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A FUGACITY APPROACH FOR ASSESSING THE BIOACCUMULATION OF HYDROPHOBIC ORGANIC COMPOUNDS FROM ESTUARINE SEDIMENT

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Abstract—The bioavailability of four sediment-spiked hydrophobic organic contaminants (HOCs; chrysene, benzo[*a*]pyrene, chlordane, and Aroclor 1254) was investigated by comparing bioaccumulation by the amphipod *Corophium colo* with uptake into a thin film of ethylene/vinyl acetate (EVA) copolymer. The EVA thin film is a solid-phase extraction medium previously identified as effective at measuring the bioavailable contaminant fraction in sediment. The present study presents the results of 11 separate treatments in which chemical uptake into EVA closely matched uptake into lipid over 10 d. For all compounds, the concentration in EVA was a good approximation for the concentration in lipid, suggesting that this medium would be an appropriate biomimetic medium for assessing the bioaccumulation of HOCs during risk assessment of contaminated sediment. For chrysene and benzo[*a*]pyrene, limitations on bioaccumulation and toxicity because of low aqueous solubility were observed. The fugacity of the compounds in lipid (f_{lip}) and in the EVA thin film (f_{EVA}) also was determined. The ratio of f_{lip} to f_{EVA} was greater than one for all chemicals, indicating that all chemicals biomagnified over the duration of the exposure and demonstrating the potential for EVA thin-film extraction to assess trophic transfer of HOCs.

Keywords—Bioavailability Solid-phase extraction Fugacity Hydrophobic organic compounds Sediment

INTRODUCTION

Industrialization throughout the last century has resulted in extensive pollution of the aquatic environment by chemicals such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides [1]. These compounds can be persistent, toxic, and bioaccumulative, and they often collectively are termed hydrophobic organic compounds (HOCs). Most HOCs are poorly soluble in water. Therefore, on release into natural bodies of water, these compounds become associated with dissolved and sedimentary organic matter and, ultimately, accumulate in the bottom sediment. Once associated with the sedimentary environment, wildlife are exposed to HOCs as a result of bioaccumulation by sediment-dwelling invertebrates and subsequent transport through the food web [1].

For HOCs to bioaccumulate, they must either exist in the dissolved state or be extracted in the gut by the digestive fluids of the organism [2,3]. Therefore, for a given chemical concentration in sediment, the bioavailable fraction may vary depending on the route of uptake. Recent investigations have revealed that desorption processes in contaminated sediment regulate the size of the bioavailable fraction and that these processes vary with the nature of the sediment and the chemical. With regard to sediment, contaminant desorption is affected by the different sorption affinities of structurally rigid versus amorphous sedimentary organic matter [4,5]; by sequestration by pyrogenic residues, such as sedimentary soot and charcoal [6]; and by deep infiltration within the sediment matrix over long compound–sediment contact times [5,7]. With regard to the chemical, differences in bioavailability have been noted between PAHs and other types of organic compounds, such as PCBs and chlorobenzenes [3,8–10], and between different types of PAHs [11].

Solid-phase extraction techniques can be used to measure the fugacity, or the escaping tendency, of a chemical, and this may be applied in assessing the bioavailability of sediment-associated contaminants [12,13]. A solid-phase extraction medium comprising a thin film of ethylene/vinyl acetate (EVA) copolymer coated to a glass surface has been advocated on the grounds that it has a small mass relative to the environmental medium of interest, a low ratio of surface area to volume, and rapid kinetics [12]. The EVA film has been applied successfully to measure the fugacity of chlorobenzenes and PCBs in biological tissue [12] as well as in spiked and field-contaminated sediment (S.V. Otton. 2004. A method to measure the sorptive capacity of sediment and plankton for selected organochlorines. Masters thesis. Simon Fraser University, Vancouver, BC, Canada) and of phenanthrene spiked into three dissimilar sediments [14].

The aim of the present study was to apply EVA thin-film extraction to assess differences in the physicochemical and ecotoxicological behavior of several HOCs associated with a single sediment. The current work builds on the recent research characterizing the behavior of a single PAH, phenanthrene, associated with several geochemically dissimilar sediments [14]. The present study investigated four hydrophobic chemicals (log K_{OW} = 5.81–6.79), including four- and five-ring PAHs (chrysene and benzo[*a*]pyrene, respectively), an organochlorine pesticide (chlordane), and a commercial mixture of PCB congeners (Aroclor 1254). The behavior of the chemicals was assessed by comparing uptake into, first, the EVA thin film and, second, the tissue of the Australian infaunal amphipod *Corophium colo*. This species is present at densities of

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Table 1. Hydrophobicity (log K_{OW}), fugacity capacity in ethylene/vinyl acetate (log Z_{EVA}) and lipid (log Z_{lip}), solubility (log S), triolein–water partitioning coefficient (log K_{TW}), and predicted maximum achievable tissue concentration for each chemical

	log K_{OW}	log Z_{EVA} (mol/m³/Pa)[a]	log Z_{lip} (mol/m³/Pa)[a]	log S (mol/L)	log K_{TW}[b]	Predicted maximum tissue residue (μmol/g/lipid)
Chrysene	5.8	7.9	7.1	−8.1	5.8	5.5
Benzo[*a*]pyrene	6.1	8.1	7.4	−8.2	6.1	7.7
Aroclor 1254[c]	7.0	6.6	6.1	−8.0	6.7	49
Chlordane	6.2	6.1	5.5	−6.9	6.2	200

[a] See *Supplemental Data*.
[b] (μmol/L)/(μmol/L), estimated from Chiou [25].
[c] Sigma-Aldrich (Sydney, NSW, Australia).

up to 9,000 individuals/m² in fresh and estuarine environments along the New South Wales (Australia) coast. An amphipod species was selected, because these organisms are subject to exposure from the aqueous phase and from ingested sediment and because several species have limited capacity to biotransform PAHs [15–17]. Therefore, given that amphipods also constitute a major food source for fish and birds, these organisms are key vectors for the mobilization of organic contaminants into the food web. Three objectives were identified for these experiments: First, to gain knowledge about the processes controlling the bioavailability of HOCs by comparing uptake into biological tissue and EVA film; second, to assess the utility of fugacity, as measured using the EVA thin-film technique, as an aid in assessing HOC bioavailability and potential for biomagnification; and third, to assess the ability of the EVA thin film as a biomimetic medium to describe HOC bioavailability accurately in laboratory-spiked and field-contaminated sediments.

MATERIALS AND METHODS

Theory

The purpose of thin-film extraction is to determine the fugacity of the test chemical in sediments by measuring its concentration in the EVA thin film. At apparent equilibrium, the fugacity (Pa) of the chemical in the thin film (f_E) equals that in the sediment (f_S):

$$f_S = f_E \quad (1)$$

Here, f_E is determined from the measured concentration (mol/m³) of the chemical in the thin film (C_E) as C_E/Z_E, where Z_E is the fugacity capacity (mol/m³/Pa) of the thin film for the test chemical. The Z_E value can be determined by various means [12]. In the present study, we determined used Z_E values by measuring the EVA–octanol partition coefficient (K_{EO}) and then determined the EVA–air partition coefficient (K_{EA}) from the product of K_{EO} and the octanol–air partition coefficient (K_{OA}; i.e., $K_{EA} = K_{EO}K_{OA}$) [14]. Because fugacity is, by definition, the partial pressure (P) of a compound in a given medium, the compound's gaseous-phase fugacity (f_A) and, therefore, Z value (Z_A), can be determined in such a system from the universal gas law:

$$P = \frac{n}{V}RT = C_A RT = f_A \quad (2)$$

where n is mass, V is volume, R is the gas constant (8.314 J/mol/K), and T is the temperature in Kelvin.

Because K_{EA} or C_E/C_A equals Z_E/Z_A, Z_E can be determined as K_{EA}/RT, and the fugacity of the chemical in the sediment can then be determined from the chemical concentration in the thin film as

$$f_S = C_E RT/K_{EA} \quad (3)$$

The purpose of the simultaneous exposure of thin films and biota to spiked sediments was to test whether the concentration and fugacity of the test chemical in the organism (specifically, in the lipid of the organism) are correlated to the fugacity in the thin films. The fugacity of the chemical in the organism's lipid (f_{lip}) was determined from the concentration in the organism as

$$f_{lip} = C_{lip}/Z_{lip} \quad (4)$$

where C_{lip} is the concentration of the chemical in amphipod lipid and Z_{lip} is the fugacity capacity of the organism's lipid for the chemical. The Z_{lip} value was assumed to approximate the fugacity capacity of octanol (Z_O), which was derived as K_{OA}/RT. The method for deriving Z_E and Z_O values has been presented elsewhere [14]. The derivation of values used in the present study is presented in the *Supplemental Data* (http://dx.doi.org/10.1897/07-457.S1), and the values themselves are provided in Table 1.

Sediments and reagents

Uncontaminated sediment was sampled in bulk from a location in the marine zone of Lake Conjola, a barrier estuary on the southeastern Australian coast. The physicochemical characteristics and contamination status of this sediment have been explored extensively using solid-state [^{13}C]nuclear magnetic resonance spectroscopy [18,19] and gas chromatography–mass spectrometry [14]. This sediment consisted of 5.23% total sedimentary organic carbon (SOC) and 93.5% mud (particle size, <63 μm) [18] and was nontoxic in previous bioaccumulation experiments [20]. Oxic (upper 2 cm) sediment was sampled using a 20- × 20-cm Birge Ekman grab deployed over the side of a boat and passed through a stainless-steel sieve (mesh size, 1.1 mm). After overlying water was removed, the sediment had a moisture content of 23%. Sediments were returned to the laboratory in 20-L polycarbonate buckets with minimal headspace and then stored at 4°C until spiking.

Benzo[*a*]pyrene, chrysene, Aroclor 1254, technical chlordane (purity, >98% for all), and Sudan IV® dye were purchased from Sigma-Aldrich (Sydney, NSW, Australia). Aroclor 1254 is a commercial mixture of 77 PCB congeners with an average of 54% chlorine by weight. Technical chlordane is a mixture of approximately 40 cyclodiene compounds within which *trans*-chlordane, *cis*-chlordane, and to a lesser extent, heptachlor dominate (on a mass basis). Ethylene/vinyl acetate

(28% vinyl acetate) copolymer and all solvents (high-performance liquid chromatography grade; purity, >99%) were purchased from Bacto Laboratories (Sydney, NSW, Australia).

Sediment spiking

Each of the four contaminants was the subject of separate experiments. The contaminant was spiked into Lake Conjola sediment at several concentrations: 0.19, 0.70, and 1.4 μmol/g dry weight for benzo[*a*]pyrene; 0.19 and 0.70 μmol/g dry weight for chrysene; 0.012, 0.037, and 0.12 μmol/g dry weight for chlordane; and 0.10, 0.38, and 0.77 μmol/g dry weight for Aroclor 1254. Molecular weights of 228.3, 252.32, 409.78, and 326.44 g/mol, respectively, were assumed from the U.S. Environment Protection Agency Dermal Permeability Coefficient Program (DERMWIN) database values (http://www.epa.gov/opptintr/exposure/pubs/episuite.htm). Spiking concentrations were selected on the basis of screening tests in which concentrations to induce low (<10% mortality), medium (~50% mortality), and high (>90% mortality) toxicity to the amphipod *C. colo* were conducted. Spiking followed the jar-rolling method described by Golding et al. [14]. Briefly, 1.2 to 11.7 ml of concentrated HOC in solvent were delivered into several open-mouth, 1-L jars, and this volume was topped up to 13 ml with acetone. For benzo[*a*]pyrene, chlordane, and Aroclor 1254, acetone was used in spiking, whereas toluene was used for chrysene, which is poorly soluble in acetone. For control treatments, pure acetone or toluene only was delivered into the jars. Glassware had been cleaned previously with phosphate-free detergent (Extran MA03®; Merck, Darmstadt, Germany), acetone, and nitric acid. The jars were placed, open, on a specially built rolling mill and rotated at 15 rpm until the acetone had evaporated to leave a uniform film of the HOC on the inner surface of the glass. Wet sediment (670–800 g) was then added as appropriate to achieve the dry-weight concentration corresponding to the added mass of the HOC. The jars were sealed with Teflon®-lined lids and returned to the rolling mill for 16 h over 2 d. Then, sediments were returned to 4°C for 28 d. This is a duration that has been identified in previous sorption studies as sufficient for spiked HOCs to establish apparent equilibrium between sedimentary organic matter and pore water (i.e., changes in solution-phase solute concentrations too small to measure over a reasonable period) [7,21,22].

Amphipod bioaccumulation experiments

Filtered seawater (salinity, ~33‰) pumped from a depth of 8 m outside the mouth of Port Hacking (NSW, Australia) was used in bioaccumulation experiments. Seawater was diluted in the laboratory to a salinity of 15 ± 0.1‰ (mean ± standard deviation throughout) using water from the Sydney (NSW, Australia) water mains. Two weeks before the beginning of the bioaccumulation experiments, 300 Australian infaunal amphipods (*C. colo*) were collected from a vertical wall of an intertidal creek feeding into the upper Hawkesbury River (NSW, Australia) and acclimated in the laboratory at 20 ± 1°C according to the methodology of the American Society for Testing and Materials guidelines [23]. Amphipods had moisture and lipid contents of 82.5 ± 2.2% and 0.54 ± 0.09% wet weight (3.04 ± 0.49% dry wt), respectively. Lipid contents were determined gravimetrically as described elsewhere [14]. Amphipods were exposed to contaminated sediment for durations of 6, 12, 24, 48, 120, and 240 h. This duration is typical for the common bioassay involving this species [23], and it was selected to match the duration of the fast-equilibrating EVA thin-film experiments.

Twelve hours before the beginning of the exposure, the sediment for each concentration was mixed thoroughly. A mass of 200 g of wet sediment was distributed into fresh, 1-L jars that were then topped up with approximately 800 ml of 15‰ seawater and allowed to settle. One jar was prepared for each time point with the exception of the 240-h time point, for which four jars were prepared to ensure sufficient tissue for analysis at the end of the experiment. Amphipods were sieved carefully from the acclimation trays, and 10 to 15 amphipods were added to each test chamber. The jars were sealed with lids, aerated using aquarium pumps (dissolved oxygen was maintained at >80%), and kept at constant illumination (maximum light intensity, 350 lux) until the end of the exposure period. Temperature was maintained at 20 ± 1°C, and no substantial increase in the salinity of overlying water was observed.

Surviving amphipods were removed from the test chambers and allowed to clear ingested sediment in 200 ml of test water for 6 to 8 h. Organisms were blotted dry, placed in polypropylene conical vials, and stored in the freezer at approximately −20°C until extraction. Dead amphipods were discarded without being extracted, because the exact time of death for these individuals could not be identified. Frozen tissue from at least three individuals was weighed using an AD-4 autobalance (PerkinElmer, Melbourne, VIC, Australia) before and after lyophilization using a Freezone® six freeze-drier (Labconco, Kansas City, MO, USA). It was necessary to pool all surviving amphipods from each treatment to ensure that tissue residues were above the detection limits for tissue extracts and to minimize the impact on the organisms' field-collection site (at present, it is not possible to culture *C. colo* in the laboratory [S. McCready, University of Sydney, NSW, Australia, personal communication]) To determine analytical reproducibility, the surviving amphipods exposed to the lowest HOC concentration for 10 d were divided into four subsamples and extracted and analyzed separately. Analyses of blank samples also were conducted.

Amphipods were extracted using a microextraction technique [17], with sample sizes between 5 and 100 mg dry weight. Samples and approximately 150 glass beads (diameter, 1 mm; Extech, Melbourne, VIC, Australia) were homogenized in 1.5 ml of polypropylene conical vials (Extech) for 5 min at 2,800 rpm using a Disruptor Genie® tissue homogenizer (Lomb, Sydney, NSW, Australia). Then, 400 μl of 4 N NaOH, 100 μl of methanol, and 500 μl of iso-octane (spiked with surrogate) were added, and samples were homogenized for another 20 min. Vials were centrifuged at 4,000 rpm for 20 min at 10°C, after which the iso-octane layer was removed. A further 500 μl of ethyl acetate and 130 μl of 12 N HCl were added to the vials, which were then homogenized and centrifuged as described before. The ethyl acetate layer was then added to the iso-octane, and the combined extract was exchanged to iso-octane under N_2.

Tissue extracts were analyzed by the accredited commercial laboratory Australian Laboratory Services (Sydney, NSW) following the U.S. Environmental Protection Agency SW846-8270B methodology [24]. For PAHs, extracts were analyzed by gas chromatography–mass spectrometry in the selected ion-monitoring mode using a HP6890 gas chromatograph (Hewlett-Packard Australia, Melbourne, VIC), a HP5973 mass spectrometer (Hewlett-Packard Australia), and a Chrompack CP-SIL gas chromatographic column (Varian, Melbourne, VIC,

Australia). For organochlorines, extracts were analyzed by dual-column gas chromatography–microcell electron-capture detection using a HP6890 gas chromatograph fitted with Gerstel 2PTV injector (Lasersan, Brisbane, QLD, Australia) and with large-volume injections (25 μl). The gas chromatographic columns were Agilent DB35ms and DB1701 fused silica capillary columns (length, 30 m; internal diameter, 0.32 mm; film thickness, 25 μm, Agilent Technologies, Melbourne, VIC, Australia). The PCB extracts were analyzed by capillary gas chromatography–mass spectrometry, and sample components were quantified against a single congener for each group of PCB homologues. The total PCB concentration is the sum of the homologue components. The compounds detected were compared to standard profiles of the commercially available Aroclors. Initial linear calibrations were conducted with five to six calibration standards. Quantitation was conducted using the primary ions for each compound, and two to three secondary ions were used for qualitative confirmation. Laboratory quality control for extract analyses consisted of a laboratory control sample analyzed in duplicate and a preparation blank per batch of samples. For the laboratory control samples analyzed for PAHs, organochlorines, and PCBs, mean recoveries were 90 to 100, 74 to 104, and 94%, respectively. No preparation blank samples had detectable contaminant concentrations. Internal standards were added to all sample extracts and calibration standards. Calibration check standards were run daily to verify that responses did not vary by more than 25%, retention times by more than 0.1 min, and internal standard responses by more than 50% from the initial calibration. Standard recovery in tissue extracts was 96 ± 4%, 86 ± 9%, and 103 ± 13% for chrysene, benzo[*a*]pyrene, and Aroclor 1254, respectively. Precision was 8, 16, 4, and 22% relative standard deviation for chrysene, benzo[*a*]pyrene, chlordane, and Aroclor 1254, respectively, for the treatments analyzed in quadruplicate. Detection limits were 0.05 to 0.1 μg/L extract for PAHs and PCBs and 0.005 to 0.01μg/L extract for organochlorines.

Thin-film extractions

Before the thin-film extractions, cylindrical glass scintillation vials (volume, 21 ml; diameter, 2 cm; Extech; see Golding et al. [14] for an image) were cleaned with phosphate-free detergent, with hexane, and twice with ultrapure water and then placed upside-down to dry. A solution of 6.1 g/L of EVA (28% vinyl acetate; density, 0.93 kg/L; Bacto Laboratories) in dichloromethane was prepared, with approximately 10 mg of Sudan IV dye (Sigma-Aldrich) added to the solution. Then, 150 μl (or 0.92 mg) of EVA were added to each vial using an adjustable pipette (Eppendorf South Pacific, Sydney, NSW, Australia). Vials then were rotated manually until the dichloromethane had evaporated, leaving a thin film (thickness, 0.05 μm) [12,14]. The uniformity of the EVA coating was determined by visual observation regarding the distribution of the dye [14]. After each vial had been coated, a final hexane rinse was performed to remove the dye and other contaminants. Vials were capped with aluminum-lined lids and stored for no more than 3 d before extraction.

Thin-film extractions involved adding contaminated sediments to thin film–coated vials at the beginning of the bioaccumulation experiment and sampling the film after 5 and 30 min as well as 1, 6, 12, 24, 48, 120, and 240 h of exposure. Uptake of contaminants from unspiked control sediment was tested only after 240 h. To sample the films, sediment was removed from the vials, after which the vials were washed with reverse-osmosis water to remove any particulate matter left in the vial. Each vial was then centrifuged for 2 min at 3,000 rpm in a Jouan CR412 centrifuge (Thermo Scientific, Milford, MA, USA) to remove leftover water. The HOCs were extracted from the EVA by adding 500 μl of hexane, capping the vial tightly, and then mixing for 20 s using a vortex mixer (Selby Scientific, Sydney, NSW, Australia). The hexane was then removed using a fine-tipped Pasteur pipette and placed in a 2-ml gas chromatographic autosampler vial (Agilent Technologies). To ensure that concentrations exceeded the analytical detection limits, extracts from the three vials prepared for each concentration were combined. The combined EVA extracts were reduced to 500 μl under N_2 and analyzed as described for tissue extracts. Therefore, whereas concentrations reported in the present study each represented three replicates, it was not possible to determine standard deviations for each data point. To determine analytical precision, thin-film extractions of 12 individual, 120 h–exposed, medium-concentration sediments were performed. Thin-film extracts of three samples were pooled to produce four independent thin-film extracts for analysis. Precision generally was high, at 6, 3, 14, and 30% relative standard deviation for chrysene, benzo[*a*]pyrene, chlordane, and Aroclor 1254, respectively. The EVA experiments ultimately were compared with tissue concentrations to explore bioavailability, but EVA measurements do not reflect any changes to HOC bioavailability because of organism burrowing behavior or to the addition of overlying water to the bioassay chambers.

Data analysis

The uptake of HOCs into EVA was analyzed using nonlinear regression (Graphpad Prism® 4.03; GraphPad Software, San Diego, CA, USA). The thin-film data were fit to a one-compartment, first-order kinetic uptake model:

$$C_E = C_{Eq}(1 - e^{-kt}) \quad (5)$$

where C_{Eq} is the film concentration at apparent equilibrium and k is the first-order uptake rate constant (1/h). The nonlinear correlation coefficient (R^2) was used to indicate the quality of fit.

Biota–sediment accumulation factors (BSAFs) were determined as

$$\text{BSAF} = \frac{C_B p_{OC}}{C_S p_{lip}} \quad (6)$$

where C_B is the concentration in the organism at 10 d (μmol/g dry wt), C_S is the concentration in the sediment (μmol/g dry wt), and p_{OC} and p_{lip} are the proportions (g/g) of organic carbon and lipid, respectively. To compare C_{lip} with C_E, C_{lip} was converted into volumetric units by multiplying by 0.827 g/cm^3 (i.e., the density of 1-octanol at 20°C).

RESULTS AND DISCUSSION

HOC toxicity

In each experiment, amphipods began burrowing immediately on addition to the test chamber. No differences in burrowing behavior were observed between the control and different sediment concentrations for each HOC or between any of the different test compounds. For all experiments, HOC tissue concentrations in amphipods exposed to control treatments were below the detection limits, and control mortality

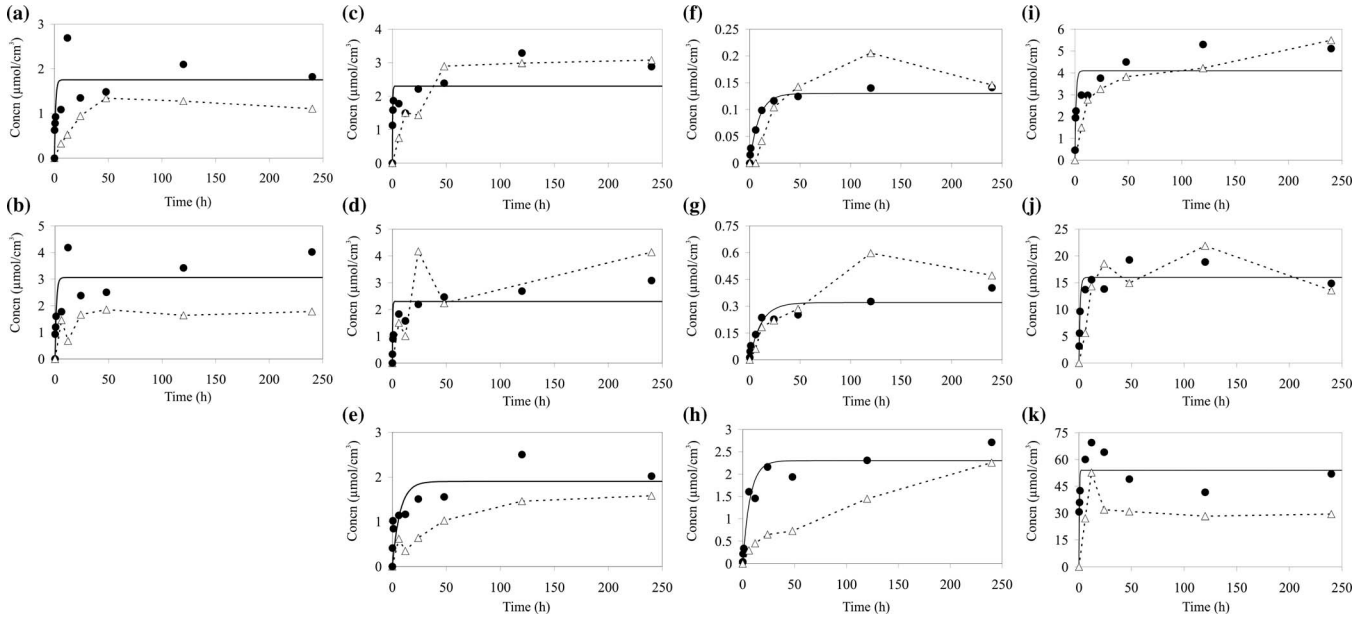

Fig. 1. Uptake of hydrophobic organic compounds into amphipod tissue (ρ) and the ethylene/vinyl acetate thin film (●) over time. Sediment concentrations increase from top to bottom for chrysene (**a** and **b**), benzo[*a*]pyrene (**c–e**), chlordane (**f–h**) and Aroclor 1254 (**i–k**; Sigma-Aldrich, Sydney, NSW, Australia). The solid line represents the uptake of the modeled hydrophobic organic compounds into ethylene/vinyl acetate (according to Eqn. 5), whereas the tissue data points (not modeled) are joined by the broken line to aid in interpretation.

was less than 20%. An increase in toxicity with sediment dose was observed for both chlordane and Aroclor 1254. For chlordane, mortality (mean ± standard deviation) at 240 h was 7.5 ± 10%, 37 ± 21%, and 80 ± 14% for low, medium, and high treatments, respectively. Corresponding values for Aroclor 1254 were 19 ± 8%, 52 ± 22%, and 72 ± 21%, respectively. Notably, all treatments involving the two hydrophobic PAHs did not exhibit toxicity significantly different from the control treatments.

The lack of any observed acute toxicity for either of the PAHs, at any exposure concentration, was explored by determining maximum achievable lipid-normalized tissue concentrations from the molar water solubility and the lipid–water distribution coefficient (Table 1). The triolein–water distribution coefficient (K_{TW}; triolein is a model lipid that shares structural similarities with triglycerides in organisms) was estimated from K_{OW} as described by Chiou [25]:

$$\log K_{TW} = 0.893(\log K_{OW}) + 0.607 \quad (7)$$

The maximum achievable concentrations for chrysene and benzo[*a*]pyrene approximate the highest observed tissue concentrations measured in the present study (Fig. 1a–e). In contrast, observed tissue concentrations of chlordane and Aroclor 1254 were below the maximum achievable concentrations. Therefore, the data suggest that the toxicity of benzo[*a*]pyrene and chrysene was, at least in part, limited by solubility, whereas the toxicity of chlordane and Aroclor 1254 was not. An important feature of this result is that the maximum achievable lipid concentrations for the PAHs are below the critical body residue of 40 to 160 μmol/g lipid identified for narcosis, the baseline mode of action by unmetabolized PAHs [17,26]. This finding is consistent with the results of screening tests in which benzo[*a*]pyrene concentrations as high as 3.2 μmol/g dry weight did not induce toxicity to *C. colo* (data not shown). Solubility-related absence of toxicity has been noted elsewhere for benzo[*a*]pyrene [17,27] and for high-molecular-weight homologues of other groups of chemicals, such as *n*-alcohols [26] and chlorobenzenes [28]. Fay et al. [17] explained this phenomenon as being behavior unique to very lipophilic compounds (log $K_{OW} > 5.5$) that are solids at environmentally relevant temperatures. For these compounds, the heat of fusion increases with hydrophobicity, resulting in a decrease in solubility without affecting lipid–water partitioning. Fay et al. [17] and others [25,27] also have suggested that lower bioaccumulation by such compounds results from an increasing incompatibility in lipid phases and membranes with increasing solute size. The inference of this result is that biota are at limited risk from toxicity caused by high-molecular-weight PAHs, but rarely do these compounds exist in the aquatic environment as single solutes. Contaminants such as PAHs generally are present as mixtures, and the components of those mixtures exhibit additive toxicity [29]. High-molecular-weight PAHs present at low environmental concentrations can still contribute toward toxicity in combination with other mixture PAHs.

HOC bioaccumulation

Figure 1 illustrates the bioaccumulation of HOCs over 10 d, whereas Table 2 presents the BSAF at 10 d. A generally asymptotic uptake pattern was observed for benzo[*a*]pyrene, chrysene, and chlordane. This profile is similar to those for the same PAHs in other species of amphipods [16,30,31] and other invertebrates [2,10]. For Aroclor 1254, the uptake profiles were marked by an early maximum in tissue concentration at approximately 12 to 24 h. This feature was most prominent in the high and medium treatments (Fig. 1j and k, respectively). This feature was notable, because it also was present in corresponding EVA data (described below).

Bioaccumulation and fugacity

For chrysene and benzo[*a*]pyrene, BSAFs declined with increasing solute concentration. This indicates that the potential for bioaccumulation decreased at higher doses. It is possible that this result is caused by increased enzyme induction

Table 2. Concentration of each chemical in sediment (C_{sed}), biota–sediment accumulation factor at 10 d ($BSAF_{10d}$), modeled concentration in ethylene/vinyl acetate (EVA) at apparent equilibrium (C_{eq}) and uptake constant (k), nonlinear correlation coefficient (r^2), fugacity in EVA (f_{EVA}) and lipid (f_{lip}), and the fugacity ratio (f_{EVA}/f_{lip})

		C_{sed} (μmol/g)	$BSAF_{10d}$	C_{eq} (μmol/cm³)[a]	k (1/h)[a]	r^2	f_{EVA} (μPa)[b]	f_{lip} (μPa)[b]	f_{EVA}/f_{lip}[b]
Chrysene	Low	0.19	0.38	1.8 (0.20)	0.96 (0.59)	0.64	0.025 ± 0.00067	0.099 ± 0.0026	3.9 ± 0.15
	Medium	0.70	0.16	3.1 (0.33)	0.83 (0.50)	0.69	0.044 ± 0.0012	0.16 ± 0.0042	3.6 ± 0.14
B[*a*]P	Low	0.19	1.1	2.3 (0.23)	3.3 (2.3)	0.64	0.018 ± 0.00060	0.13 ± 0.0044	7.1 ± 0.23
	Medium	0.70	0.37	2.3 (0.19)	0.69 (0.32)	0.83	0.018 ± 0.00060	0.17 ± 0.0059	9.7 ± 0.32
	High	1.4	0.07	1.9 (0.27)	0.14 (0.088)	0.58	0.015 ± 0.00050	0.066 ± 0.0022	4.5 ± 0.17
Chlordane	Low	0.012	0.71	0.13 (0.0044)	0.11 (0.014)	0.98	0.11 ± 0.0080	0.45 ± 0.033	4.1 ± 0.39
	Medium	0.037	0.71	0.32 (0.028)	0.089 (0.029)	0.89	0.27 ± 0.020	1.5 ± 0.11	5.4 ± 0.28
	High	0.12	1.1	2.3 (0.14)	0.14 (0.037)	0.94	1.9 ± 0.14	7.0 ± 0.51	3.6 ± 0.17
Aroclor 1254[c]	Low	0.10	3.0	4.1 (0.34)	0.96 (0.42)	0.82	1.1 ± 0.046	4.4 ± 0.18	3.9 ± 0.18
	Medium	0.38	1.9	16 (0.85)	0.92 (0.26)	0.91	4.4 ± 0.18	11 ± 0.44	2.4 ± 0.14
	High	0.77	2.0	54 (4.5)	3.1 (1.7)	0.71	15 ± 0.60	23 ± 0.95	1.6 ± 0.090

[a] Values in parentheses are standard errors.
[b] Values are presented as the mean ± standard deviation.
[c] Sigma-Aldrich (Sydney, NSW, Australia).

at higher PAH concentrations in sediment, leading to greater biotransformation and lower BSAFs [32]. No effect of dose on the BSAF was observed for chlordane or Aroclor 1254, which generally are not metabolized. For the latter chemical, BSAFs were high, but they were not dissimilar to values reported elsewhere of 3.39 (mol/g lipid)/(mol/g SOC) for Aroclor 1254 with earthworms [33] and for hydrophobic PCB congeners with various invertebrates [10,34]. Because PCBs are lipophilic and difficult to metabolize, this group of compounds is especially notorious for bioaccumulation [35]. For chrysene and benzo[*a*]pyrene, BSAFs generally were less than 1 (mol/g lipid)/(mol/g SOC). Values reported in the present study are either within or above the range of BSAFs reported for other amphipod species [36] but are closest to those reported for *Corophium volutator* [16].

The BSAF has been used elsewhere to identify the potential for sediment-associated compounds to biomagnify [37]. In such applications, biomagnification is implied if C_{lip} is greater than the concentration in sedimentary organic carbon (i.e., BSAF > 1). This is correct as long as lipids and SOC exhibit a similar sorptive capacity (i.e., the fugacity capacities of lipids and SOC are the same). Because of the variety in composition and properties of lipids and organic matter in sediments, this is unlikely to occur in most cases. Therefore, it is more appropriate to compare the chemical fugacities in lipids and sediments to determine the equilibrium status of the chemical and the occurrence of biomagnification where $f_{lip} f_{SOC}$. Because f_{SOC} can be measured using the EVA thin-film technique as f_{EVA}, a more insightful measure of the bioavailability and potential for biomagnification of a sediment-associated contaminant may be provided by exploring the ratio of f_{lip} to f_{EVA}. Table 2 shows that in contrast to BSAFs, f_{lip}/f_{EVA} values for all compounds were greater than one. This indicates that biomagnification of these compounds from the sediment occurred. Biomagnification of PAHs through the food web generally is considered to be uncommon, although it can occur in species at lower trophic levels that are not able to effectively metabolize these compounds [38,39]. The latter may, indeed, be the case for *C. colo*. The inconsistency between BSAF and f_{lip}/f_{EVA} observed in the present study is an important finding, and it may suggest that a fugacity approach using the EVA thin film would be an insightful means for assessing trophic transfer of HOCs.

EVA as a biomimetic medium

The EVA uptake curves in Figure 1 show a remarkable similarity to the uptake curves for *C. colo*. The similarity is most prominent for Aroclor 1254, for which the early maximum observed in tissue concentrations in the medium- and high-concentration treatments were mimicked in the EVA concentration data. The similarity between corresponding uptake profiles also was evident for the other compounds. Figure 2 shows HOC concentration in lipid and in EVA for all corresponding time points and describes a linear, approximately 1:1 relationship. Collectively, the relationship between all corresponding tissue and EVA concentrations yielded a correlation coefficient of 0.92 ($n = 65$). This suggests that the concentration in EVA is directly proportional to the concentration in lipid for these compounds. This result indicates that EVA and amphipod lipid are sufficiently similar with respect to structure and composition that the toxicokinetic behavior of the two media are comparable.

As a biomimetic medium, EVA may be a valuable tool in

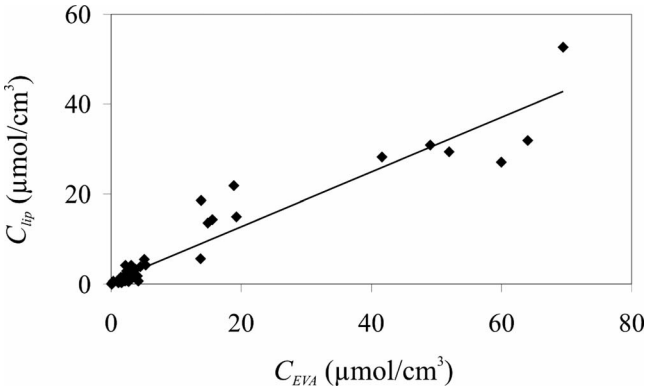

Fig. 2. Relationship between tissue (C_{lip}) and ethylene/vinyl acetate (C_{EVA}) concentrations for all chemicals. Data exclude experiment durations of t = 30, 60, and 120 min, at which times ethylene/vinyl acetate but not tissue concentrations were measured.

residue-based hazard assessments, which in recent years have been argued to be more meaningful than exposure-based approaches (F.A.P.C. Gobas et al., *SETAC Globe,* 2001, 2:33–34). In a residue-based approach, the critical measurement is the chemical concentration at the target site in the organism (e.g., membrane lipid for narcotic compounds) rather than the chemical concentration in the exposure medium (e.g., sediment or water). This approach is argued to be more indicative of the ecological threat presented by an environmental contaminant, because questions of bioavailability are explicitly considered [40]. In this context, because EVA approximates lipid-normalized tissue concentrations of hydrophobic chemicals, this medium may be useful as a substitute for bioassays involving invertebrates or other lower-trophic-level organisms. The EVA technique does have limitations: It is most applicable in exploration of HOCs that are not metabolized and for organisms that have poor metabolic capabilities. Because the EVA film, however, does not mimic physiological and behavioral processes, such as burrow construction or biotransformation, this medium may provide a easy-to-interpret method for predicting bioaccumulation that is not confounded by species-specific factors.

SUPPORTING INFORMATION

Table S1. Literature-averaged log K_{OA} values and measured K_{EO} for 2, 7, and 60 d for polycyclic aromatic hydrocarbons (PAHs), organochlorines, and polychlorinated biphenyls (PCBs).

Table S2. Fugacity capacity of polycyclic aromatic hydrocarbons (PAHs), organochlorine compounds, and polychlorinated biphenyls (PCBs) in ethylene/vinyl acetate (Z_{EVA}) and 1-octanol (Z_{oct}).

Both found at DOI: 10.1897/07-457.S1 (120 KB PDF).

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