes disrupts normal function [12]. It is assumed that narcosis does not involve a specific chemical reaction or unique receptor but rather that the general membrane disturbance resulting from the presence of a chemical leads to decreased organism activity and ultimately death [13].

The CBR model predicts that acute narcotic toxicity results from a near-constant body residue of 2 to 8 μmol of chemical/g wet tissue, independent of the LC50 and the chemical's structure [5]. Although this prediction has been supported with experimental data [14,15], acute narcotic CBRs display somewhat larger ranges that appear to be species specific [16]. Normalizing CBRs to the lipid content of organisms can account for some, but not all, of this interspecies variation [15–17]. Time to death has also been found to influence the CBRs obtained [17].

The main focus of this study was to determine the CBR for *A. abdita*, a marine amphipod commonly used in sediment toxicity tests. The contaminant classes of interest in this study were polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCBs), and alkylphenols. The PAH and PCB compound classes were chosen based on their prevalence in contaminated sediments in the environment and their presumed

* To whom correspondence may be addressed (anne.mcelroy@sunysb.edu).

mode of acute toxic action (narcosis) [15,18]. Because PAHs may also act via specific modes of toxicity (e.g., the production of toxic metabolites), we also determined the ability of *A. abdita* to metabolize PAHs. Alkylphenols were included in this study because they are a widely used contaminant class of recent regulatory interest due to their estrogenic activity [19,20]. The mode of acute toxic action for alkylphenols has not been studied explicitly [21].

MATERIALS AND METHODS

Chemicals

The following chemicals were used in this study: benzo[*a*]pyrene (B*a*P) (G-[^{3}H]B*a*P, 2.0 TBq/mmol, from Amersham Life Sciences, Arlington Heights, IL, USA; unlabeled, originally 98% pure B*a*P, from Sigma, St. Louis, MO, USA), benz[*a*]anthracene (B*a*A) (12-[^{14}C]B*a*A, 1.8 GBq/mmol, from Amersham; unlabeled, 95% pure B*a*A, from Sigma), 2,2′,4,4′-tetrachlorobiphenyl (TCB) (U-[^{14}C]TCB, 3.9 GBq/mmol, 95% pure TCB, from Sigma; unlabeled, 99% pure TCB, from Ultra Scientific, North Kingstown, RI, USA), and 4-nonylphenol (technical mix of isomers from Fluka, Milwaukee, WI, USA). All solvents used were high purity gas chromatography-grade Burdick and Jackson (VWR Scientific Products, Bridgeport, NJ, USA).

Purification of [^{3}H]BaP

Due to relatively rapid autoradiolysis, the [^{3}H]B*a*P was repurified prior to use using a 24-cm × 11-mm 5% deactivated alumina column. Elution with 25 ml of 30% dichloromethane in hexane resulted in B*a*P that was >99% pure, as verified by reverse phase high-performance-liquid chromatography. Prior to use, the [^{14}C]benz[*a*]anthracene was also checked by high-performance liquid chromatography and determined to be >99% pure.

Animals

Juvenile *A. abdita* (0.7–1.2 mm length), collected from the Narrow River, Rhode Island, USA, were purchased from Eastern Aquatic Biosupply (Newport, RI, USA) and shipped to our laboratory via overnight express service in Narrow River sediment. Wet, dry, and lipid weights were determined on pooled amphipod samples. Wet and dry weights were determined using pools of eight live amphipods. Samples were weighed on a Cahn microbalance (±0.0001 mg precision; Thermo Instruments, Waltham, MA, USA), dried in an oven at 55°C for 4 d, and weighed again. Mean wet weight ± SD was 0.476 ± 0.097 mg/amphipod and mean dry weight was 0.109 ± 0.016 mg/amphipod. Mean amphipod lipid content was 10.4% (±1.3% SD, n = 4) on a dry weight basis.

For use in toxicity experiments, amphipods were sieved out of the sediment within 1 h of their arrival with a 500-μm sieve using seawater (approximately 26‰ salinity) at temperatures close to the shipping water temperature. Amphipods were kept in seawater at 20°C, separated into groups of 15, and added to exposure jars as soon as possible after arrival (<4 h).

Lipid analysis

Pools of five *A. abdita* were used for lipid analysis. Lipid analysis was conducted using a modification of the Herbes and Allen approach [22]. Tissues were extracted three times in 0.1-ml vials with a chloroform:methanol (2:1) solution by pulverizing with 1-mm glass beads in a Mini Bead-Beater® (Biospecs Products, Bartlesville, OK, USA) for 60 s. Suspensions were then centrifuged and supernatant removed as the extract. A 0.9% NaCl solution was added to the combined extracts and the sample again subjected to the Mini Bead-Beater. After centrifugation, the chloroform phase was transferred to a preweighed tin cup for lipid measurement. Samples were weighed on a Cahn microbalance after being dried in a 55°C oven overnight.

Sediment labeling

Spiking solutions of pure compounds (TCB, nonylphenol [NP], B*a*P, B*a*A) and of mixtures (B*a*P/TCB, B*a*P/NP, TCB/NP) were prepared using either unlabeled compounds or a mixture of radiolabeled and unlabeled compounds. All solutions were designed to achieve the desired nominal sediment concentrations and a minimum radioactive signal of 0.1 kBq/g wet sediment. Binary mixtures of equal concentrations of two contaminants were designed based on results (lethal residues for 50% of the population, LR50s) from the individual compound toxicity tests. Mixture concentrations were designed to achieve body burdens resulting in 50% mortality of *A. abdita,* assuming additive toxic effects of the compounds. If the compounds in the binary mixtures were acting antagonistically (less than additive), it was assumed that the resulting toxic body burdens would be significantly higher than those determined in individual compound toxicity tests. Conversely, if the compounds in these mixtures were acting synergistically (more than additive), it was assumed that the resulting toxic body burdens would be significantly lower than those determined in the individual compound toxicity tests.

Spiking solutions were added to solvent-rinsed amber glass jars with Teflon®-lined lids. The jars were coated with the appropriate compound by evaporating the carrier solvent under N_2 just until dryness. Sediment from a central Long Island Sound, USA, reference site and filtered seawater were added to each jar to create a 50% sediment:water slurry. Jars were shaken for 7 d on an orbital shaker (VWR Scientific Products) at 125 rpm and hand shaken vigorously once per day for 30 s to ensure complete resuspension of sediment. After 7 d, the sediments were centrifuged at 2,145 rpm for 7 min on a tabletop centrifuge to remove excess seawater, were homogenized, and were added to exposure chambers in 5-g aliquots.

Sediment toxicity tests

In a modified version of the standard U.S. Environmental Protection Agency amphipod toxicity test (miniaturized by Ho et al. [23]), *A. abdita* were exposed to contaminant-labeled sediment (described above) in glass jars containing 5 g wet sediment and 60 ml of overlying filtered seawater for 10 d. They were allowed to burrow into the sediment for at least 1 h before gentle aeration of the water column was introduced through a Pasteur pipette. The temperature was monitored daily and maintained at 20 ± 1°C. Salinity and total ammonia levels were checked at the beginning and end of each experiment and found to range between 29 and 32‰ and 0 and 1 ppm, respectively. Dissolved oxygen measured with a microoxygen electrode (MI-730; Microelectrodes, Bedford, NH, USA) was found to be at 100% (±2%) saturation throughout one experiment and was not routinely measured. At the conclusion of each test, amphipods were sieved out of the sediment with a 250-μm sieve. The number of live amphipods found at the conclusion of each experiment was quantified, and all live organisms were saved for body burden analysis. Although some dead organisms were retrieved, most were not recovered

due to rapid tissue degradation. Because the time of death was unknown, the few amphipod carcasses recovered were not saved for contaminant analysis. Percent mortality resulting from each treatment was determined. Mean control mortality was ≤13% in all experiments except for the first (37%). This high control mortality in the first experiment was probably due to temperature control problems (22 ± 3°C). However, the first experiment did not result in any data that were used to determine critical body residues.

Sediment chemistry analysis

Aliquots of sediments for chemical analysis were taken 1 d before the beginning of each toxicity test, concurrently with sediment addition to exposure jars. All sediment samples containing unlabeled compounds were stored at −20°C until analyzed. These sediments (approximately 3 g wet) were spiked with surrogate standards (octylphenol, *p*-terphenyl, 2,4,5-trichlorobiphenyl, 2,2′,3,4,5,6′-hexachlorobiphenyl) delivered in iso-octane and were allowed to air dry. Dried samples were pulverized with a mortar and pestle and Soxhlet extracted with hexane for at least 16 h. Sediment extracts were reduced to 10 ml, and potential interfering compounds were removed via solid phase extraction through an aminopropylsilica cartridge (Alltech Associates, Deerfield, IL, USA). Nonylphenol was eluted from the soil phase extraction cartridge with hexane: acetone (3:1). Sediment extracts were exchanged to iso-octane, injection standards (tribromophenol, octachloronaphthalene, *n*-hexadecane) were added, and the extracts were volume-reduced to 500 µl. Extracts were injected in splitless mode on a Hewlett Packard 5890 Series II gas chromatography (with a 30-m DB-5 column, 0.32-mm internal diameter and 0.25-µm film thickness; Hewlett Packard, Avondale, PA, USA) equipped with an electron-capture detector (for PCB detection) or a flame ionization detector (for PAH and NP detection). The temperature program used with the electron-capture detector was 40°C for 2 min, ramping at 30°C/min to 120°C, then at 2°C/min to 240°C, and then being held for 10 min. The temperature program used with the flame ionization detection was 40°C for 2 min, ramping at 30°C/min to 120°C, then at 2°C/min to 150°C, then at 5°C/min to 270°C, and then being held for 15 min.

Measured sediment contaminant concentrations averaged 67% of the nominal concentrations. Differences in measured and nominal concentrations were compound dependent and were as follows: $BaP_{measured} = 0.84BaP_{nominal}$, $BaA_{measured} = 0.43BaA_{nominal}$, $TCB_{measured} = 0.63TCB_{nominal}$, and $NP_{measured} = 0.70NP_{nominal}$. The reasons for departures between nominal concentrations and those measured was not determined. Calculations of biota sediment accumulation factors (BSAFs) reported in this work rely on the actual measured concentrations.

Aliquots of sediments containing radiolabeled compounds were taken for chemical analysis 1 d before each exposure began and at the conclusion of each experiment. These sediment samples (≤0.2 g wet wt) were added to scintillation vials with 500 µl of tissue solubilizer (Solvable, Packard, Meriden, CT, USA). Samples were allowed to dissolve overnight at 60°C, at which point 10 ml of scintillation fluid (Ultima Gold XR, Packard) was added. Samples were left at room temperature for at least 2 h to reduce chemiluminescence prior to counting in a liquid scintillation counter (LKB Wallac 1217 Rackbeta LSC, Wallac, Turku, Finland). For samples containing ^{14}C-labeled compounds, the external standards ratio method was used to correct for quench. For samples containing ^{3}H-labeled compounds, internal standards ([^{3}H]toluene) were used for quench correction. All sample counts were corrected for background.

Tissue chemistry analysis

Polycyclic aromatic hydrocarbon metabolism. To analyze the ability of *A. abdita* to metabolize PAHs to polar metabolites, surviving amphipods that had been exposed to radiolabeled B*a*P or B*a*A were extracted using a modification of Collier's method [24]. Organisms were rinsed in filtered seawater, blotted dry, and stored by replicate groups in 2-ml polypropylene vials (Biospec) at −20°C until analysis. Briefly, approximately 50 one-mm glass beads were added to the thawed sample in 2-ml polypropylene vials. Hexane and 2 N NaOH (in 25% ethanol) were added in a 5:2 ratio. Samples were beaten in the Mini Bead-Beater at 3,800 rpm for 30 s and 60 s, then spun for 15 min at 2,145 rpm. The hexane layer, which contained parent PAH, was removed and placed in a scintillation vial. This extraction was repeated, the hexane extracts combined, gently blown to dryness under N_2, and scintillation fluid added. The aqueous phase, which contained any water-soluble PAH metabolites formed, was transferred to a scintillation vial, 2 N hydrochloric acid was added to neutralize the base, and scintillation fluid was added. Radioactivity was measured in each phase on a liquid scintillation counter using internal standards for quench correction and counting efficiency.

Body burden analysis. Live organisms exposed to only unlabeled compounds (all NP exposures, NP/B*a*P, and NP/TCB) were rinsed in filtered seawater, blotted dry, and stored in glass vials at −20°C until analysis. Organisms were stored, extracted, and analyzed in pools of 2 to 15 individuals from the same exposure jar. For extraction of contaminants, organisms were added to 0.1-ml glass conical microvials with approximately 15 one-mm glass beads, 40 µl 4 N sodium hydroxide, 10 µl methanol, and 50 µl iso-octane spiked with surrogate standards (2,4,6-tribromophenol, *p*-terphenyl, 2,4,5-trichlorobiphenyl, 2,2′,3,4,5,6′-hexachlorobiphenyl). Tissues were pulverized by beating for 100 s at 3,800 rpm in a Mini Bead-Beater, allowed to sit for 5 min, and centrifuged at 6,000 rpm for 20 min at 10°C. The iso-octane layer was removed and tissues were extracted again with 50 µl of ethyl acetate. Samples were slightly acidified by addition of 13 µl of 12 N HCl and beaten and centrifuged as before. The ethyl acetate layer was removed and added to the iso-octane extract. Combined tissue extracts were volume reduced under N_2, exchanged to iso-octane, stored at −20°C, and analyzed as described above for sediments.

Live organisms exposed to radiolabeled TCB and the TCB/B*a*P mix were rinsed with filtered seawater, blotted dry, and added to scintillation vials (in pools of 1–15 individuals from the same exposure jar) with 500 µl of tissue solubilizer. Samples were prepared and analyzed as described above for radiolabeled sediments.

In order to determine the background levels of PAHs, PCBs, and nonylphenol in *A. abdita,* control organisms were extracted as described above and analyzed for these contaminant classes. Control amphipods had total PCB levels similar to those of procedural blanks (≤1 µg/g wet tissue). Control amphipods had a maximum total PAH concentration of 16 µg/g wet tissue based on quantification of peaks with retention times similar to those in a standard mixture of 25 PAHs. Control

amphipods had nonylphenol levels below the detection limits of the instrument.

Biota sediment accumulation factors (BSAFs, lipid and sediment organic carbon-normalized accumulation factors) were calculated using body burdens of survivors measured at the end of the 10-d exposures and compound concentrations in sediments spiked with individual compounds. The BSAFs were calculated as

$$\text{BSAF} = [(\mu\text{g compound in organism/g dry tissue}) \div (\text{g lipid/g dry tissue})] \div [(\mu\text{g compound in sediment/g dry sediment}) \div (\text{g organic carbon/g dry sediment})] \quad (1)$$

Statistical treatment of the data

Lethal residues for 50% of the population (LR50s) were determined using a modification of the graphical method [2], substituting contaminant body burdens for sediment contaminant concentrations. The term LR50 is used instead of the more traditional LD50 (lethal dose for 50% of the population) to emphasize the relationship between toxicity and body residues. Due to the narrow range of body burdens where partial toxicity was observed, a linear regression was used. Regressions and 95% confidence intervals were determined using the SigmaPlot® graphical program (Jandel Scientific, San Rafel, CA, USA).

RESULTS

Sediment toxicity tests

Large differences in toxicity were observed between the compound classes studied. *Ampelisca abdita* exposed to NP or TCB exhibited a strong, but narrow, dose–response relationship between sediment concentrations and survival, with mortality reaching 100% at nominal concentrations above 347 μg/g dry sediment and 710 μg/g dry sediment, respectively (Fig. 1). In contrast, *A. abdita* exposed to B*a*P or B*a*A failed to exhibit mortality levels above controls even at the highest sediment concentrations tested (1,280 μg/g dry sediment for B*a*P; the maximum sediment concentration of B*a*A was only 43 μg/g dry sediment; data not shown) (Fig. 1). Body residues were determined for organisms only from those treatments resulting in partial mortality, as analysis could only be done on surviving organisms.

Body residues and LR50 calculations

For both PAHs examined, body burdens consisted primarily of the parent compound. Amphipods exposed to benzo[*a*]pyrene for 10 d contained >90% unmetabolized parent compound, with a small fraction (8.3 ± 1.9% SD) of the body burden being polar metabolites. Similarly, in the experiment in which *A. abdita* were exposed to benz[*a*]anthracene, most of the body burden on day 10 was isolated as unmetabolized parent compound (87.2 ± 5% SD).

The most sensitive portion of the toxicity/body burden relationships for each compound is plotted in Figure 2 using percent mortality corrected for control mortality plotted against body burden. Figure 2 shows the regressions for amphipod mortality versus contaminant body burden (parent plus metabolites) for nonylphenol, 2,4,2′,4′-tetrachlorobiphenyl, and benzo[*a*]pyrene. Based on linear regressions between these parameters, the body residue associated with 50% mortality

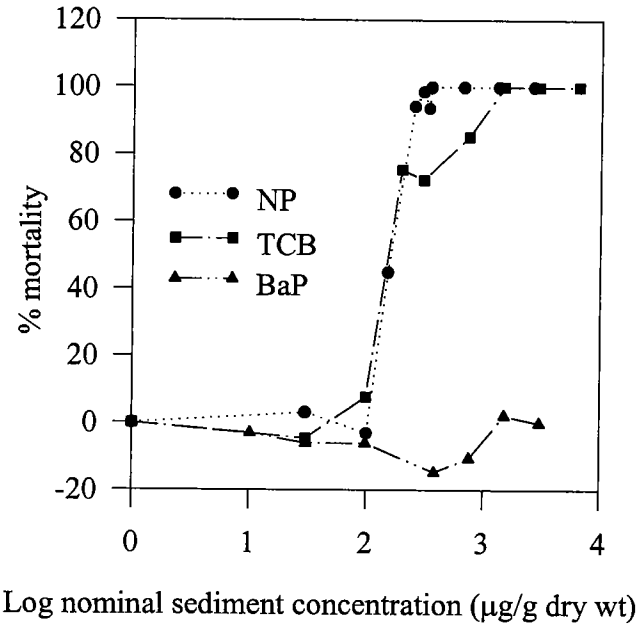

Fig. 1. Concentration of nonylphenol (●), 2,2′,4,4′- tetrachlorobiphenyl (■), and benzo[*a*]pyrene (▲) in sediments versus percent mortality (corrected for control mortality) in *Ampelisca abdita* after 10-d exposures.

(LR50) was estimated for each compound. The LR50s for NP and TCB were estimated to be 1.1 μmol/g wet tissue (95% confidence interval [CI], 0.8–1.4) and 0.57 μmol/g wet tissue (95% CI, 0.25–2.2), respectively (Table 1). On a lipid-weight basis, these LR50s for NP and TCB are 35 and 18 μmol/g lipid for NP and TCB, respectively. The LR50s for B*a*P and B*a*A could not be determined because there was no significant mortality at any exposure level.

The LR50 for each of the binary mixtures (TCB/B*a*P, NP/B*a*P, TCB/NP) was determined in the same way as for the individual compounds. Nominal dosing concentrations for mixtures containing B*a*P were chosen to bracket the expected LR50 for TCB or NP, using equivalent concentrations for B*a*P (see Methods). Table 1 summarizes the LR50s for the binary mixtures and shows the relative contribution of each compound to the total body burden at 50% mortality. The LR50s for the mixtures were not significantly different than the LR50s for the individual compounds (NP and TCB).

In order to compare this work to other studies measuring bioaccumulation, 10-d BSAFs were also calculated for NP, TCB, B*a*P, and B*a*A. Values presented in Table 2 represent the mean BSAF calculated from body burdens accumulated from three to five different sediment contaminant concentrations. Lipid-normalized body burdens were found to be positively correlated with organic carbon-normalized sediment contaminant concentrations (r^2, 0.66–0.98), and the BSAFs calculated showed no consistent variation with sediment contaminant concentrations.

DISCUSSION

We used the critical body residue approach to assess the toxicity of sediment-associated contaminants to *A. abdita*, a species widely used in sediment toxicity tests. The lethal body burdens (LR50s) of nonylphenol and 2,2′,4,4′-tetrachlorobiphenyl for *A. abdita* in this study were similar (1.1 and 0.57 μmol/g wet tissue, respectively) and fall near or below the range of CBRs predicted for acute narcosis (2–8 μmol/g wet

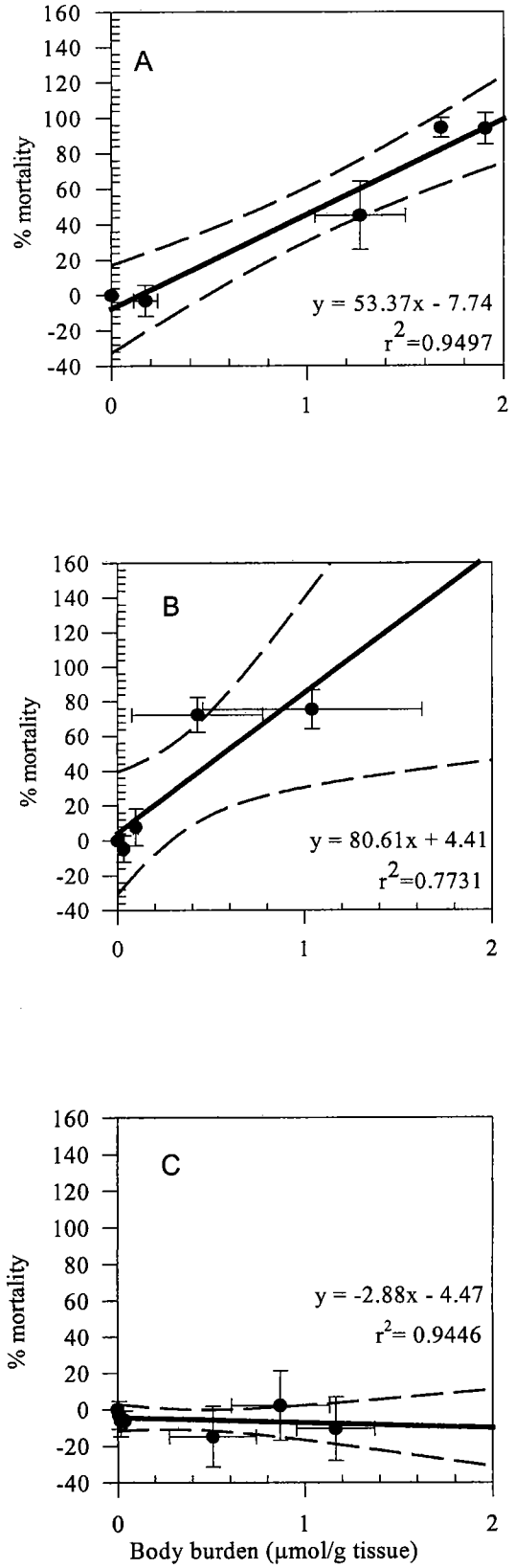

Fig. 2. Body burdens of (**A**) nonylphenol (estimated lethal residues for 50% of the population [LR50] = 1.1 μmol/g wet tissue), (**B**) 2,2′,4,4′-tetrachlorobiphenyl (estimated LR50 = 0.57 μmol/g wet tissue), and (**C**) benzo[*a*]pyrene in *Ampelisca abdita*. Dashed lines represent 95% confidence intervals. Error bars represent standard deviations around mean body burden (horizontal) and mean percent mortality (vertical). Percent mortality is corrected for control mortality.

Table 1. Lethal residues for 50% of the population (LR50s) for sediment exposures of *Ampelisca abdita* to individual compounds and binary mixtures

Treatment[a]	LR50 (μmol summed compounds/g) (95% CI)	Tissue levels of each compound at LR50 (95% CI)
NP	1.1 (0.8–1.4)	—
TCB	0.57 (0.25–2.2)	—
B*a*P/TCB	1.53 (0.9–13.6)	B*a*P: 0.17 (0.11–0.38) TCB: 1.35 (0.8–>15)
B*a*P/NP	0.60 (0–>40)	B*a*P: 0.09 (0.02–>2) NP: 0.55 (0–>2)
NP/TCB	1.31 (0.8–1.8)	NP: 0.78 (0.3–1.8) TCB: 0.55 (0.4–0.65)

[a] NP = nonylphenol; TCB = tetrachlorobiphenyl; B*a*P = benzo[*a*]-pyrene.

tissue or 63–255 μmol/g lipid for an organism with 3.14% lipid) [5].

Studies with both invertebrates and vertebrates exposed to various PCBs have reported LR50s similar to those we found for TCB. Borgmann et al. [25] reported LR50s of 0.1 to 0.6 μmol/g wet tissue (approx. 4–25 μmol/g lipid) in aqueous exposures of the freshwater amphipod *Hyalella azteca* to 2,5,2′,5′-tetrachlorobiphenyl and Aroclor 1242 [25]. The LR50s of 0.5 to 0.9 μmol/g wet tissue (approx. 21–38 μmol/g lipid) were reported for *H. azteca* exposed to a variety of PCB congeners [26]. The LR50s for fathead minnows exposed to PCBs were 0.4 to 2.3 μmol/g wet tissue, with high intraspecies differences only partially removed by accounting for lipid contents [15].

We are aware of no published CBR work with longer chain alkylphenols in sediment exposures. However, some researchers have analyzed body residues of other substituted phenols in relation to toxic effects. McCarty et al. [27] encountered a wide range of acute CBRs in exposures of the freshwater amphipod *Diporeia* (formerly *Pontoporeia hoyi*) to 30 substituted phenols. The LR50 for pentachlorophenol was 0.91 μmol/g wet tissue (approx. 15 μmol/g lipid) [27], similar to our value of 1.1 μmol/g for NP in *A. abdita*. The LR50s for fish exposed to various chlorophenols through the water have been reported to vary between 0.2 and 2 μmol/g wet tissue [28,29].

The nonylphenol and 2,2′,4,4′-tetrachlorobiphenyl data from the present study suggest that these compounds are likely acting via narcosis in *A. abdita* at body residues slightly lower than the range predicted by the CBR model. However, the CBRs we found for NP and TCB are consistent with CBRs in the literature for similar compounds and organisms. Although lipid normalization decreases differences in CBR between individuals and between species [15], acute narcotic CBRs still show some interspecific variation [16]. The range predicted

Table 2. Ten-d biota sediment accumulation factors (lipid and organic carbon normalized) for *Ampelisca abdita*[a]

Compound	*n*	Mean BSAF (SD)
B*a*P	6	0.147 (0.071)
B*a*A	3	0.126 (0.095)
TCB	5	0.415 (0.288)
NP	4	0.933 (0.451)

[a] *n* = number of analyses; BSAF = biota sediment accumulation factors; SD = standard deviation; B*a*P = benzo[*a*]pyrene; B*a*A = benzo[*a*]anthracene; TCB = tetrachlorobiphenyl; NP = nonylphenol.

Table 3. Physicochemical properties of compounds and predicted maximum lipid-normalized body burdens

Compound	log S (mol/L)	log K_{ow}	log K_{tw}[a]	Predicted BR_{max}[b] (μmol/g lipid)	Measured BR_{max} (μmol/g lipid)
B*a*A	−7.31[c]	5.96[c]	5.88	35	0.6
B*a*P	−8.22[c]	6.50[c]	6.41	17	37
TCB	−7.06[c]	6.18[c]	6.08	100	35
NP	−4.61[d]	4.20[e]	4.38	590	61

[a] Triolein-water partition coefficient, calculated using Equation 2.
[b] BR_{max} = highest measured (experimental) or predicted (estimated solubility-limited saturation) body residues.
[c] [46].
[d] [47].
[e] [48].

by the CBR model appears to be too narrow to accommodate the range of LR50s observed. Our data and those discussed above [15,25–29] clearly show that some species may suffer acute narcotic toxicity at consistently lower body burdens than the 2 μmol/g wet tissue predicted by the CBR model. Given the interspecific differences in CBR, it is important to experimentally determine the CBR for individual species using model compounds with known modes of toxic action.

In contrast to NP and TCB, acute B*a*P toxicity was not observed in this study despite high sediment exposure concentrations and body burden accumulations that were comparable to that of the other target compounds (up to 1.17 μmol/g wet tissue or 37.3 μmol/g lipid). The lack of toxicity for this high molecular weight PAH is consistent with reports from other studies with very low solubility organic compounds due to limitations related to aqueous solubility [13]. The maximum achievable lipid-normalized body burdens of nonionic organic compounds can be estimated from the product of the lipid-water coefficient and the aqueous solubility (S). Bioaccumulation becomes limited by solubility for very high K_{ow} compounds, especially those with high melting points, for three reasons [30]: (1) the heat of fusion of compounds that are solids at environmentally relevant temperatures increases with melting point and results in a drop in aqueous solubility without affecting lipid-water partitioning; thus, for compounds that are solids, solubility drops faster than hydrophobicity as K_{ow} increases; (2) lipid-water partition coefficients appear to increase less than linearly with increasing K_{ow} (note that the slope in Eqn. 2 below is less than unity); and (3) for very high K_{ow} organic compounds (log $K_{ow} > 5.5$), there is an increasing incompatibility in lipid phases/membranes with increasing solute size/K_{ow} [30], leading to lower accumulation than that predicted from extrapolation of regressions based on data for lower K_{ow} compounds. To illustrate the effect of solubility limitations on bioaccumulation with the compounds from this study, the lipid-water partition coefficients are estimated from the linear free energy relationships between the triolein-water partition coefficient (K_{tw}) and K_{ow} [30] as

$$\log K_{tw} = 0.893 \log K_{ow} + 0.607 \quad (2)$$

Based on literature estimates of aqueous solubility and K_{ow}, the maximum achievable lipid-normalized body burdens are estimated for single solute exposures in Table 3. The lack of toxicity of benzo[*a*]pyrene is consistent with the prediction of a maximum lipid-normalized body burden of 17 μmol/g lipid. Note that the measured maximum body burdens for B*a*P never exceeded 37 μmol/g lipid, reasonably close to that predicted. Tetrachlorobiphenyl and nonylphenol accumulation is predicted to be less limited by solubility (100 and 590 μmol/g lipid, respectively). Acute narcosis occurred at body burdens below these estimates, and maximal measured body burdens for these two compounds did not approach solubility-limited accumulation (Table 3). It is of interest that the maximum measured body burdens of benzo[*a*]pyrene were similar to those that caused toxicity for nonylphenol and tetrachlorobiphenyl in this study. It is unclear why this was so, although B*a*P body burdens apparently approached solubility-limited accumulation. It is possible that B*a*P has a lower intrinsic toxicity than either TCB or NP due to a difference in toxic mode of action or that the more planar B*a*P disrupts structured membranes to a lesser extent.

While high molecular weight PAHs are not acutely toxic in single solute exposures, they can contribute to narcosis in mixtures [31,32]. However, PAHs with higher solubility can display toxicity. Replicate experiments with pyrene and *Diporeia* [33] and with fluoranthene and *Leptocheirus plumulosus* [34] provided LR50 estimates of 6.3 and 9.4 and of 0.7 and 2.4 μmol/g wet tissue, respectively.

Because PAHs can be metabolized by many organisms, body burden analysis should consider concentrations of both metabolites and parent compound. The differential ability to metabolize PAHs among and within taxa has been well demonstrated [35,36; A.E. McElroy, unpublished data]. Even between different amphipod species, clear differences have been observed. For example, *Diporeia* does not significantly metabolize PAHs, while *Hyalella azteca* does [10]. The marine amphipod *Rhepoxynius abronius* is capable of significant B*a*P metabolism (70% of parent converted to metabolites), while *Eohaustorius washingtonianus* is much less efficient (27% of parent converted to metabolites) [37]. The present study demonstrates that *A. abdita* has very limited ability to metabolize B*a*P and B*a*A (<13% of parent converted to metabolites). Metabolic ability is an important consideration in CBR research because the acute toxicity of PAH metabolites is unknown. If some PAH metabolites are acutely toxic and an organism has the ability to produce significant amounts of metabolites (as many do), toxic body residues (parent and metabolites) could be achieved, even with less soluble but metabolizable PAHs. Because metabolites may not be acting via narcosis, determination of a species' metabolic ability is also important to ensure that the CBR approach is appropriate in each scenario.

An attempt was made to assess the use of the CBR approach for mixtures by exposing *A. abdita* to binary mixtures of the compounds tested in this study. Because narcotics are thought to act in an additive fashion for acute toxicity [7,31,32,38,39], it was expected that the narcotic lethal body burdens of the binary mixtures would be equivalent to the individually determined lethal body burdens. Unfortunately, the confidence intervals around these mixture LR50s are quite large and no LR50 could be determined for B*a*P or B*a*A. Therefore, we cannot really make any conclusions about additivity from our data. More complex mixtures should be used to assess the toxic effects of multiple compounds because they are more environmentally realistic and should produce more consistent results.

Most work on narcosis has focused on chemicals that are smaller than the target chemicals examined in this study. It is possible that the shape and number of degrees of freedom (i.e.,

flexible carbon-carbon sigma bonds) of narcotic chemicals affects the ability of membranes to accommodate them at different concentrations before changes in membrane fluidity/function occur. More work is needed to determine factors that affect both interspecies sensitivity to narcotic chemicals and mechanisms that might lead to significant differences in CBRs for different chemical classes for which acute toxicity is apparently controlled by nonspecific narcosis.

The lipid- and sediment organic carbon-normalized bioaccumulation factors observed for *A. abdita* with the compounds in this study (Table 2) are consistent with other BSAFs reported in the literature for similar compounds and species despite the relatively short period of exposure. We observed 10-d BSAFs of 0.126 (±0.095 SD) and 0.147 (±0.071 SD) for B*a*A and B*a*P, respectively. Ferguson and Chandler [40] found BSAFs using field-collected *Streblospio benedicti* of 0.13 to 0.30 for B*a*A and 0.08 to 0.14 for B*a*P, while Meador et al. [41] observed 10-d BSAFs of 0.2 and 0.25 for B*a*A and B*a*P, respectively, in the polychaete *Armandia brevis*. Amphipods (*Diporeia* and *Hyalella*) have displayed 17-d BSAFs of 0.05 to 0.75 for fluoranthene [42]. For 2,2′,4,4′-tetrachlorobiphenyl, we observed a BSAF of 0.42 (±0.29 SD) with *A. abdita*. This is similar to the BSAFs of 0.31 [43] for 2,2′,4,4′-tetrachlorobiphenyl and 0.21 to 0.42 [44] for 2,4,5,2′,4,′5′-hexachlorobiphenyl for the polychaete *Nephtys incisa*. However, the BSAF for 2,4,2′,4′-tetrachlorobiphenyl determined for the deposit-feeding bivalve *Yoldia limatula* in that study was significantly higher, between 0.9 and 3 [44]. The BSAF for nonylphenol was 0.93 (±0.45 SD) in this study and, to our knowledge, is the first BSAF reported for this compound.

The work completed in this study represents a significant first step in assessing critical body residues in *A. abdita*. However, additional CBR research should be conducted with *A. abdita* due to this organism's environmental and economic importance. First, the CBR estimate for individual narcotic chemicals in *A. abdita* should be better constrained through laboratory exposures with additional compounds of known mode of toxic action. Second, the additive toxicity of sediment-associated mixtures should be further tested by exposing *A. abdita* to more complex mixtures. Data obtained from complex mixture experiments could provide a better estimate of how well the additive narcosis model can predict the toxicity of mixtures. Body residue analysis of amphipods exposed to contaminated sediments from the field would provide a more environmentally realistic exposure to mixtures and generate CBRs more applicable to risk assessment of contaminated sediments. Finally, body burdens in field-collected *A. abdita* should be analyzed. Comparisons between laboratory-determined CBRs for single compounds and body burdens of contaminant mixtures accumulated in the field should indicate whether contaminated sediments are likely to produce acute toxicity in test organisms and which contaminants are likely to contribute most to toxicity. Comparisons between laboratory-derived CBRs and accumulated body burdens of narcotic chemicals in field-collected organisms should indicate how close sediments are to toxic levels at a particular site. Consideration should be given to experimental and field conditions when comparing laboratory-determined CBRs and body burdens in field-collected organisms [45]. Ultimately, species-specific CBR measurements could be used to derive sediment and tissue quality criteria [7]. If body residue-effect relationships can be better defined for this species, the ability to interpret existing data and predict potential adverse impacts in advance will be significantly improved.

CONCLUSIONS

This study represents the first CBR measurements for the important marine species *A. abdita*. The CBR values observed here for 2,2′,4,4′-tetrachlorobiphenyl and nonylphenol are near the low end of those predicted for acute narcotic toxicity. This is consistent with *A. abdita* being a sensitive species for acute toxicity in sediment toxicity tests. In addition, the nonylphenol CBRs observed in this study indicate that this compound's mode of acute toxicity is likely narcosis. The microextraction technique used here allows analysis of extremely small samples, facilitating the use of CBRs as additional and accessible endpoints for inclusion in the small animal, standard toxicity tests currently in use. The toxicological significance of body residues in field organisms can be assessed by comparison to laboratory-measured CBRs, complementing more conventional approaches for risk assessment of contaminated sediments.

Acknowledgement—This project was supported by the Hudson River Foundation, New York, NY, USA. We are grateful to Kay Ho for demonstrating and providing technical advice for conducting the miniaturized 10-d amphipod toxicity test. This is contribution 1172, Marine Sciences Research Center, Stony Brook, New York, USA.

REFERENCES

1. Ingersoll CG. 1995. Sediment tests. In Rand GM, ed, *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment.* Taylor & Francis, Washington, DC, pp 231–255.
2. U.S. Environmental Protection Agency. 1994. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. Technical Report. Office of Research and Development, Narragansett, RI.
3. Franz DR, Tanacredi JT. 1992. Secondary production of the amphipod *Ampelisca abdita* Mills and its importance in the diet of juvenile winter flounder *Pleuronectes americanus* in Jamaica Bay, New York. *Estuaries* 15:193–203.
4. National Research Council. 1997. *Contaminated Sediments in Ports and Waterways: Cleanup Strategies and Technologies.* National Academy, Washington, DC, USA.
5. McCarty LS, Mackay D, Smith AD, Ozburn GW, Dixon DG. 1992. Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: Neutral narcotic organics. *Environ Toxicol Chem* 11:917–930.
6. Niimi AJ, Kissoon GP. 1994. Evaluation of the critical body burden concept based on inorganic and organic mercury toxicity to rainbow trout (*Oncorhynchus mykiss*). *Arch Environ Contam Toxicol* 26:169–178.
7. McCarty LS, Mackay D. 1993. Enhancing ecotoxicological modeling and assessment. *Environ Sci Technol* 27:1719–1728.
8. Hickie BE, McCarty LS, Dixon DG. 1995. A residue-based toxicokinetic model for pulse-exposure toxicity in aquatic systems. *Environ Toxicol Chem* 14:2187–2197.
9. Mackay D, Puig H, McCarty LS. 1992. An equation describing the time course and variability in uptake and toxicity of narcotic chemicals in fish. *Environ Toxicol Chem* 11:941–951.
10. Kane Driscoll S, Landrum PF. 1997. A comparison of equilibrium partitioning and critical body residue approaches for predicting toxicity of sediment-associated fluoranthene to freshwater amphipods. *Environ Toxicol Chem* 16:2179–2186.
11. van Wezel AP, Schmitz MGJ, Tielens AGM. 1997. Acetylcholinesterase and ATPase activities in erythrocyte ghosts are not affected by 1,2,4-trichlorobenzene: Implications for toxicity by narcotic chemicals. *Environ Toxicol Chem* 16:2347–2352.
12. Rand GM, Wells PG, McCarty LS. 1995. Introduction to aquatic toxicology. In Rand GM, ed, *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment.* Taylor & Francis, Washington, DC, USA, pp 3–67.
13. van Wezel AP, Opperhuizen A. 1995. Narcosis due to environ-

mental pollutants in aquatic organisms: Residue-based toxicity, mechanisms and membrane burdens. *Crit Rev Toxicol* 25:255–279.
14. Sijm DTHM, Schipper M, Opperhuizen A. 1993. Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. *Environ Toxicol Chem* 12:1117–1127.
15. van Wezel AP, de Vries DAM, Kostense S, Sijm DHTM, Opperhuizen A. 1995. Intraspecies variation in lethal body burdens of narcotic compounds. *Aquat Toxicol* 33:325–342.
16. Di Toro D. 1998. Predicting the toxicity of PAH mixtures in field collected sediments. *Abstracts,* 19th Annual Meeting, Society of Environmental Toxicology and Chemistry, Charlotte, NC, USA, November 13–19, p 26.
17. de Maagd PJG, van de Klundert ICM, van Wezel AP, Opperhuizen A, Sijm DTHM. 1997. Lipid content and time-to-death-dependent lethal body burdens of naphthalene and 1,2,4-trichlorobenzene in fathead minnow (*Pimephales promelas*). *Ecotoxicol Environ Saf* 38:232–237.
18. Albers PH. 1995. Petroleum and individual polycyclic aromatic hydrocarbons. In Hoffman DJ, Rattner BA, Burton GA Jr, Cairns J Jr, eds, *Handbook of Ecotoxicology.* CRC, Boca Raton, FL, USA, pp 330–353.
19. Renner R. 1997. European bans on surfactant trigger transatlantic debate. *Environ Sci Technol* 31:316A–320A.
20. Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP. 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environ Toxicol Chem* 15:194–202.
21. Schüürmann G. 1991. Acute aquatic toxicity of alkyl phenol ethoxylates. *Ecotoxicol Environ Saf* 21:227–233.
22. Herbes S, Allen C. 1983. Lipid quantification of freshwater invertebrates: Method modification for microquantitation. *Can J Fish Aquat Sci* 40:1315–1317.
23. Ho KT, McKinney RA, Kuhn A, Pelletier MC, Burgess RM. 1997. Identification of acute toxicants in New Bedford Harbor sediments. *Environ Toxicol Chem* 16:551–558.
24. Collier T. 1978. Disposition and metabolism of naphthalene in rainbow trout (*Salmo gairdneri*). MS thesis. University of Washington, Seattle, WA, USA.
25. Borgmann U, Norwood WP, Ralph KM. 1990. Chronic toxicity and bioaccumulation of 2,5,2′,5′- and 3,4,3′,4′-tetrachlorobiphenyl and Aroclor® 1242 in the amphipod *Hyalella azteca. Arch Environ Contam Toxicol* 19:558–564.
26. Landrum P. 1998. Comparison among three invertebrate species of the body residue for lethal and sublethal effects. *Abstracts,* 19th Annual Meeting, Society of Environmental Toxicology and Chemistry, Charlotte, NC, USA, November 13–19, p 10.
27. McCarty LS, Mackay D, Smith AD, Ozburn GW, Dixon DG. 1993. Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: Polar narcotic organics. *Ecotox Environ Saf* 25:253–270.
28. van Wezel AP, Punte SS, Opperhuizen A. 1995. Lethal body burden of polar narcotics: Chlorophenols. *Environ Toxicol Chem* 14:1579–1585.
29. Kishino T, Kobayashi K. 1995. Relation between toxicity and accumulation of chlorophenols at various pH, and their absorption mechanisms in fish. *Water Res* 29:431–442.
30. Chiou CT. 1985. Partition coefficients of organic compounds in lipid-water systems and correlations with fish bioconcentration factors. *Environ Sci Technol* 19:57–62.
31. Spehar R, et al. 1998. Chronic exposure of freshwater and saltwater invertebrates to sediment containing a mixture of high I_{ow} PAHs. *Abstracts,* 19th Annual Meeting, Society of Environmental Toxicology and Chemistry, Charlotte, NC, USA, November 13–19, pp 25–26.
32. Landrum PF, Eadie BJ, Faust WR. 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* sp. *Environ Toxicol Chem* 10:35–46.
33. Landrum PF, Dupuis WS, Kukkonen J. 1994. Toxicokinetics and toxicity of sediment-associated pyrene and phenanthrene in *Diporeia* spp.: Examination of equilibrium-partitioning theory and residue-based effects for assessing hazard. *Environ Toxicol Chem* 13:1769–1780.
34. Kane Driscoll SB, Schaffner LC, Dickhut RM. 1998. Toxicokinetics of fluoranthene to the amphipod, *Leptocheirus plumulosus,* in water-only and sediment exposures. *Mar Environ Res* 45:269–284.
35. Livingstone DR. 1991. Organic xenobiotic metabolism in marine invertebrates. In Gilles R, ed, *Advances in Comparative and Environmental Physiology,* Vol 7. Springer-Verlag, Berlin, Germany, pp 46–183.
36. Kane Driscoll S, McElroy AE. 1996. Bioaccumulation and metabolism of benzo[*a*]pyrene in three species of polychaete worms. *Environ Toxicol Chem* 15:1401–1410.
37. Reichert WL, Eberhart BL, Varanasi U. 1985. Exposure of two species of deposit-feeding amphipods to sediment-associated [^{3}H]benzo[a]pyrene: Uptake, metabolism and covalent binding to tissue macromolecules. *Aquat Toxicol* 6:45–56.
38. Swartz R, Schults D, Ozretich R, Lamberson J, Cole F, DeWitt T, Redmond M, Ferraro S. 1995. ΣPAH: A model to predict the toxicity of polynuclear aromatic hydrocarbon mixtures in field-collected sediments. *Environ Toxicol Chem* 14:1977–1987.
39. Verhaar HJM, Busser FJM, Hermens JLM. 1995. Surrogate parameter for the baseline toxicity content of contaminated water: Simulating the bioconcentration of mixtures of pollutants and counting molecules. *Environ Sci Technol* 29:726–734.
40. Ferguson PL, Chandler GT. 1998. A laboratory and field comparison of sediment polycyclic aromatic hydrocarbon bioaccumulation by the cosmopolitan estuarine polychaete *Streblospio benedicti* (Webster). *Mar Environ Res* 45:387–401.
41. Meador JP, Casillas E, Sloan CA, Varanasi U. 1995. Comparative bioaccumulation of polycyclic aromatic hydrocarbons from sediment by two infaunal invertebrates. *Mar Ecol Prog Ser* 123: 107–124.
42. Kane Driscoll S, Harkey GA, Landrum PF. 1997. Accumulation and toxicokinetics of fluoranthene in sediment bioassays with freshwater amphipods. *Environ Toxicol Chem* 16:742–753.
43. Lake JL, Rubinstein NI, Lee H, Lake CA. 1990. Equilibrium partitioning and bioaccumulation of sediment-associated contaminants by infaunal organisms. *Environ Toxicol Chem* 9:1095–1106.
44. Means JC, McElroy AE. 1997. Bioaccumulation of tetrachlorobiphenyl and hexachlorobiphenyl congeners by *Yoldia limatula* and *Nephtys incisa* from bedded sediments: Effects of sediment- and animal-related parameters. *Environ Toxicol Chem* 16:1277–1286.
45. van Wezel AP, Jonker MTO. 1998. Use of the lethal body burden in the risk quantification of field sediments: Influence of temperature and salinity. *Aquat Toxicol* 42:287–300.
46. Schwarzenbach RP, Gschwend PM, Imboden DM. 1993. *Environmental Organic Chemistry.* John Wiley & Sons, New York, NY, USA.
47. Ahel M, Giger W. 1993. Aqueous solubility of alkylphenols and alkylphenol polyethoxylates. *Chemosphere* 26:1461–1470.
48. Ahel M, Giger W. 1993. Partitioning of alkylphenols and alkylphenol polyethoxylates between water and organic solvents. *Chemosphere* 26:1471–1478.