

1-1-2006

Application of surrogate methods for assessing the bioavailability of PAHs in sediments to a sediment ingesting bivalve

Stuart L. Simpson
CSIRO, stuart.simpson@csiro.au

Victoria L. Burston
University of Wollongong

Dianne F. Jolley
University of Wollongong, djolley@uow.edu.au

Kim Chau
NSW Department of Environment and Conservation

Follow this and additional works at: <https://ro.uow.edu.au/scipapers>



Part of the [Life Sciences Commons](#), [Physical Sciences and Mathematics Commons](#), and the [Social and Behavioral Sciences Commons](#)

Recommended Citation

Simpson, Stuart L.; Burston, Victoria L.; Jolley, Dianne F.; and Chau, Kim: Application of surrogate methods for assessing the bioavailability of PAHs in sediments to a sediment ingesting bivalve 2006, 2401-2410.
<https://ro.uow.edu.au/scipapers/3706>

Application of surrogate methods for assessing the bioavailability of PAHs in sediments to a sediment ingesting bivalve

Abstract

The usefulness of two surrogate methods for rapidly determining the bioavailability of PAHs in hydrocarbon-contaminated marine sediments was assessed. Comparisons are made between the PAHs accumulated by the benthic bivalve, *Tellina deltoidalis*, and the extractable-PAHs determined using a 6-h XAD-2 resin desorption method and a 4-h gut fluid mimic (GFM) extraction method. There were significant positive relationships between PAH bioaccumulation by the bivalves and sediment PAH concentrations. These relationships were not improved by normalising the sediment PAH concentrations to the organic carbon concentration. The average percentage lipid content of the bivalves was $1.47 \pm 0.22\%$ and BSAFs for total-PAHs ranged from 0.06 to 0.80 (kg OC/kg lipid). The XAD-2 and GFM methods both extracted varying amounts of PAHs from the sediments. Low concentrations of PAHs were extracted by the GFM method (0.2–3.6% of total-PAHs in sediments) and the GFM results were inadequate for generalising about the bioavailability of the PAHs in the sediments. The XAD-2 method extracted greater amounts of PAHs (3–34% of total-PAHs in sediments), however, the total-PAH concentrations in the sediments provided a better, or equally good, prediction of PAH bioaccumulation by *T. deltoidalis*. The results indicated that these methods required further development before they can be applied routinely as surrogate methods for assessing the bioavailability of PAHs in sediments. Future research should be directed towards lowering detection limits and obtaining comparative data for a greater range of sediment types, contaminant classes and concentrations, and organisms of different feeding guilds and with different gut chemistry.

Keywords

Application, surrogate, methods, for, assessing, bioavailability, PAHs, sediments, sediment, ingesting, bivalve, CMMB

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Simpson, S. L., Burston, V. L., Jolley, D. F. & Chau, K. (2006). Application of surrogate methods for assessing the bioavailability of PAHs in sediments to a sediment ingesting bivalve. *Chemosphere*, 65 (11), 2401-2410.

Application of surrogate methods for assessing the bioavailability of PAHs in sediments to a sediment ingesting bivalve

Stuart L. Simpson^{a,*}, Victoria L. Burston^{a,b}, Dianne F. Jolley^b, and Kim Chau^c

^aCentre for Environmental Contaminants Research, CSIRO Energy Technology, Private Mailbag 7, Bangor, NSW 2234, Australia

^bGeoQuEST, Department of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia

^cEnvironmental Forensics and Analytical Science, Department of Environment and Conservation (NSW), PO Box 29, Lidcombe 1825, Australia

Abstract

The usefulness of two surrogate methods for rapidly determining the bioavailability of PAHs in hydrocarbon-contaminated marine sediments was assessed. Comparisons are made between the PAHs accumulated by the benthic bivalve, *Tellina deltoidalis*, and the extractable-PAHs determined using a 6-h XAD-2 resin desorption method and a 4-h gut fluid mimic (GFM) extraction method. There were significant positive relationships between PAH bioaccumulation by the bivalves and sediment PAH concentrations. These relationships were not improved by normalising the sediment PAH concentrations to the organic carbon concentration. The average percentage lipid content of the bivalves was $1.47 \pm 0.22\%$ and BSAFs for total-PAHs ranged from 0.06 to 0.80 (kg OC/kg lipid). The XAD-2 and GFM methods both extracted varying amounts of PAHs from the sediments. Low concentrations of PAHs were extracted by the GFM method (0.2-3.6% of total-PAHs in sediments) and the GFM results were inadequate for generalising about the bioavailability of the PAHs in the sediments. The XAD-2 method extracted greater amounts of PAHs (3-34% of total-PAHs in sediments), however, the total-PAH concentrations in the sediments provided a better, or equally good, prediction of PAH bioaccumulation by *T. deltoidalis*. The results indicated that these methods required further development before they can be applied routinely as surrogate methods for assessing the bioavailability of PAHs in sediments. Future research should be directed towards lowering detection limits and obtaining comparative data for a greater range of sediment types, contaminant classes and concentrations, and organisms of different feeding guilds and with different gut chemistry.

Keywords: Bioaccumulation, PAHs, sediments, XAD-2, gut fluid

Word Count: 7000. Five tables, three figures

1. Introduction

Assessment of the bioavailability and bioaccumulation of hydrophobic organic contaminants (HOCs) (e.g. polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs)) by benthic organisms living in contaminated sediments has generally been undertaken using organisms collected from field sites or by laboratory-based bioaccumulation assays (Moore et al., 2005). Concentrations of HOCs in sediments show a wide variability due to differences in bioavailability that relate to organism physiology and sediment characteristics (Moore et al., 2005; Cornelissen et al., 2005). The bioavailability of HOCs is complicated by the large range of different types of particulate organic carbon (OC) phases to which HOCs may bind (Luthy et al., 1997; Gustafsson et al., 1997; Ahrens and Hickey, 2003; Fredrickson et al., 2004; Cornelissen et al., 2005), the concentrations of these OC phases, and the properties of the HOCs that determine their binding strength and desorption rates (Cornelissen et al., 1997; Van den Heuvel and van Noort, 2005; Kukkonen et al., 2003; Cornelissen et al., 2005).

Traditionally the bioavailability of sediment-bound HOCs has been determined using field-collected organisms or bioaccumulation assays of 2-6 week test duration (Moore et al., 2005). Recently, a number of surrogate (biomimetic) methods have been proposed that may be more rapid and less expensive than traditional methods (MacRae and Hall, 1998; Cornelissen et al., 2001; Lei et al., 2004; Voparil and Mayer, 2004; Vinturella et al., 2004). Semipermeable membrane devices (SPMDs) and solid-phase microextraction fibers have been used mostly for examining HOC in waters (Macrae and Hall, 1998; van der Wal et al., 2004; Stuer-Laurideson, 2005). Leppänen and Kukkonen (2000) demonstrated that small SPMDs buried in sediment spiked with PAHs were useful mimics of PAH uptake by oligochaete worms. Passive samplers constructed from polyethylene (PEDs) have been demonstrated to mimic the uptake of PAHs by benthic polychaetes (Vinturella et al., 2004).

Tenax[®] TA and Amberlite[®] XAD-2 resin desorption methods have been used to examine the lability and bioavailability of sediment-sorbed HOCs (Cornelissen et al., 1997, 2001; Kraaij et al., 2002; ten Hulscher et al., 2003; Kukkonen, et al., 2003; Lei et al., 2004; Moermond et al., 2005; van den Heuvel and van Noort, 2005). HOCs that rapidly desorb from sediments exhibit linear desorption kinetics and are considered to be the bioavailable fraction (Cornelissen et al., 1997; 2001; Kukkonen, et al., 2003; ten Hulscher et al., 2003). Cornelissen et al. (2001) determined that a 6-h Tenax extraction of field-contaminated sediments was about half the rapidly desorbing fraction of PAHs, and could possibly be used to estimate the bioavailable fraction of the sediment. Several researchers have found that biota-sediment accumulation factors (BSAFs) measured for organisms are better explained by the rapidly desorbing fraction of PAHs, as determined using short-duration Tenax extractions, than by total PAH (e.g. Soxhlet extractable) concentrations in sediments (Kraaij et al., 2001, 2002; ten Hulscher et al., 2003; Moermond et al., 2005). These results help explain the variability in BSAFs caused by stronger binding of PAHs by sediment phases (such as black carbon) and challenge the

validity of using equilibrium partitioning models based on total PAH concentrations for prediction of bioaccumulation (Moore et al., 2005).

The solubilization of PAHs from sediments is greater in the digestive fluids of deposit-feeding organisms than in seawater due to surfactant micelles in the digestive fluids that enhance solubility (Weston and Mayer, 1998; Voparil and Mayer, 2000). Because many benthic invertebrates ingest large amounts of sediment, and digestive fluids are retained longer than sediments in their guts, for some organisms, PAH uptake by this exposure pathway may be greater than that from direct body adsorption or water filtration. The bioavailability of particle-associated HOCs to benthic organisms can be estimated using digestive fluid (extracted from organisms) or digestive fluid mimics (Weston and Mayer, 1998; Voparil and Mayer, 2000, 2004; Ahrens and Hickey, 2003; Voparil et al., 2004; Nakajima et al., 2005). However, because the collection of sufficient volumes of gut fluids from organisms is difficult and time consuming, the preparation of synthetic gut fluid mimics (GFM) from commercially available surfactants and enzymes has been proposed (Voparil and Mayer, 2004; Nakajima et al., 2005). Voparil and Mayer (2004) found that the PAHs extracted by a combination of sodium taurochlorate and bovine serum albumin (BSA) correlated well with the PAHs extracted by benthic polychaete, *Arenicola marina*, gut fluids.

With increased emphasis on the use of multiple lines of evidence (LOE) in assessing sediment quality, decision frameworks that include bioaccumulation in combination with other LOEs (chemistry, toxicity) in a weight-of-evidence framework are increasingly being adopted (Chapman et al., 2002; Simpson et al., 2005). This study assesses the usefulness of two surrogate methods as LOE for determining the bioavailability and bioaccumulation of PAHs in marine sediments. Comparisons are made between the fraction of PAHs determined using 6-h XAD-2 and GFM extraction methods and the PAHs accumulated by the benthic bivalve, *Tellina deltoidalis*. The application of the surrogate methods in sediment quality assessment is discussed.

2. Methods

2.1. Waters and sediments

Clean seawater was collected from Port Hacking, Sydney, New South Wales (NSW), Australia, membrane filtered (0.45 µm), and acclimated to a temperature of 21±1°C. Where necessary, the salinity of the filtered seawater was adjusted to the test salinity of 30 ‰ using Milli-Q deionised water (18 MΩ; Milli-Q Academic Water System). Control sediments with negligible contamination were collected from estuarine sites at Bonnet Bay (silty) and Grays Point (sandy), Sydney, Australia, as described previously (King et al., 2004).

Contaminated sediments were collected from two sites in NSW, Australia; the Hunter River, Newcastle, and Duck Creek, Parramatta River, Sydney, with recognised hydrocarbon contamination. Ten surface sediment samples (0-20 cm depth) were collected at individual locations from a boat using

a sediment corer operated by using a 3 m pole. Sediments were homogenised in the field in a stainless steel container using a stainless steel spoon, and 1-2 kg of sediment transferred to a glass jar, then chilled on ice for transport to the laboratory. Within 24 h of collection, sediments were stored in a cool room at 4°C, until analysed or used in tests.

2.2. General methods and reagents

All glass and plasticware for chemical analyses were cleaned by soaking in 10% (v/v) HNO₃ (BDH, Analytical Reagent grade) for a minimum of 24 h followed by thorough rinsing with Milli-Q water. All glassware used for analyses of HOCs and extraction techniques was cleaned by rinsing with nanograde acetone and dichloromethane (DCM) (Suprasolv, Merck) before use. All glass and plasticware used for bioaccumulation tests was cleaned in a dishwasher (Gallay Scientific Pty Ltd) programmed for a phosphate-free detergent wash (Clean A, Gallay Scientific Pty Ltd), a dilute acid wash (1% HNO₃), followed by thorough rinsing with Milli-Q water. Clean sand was prepared by heating sand for 2 h at 400 °C in muffle furnace to remove volatile organics.

Procedures for sediment size fractionation (63 µm sieves), total organic carbon (TOC by high temperature TOC analyser) and speciation of organic carbon into biogenic/diagenetic organic carbon (OC, loss on ignition at 375 °C) and combustion-derived black carbon (BC, loss on ignition at 1050 °C) forms have been described previously (Simpson et al., 2006). Analyses of total petroleum hydrocarbons (TPHs) followed USEPA methods 8260/8015 (USEPA, 1996). Analyses of PAHs were made by gas chromatography-mass spectrometry (GC-MS) using a 6890N Network GC system (Agilent Technologies) with a split/splitless injector, a HP-5MS capillary column (30 m × 250 µm × 0.25 µm) (Agilent Technologies 19091s-4343), 1.3 mL/min flow rate of helium, maximum temperature of 350 °C and a 5973 Network (Agilent Technologies) mass selective detector. The 16 PAHs analysed comprised the six low molecular weight (low-MW) PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene) and the ten high-MW PAHs (fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)-fluoranthene, benzo(k)-fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(ah)anthracene, and benzo(ghi)perylene). The mass spectrometer was operated under both scan mode (USEPA method 1618) (USEPA, 1996), and selected ion monitoring (SIM) mode (USEPA Method 610) (USEPA, 1996). The sediment and gut fluid mimic (GFM) extracts were analysed both in scan and SIM mode, while the bivalves were only analysed in SIM mode and the XAD-2 extracts were only analysed in scan mode. An internal standard containing d12-chrysene and d12-perylene was spiked into each extract immediately before analyses.

2.3. Bivalve bioaccumulation tests

The bivalves, *Tellina deltoidalis*, were collected from estuarine sand and mud flats of Lane Cove River, NSW, Australia (King et al., 2004). Approximately 300 adult bivalves (10-20 mm in length) were collected at low tide from the top 10 cm of sediment using a shovel and a 1 mm mesh stainless steel sieve. The bivalves were placed into plastic containers (16×12×4 cm) with water and a 3 cm

thick layer of sediment, collected in situ, and transported to the laboratory. Bivalves were kept in a temperature-controlled laboratory at 21 ± 2 °C in plastic holding trays (40×30×10 cm, 100 bivalves/tray) containing <1.1 mm sieved sediment to a depth of 3 cm and approximately 5 cm of overlying seawater (30 ‰ salinity). The overlying water in trays was continuously gently aerated and trays were covered with foil to minimise light disturbances to the bivalves. Bivalves were isolated from the holding sediment by gentle sieving through 1.1 mm sieves immediately prior to test commencement.

Bivalve bioaccumulation tests were conducted in 1 L glass beakers for 14 d in an environmental chamber (21 ± 1 °C). Each beaker contained 250 g of homogenised sediment, 600 mL seawater and ten bivalves (randomly added). The beakers had acrylic lids to minimise evaporation of seawater and Milli-Q water was added, as necessary, to maintain the seawater at the original volume and salinity. Dissolved oxygen (DO) concentrations were maintained at >85% saturation (monitored daily) by a gentle bubbling the seawater with air. The salinity of 30 ± 1 ‰ and pH of 8.0 to 8.2 was maintained in the seawater throughout the test. Three times a week ~70% of the seawater was gently removed and replaced with new seawater. Control sediments contained a 50:50 mixture of the silty and sandy control sediments. Replicate tests were conducted on each sediment.

The test was terminated after 14 days and the contents of each beaker were sorted until all bivalves (dead or alive) were recovered. Alive bivalves were washed with Milli-Q water and placed in 1 L beakers containing 800 mL of seawater to depurate for 48 h. After 24 h of depuration, the seawater and all visible faecal matter were removed and new seawater added. The soft tissues of the bivalves were shucked with a stainless steel scalpel. The soft tissue was patted dry with tissue paper (Kimwipes, Kimberley Clark), weighed, and stored in the freezer until analysis (within two weeks of test completion).

Bivalve lipid and moisture analyses were made in triplicate on three composite samples comprising of ~1 g wet bivalve tissue (20 bivalves) which were dried and ground with sufficient sodium sulfate to obtain a free flowing powder. These were added to a cellulose extraction thimble, Soxhlet extracted with a 1:1 acetone:DCM solvent for ~80 cycles. The extracted lipids were weighed after desiccation to determine percentage lipid content. Percent moisture content of bivalve tissues was determined by gravimetry after drying the soft tissue of the bivalves in the oven (110 °C) overnight.

2.4. Analysis of PAHs in sediment, bivalve, XAD-2 and GFM extracts

With each batch of samples, a blank and two quality control (QC) samples were extracted and analysed to determine method detection limits and recoveries of PAHs. The blanks contained Milli-Q water spiked with d10-phenanthrene. The QC samples containing sand for the sediment, XAD-2 and GFM analyses, or bivalve tissues (from control sediments) for bivalve analyses. All samples were extracted and analysed separately in triplicate. The QC samples were spiked with the 10 mg/L QC solution containing the surrogate standard d10-phenanthrene and 16 PAHs.

Extraction and analysis of PAHs from sediments and bivalves followed the USEPA method 3550B (USEPA, 1996). Wet sediment, equivalent to 2.0 g dry weight, was spiked with d10-phenanthrene, then sufficient anhydrous sodium sulfate was mixed with the sediments until the mixture resembled a free flowing powder. This sediment mixture was extracted with 1:1:1 acetone:DCM:hexane (Suprasolv, Merck) by sonication for 3 minutes (Sonic disruptor, Branson 450; ½ inch probe, 50% duty cycle). For each sample, sonication extraction was repeated three times and solvent extracts combined into a turbo evaporation tube. The solvent extracts were concentrated to 5.0 mL in a solvent concentrator at 45 °C (Turbovap ZW640-3, Zymark) and stored at 4° C until analysis.

The frozen soft tissue masses of the bivalves were defrosted, homogenized using a mortar and pestle and, using the method described for the sediment extractions, dried with sodium sulfate, solvent extracted and concentrated to 7 mL. The concentrated extract was centrifuged (5 min at 1500 g) to form a lipid pellet and solvent supernatant. The solvent layer was decanted, made up to 5.0 mL with additional solvent, and combined with silica gel to adsorb polar organic compounds and excess solubilised lipids (USEPA method 3630C). Samples were stored at 4 °C until analysis.

For the XAD-2 extractions, 1.0±0.05 g (dry weight equivalent) of the wet test sediment and 1.0 g of XAD-2 Amberlite® (Supelco, USA) resin were added to 25 mL of Milli-Q water containing mercury(II) chloride (5 mg) and sodium azide (32 mg) (to inhibit microbiological activity) in a 50 mL Pyrex centrifuge tube. Previous studies have found that a 1:1 ratio of XAD-2 to sediment is sufficient for the XAD-2 not to become saturated with PAHs (Lei et al., 2004). Samples were buffered between pH 6-7.5 with a potassium phosphate/sodium hydroxide buffer, spiked with d10-phenanthrene, and then shaken on a rotary shaker (100 rpm) for 6 h in the dark at room temperature. The resin, which was less dense than the sediment, was separated from the sediment by centrifuging at 3000 g for 10 min. The resin was recovered from the surface by vacuum into a pasture pipette (>95% recovery) and transferred to a centrifuge tube. Sufficient sodium sulfate was added to each XAD-2 resin sample to remove residual water before the PAHs were extracted into 10 mL of DCM in an ultrasonic bath (50 Hz) for 30 min. Samples were stored at 4 °C until analysis. All samples were extracted and analysed separately in triplicate.

The GFM method followed that previously described by Voparil et al. (2004). A 2 mL cocktail of 15.6 mM sodium taurocholate and 5 g/L bovine serum albumin was added to 1.0±0.05 g (dry weight equivalent) of the wet test sediment in 50 mL Pyrex centrifuge tubes. Samples were continually mixed in the dark for 4 h on a rotary shaker (100 rpm), then centrifuged (3000 g for 10 min). The supernatant (cocktail solution) was decanted and filtered through a 0.45 µm PTFE filter to a vial. The PAHs in the filtrate were vigorously extracted by liquid-liquid extraction with DCM in a separating funnel with 3 × 50 mL portions of DCM, which were collected, dried in a column of sodium sulfate and pooled. If an emulsion occurred upon shaking, the DCM layer was collected and shaken with a further 20 mL of 1:1:1 DCM:hexane:acetone solution, and the solvent layer collected. The extracts were evaporated

under nitrogen to 2 mL and stored at 4 °C until analysis. Two sediments (HR 2 and DC 7) were extracted and analysed separately in triplicate.

The 6-h and 4-h extraction times for the XAD-2 and GFM methods, respectively, were chosen based on the results of past studies (Voparil and Mayer, 2004; Cornelissen et al., 2001; Lei et al., 2004) and were intended to provide a rapid estimate of the bioavailable fraction of PAHs in the sediments. Past studies using Cd and Cu radioisotopes have indicated that the gut passage time of sediments in *T. deltoidalis* is approximately 8 h (King et al., 2005).

3. Results and Discussion

3.1. Quality assurance data for PAH analyses

For sediment, XAD-2 and GFM analyses, the blanks (sand) were below the method detection limits of 0.10, 0.15 and 0.03 mg PAH/kg sediment dry weight, respectively, except for naphthalene in the XAD-2 which was found to be a contaminant in the resin. Bivalve tissue blanks were below the method detection limits of 0.10 mg PAH/kg tissue dry weight. Recoveries of d10-phenanthrene spiked into sediment, XAD-2 and bivalve tissue samples before extraction were 90-110% of the expected values. For the sediment extractions, recoveries of PAHs spiked into the sediments were 70-130%. For XAD-2 and GFM extractions, spike recoveries of 16 PAHs were 47-76% (XAD-2) and 52-67% (GFM) for the six low-MW PAHs, and 41-71% (XAD-2) and 75-101% (GFM) for the eight high-MW PAHs. The lower recovery of PAHs achieved for the XAD-2 extraction method may have been in part due to the single extraction (DCM) used for the analyses, compared to the triplicate extractions (1:1:1 acetone:DCM:hexane) used for the sediment analyses. For the bivalve tissue extractions, spike recoveries of the 16 PAHs were 77-111% for the six low-MW PAHs and 91-129% for the eight high-MW PAHs other than pyrene (145%) and indeno(1,2,3-cd)-fluoranthene (136%).

3.2. Test sediment properties and contaminant concentrations

The concentrations of PAHs and TPHs in the sediments are shown in Table 1. Past studies have shown that the Hunter River (HR) sediments also contained high concentrations of lead (30-400 mg/kg) and zinc (100-5000 mg/kg), moderate concentrations of other metals (20 mg/kg As, 5 mg/kg Cd, 60 mg/kg Cr, 10-80 mg/kg Cu, 1 mg/kg Hg, and 10-50 mg/kg Ni), while other organics were generally below analytical detection limits (BTEXs 0.5-1 mg/kg, OC pesticides 0.001-0.01 µg/kg, PCBs 0.01-0.1 mg/kg) (Simpson et al., 2006). The low-MW and high-MW PAHs comprised (mean±SD) 31±18% and 69±18% of the total PAHs in the sediments, respectively. As a percent of the total PAHs, fluoranthene (13±4%) and pyrene (15±5%) had the highest concentrations. There was a significant relationship ($p<0.01$) between the concentrations of total-PAHs and TPHs in the sediment: $\text{TPHs} = 12.3 \times \text{total-PAHs mg/kg}$ ($r^2 = 0.74$), indicating a possible common source for these contaminants.

The Duck Creek (DC) sediments had finer particle sizes and lower TOC concentrations than the HR sediments (Table 1). The high TOC of the HR sediments was due to both fine coal and the high oil content of these sediments. There was a significant correlation ($p < 0.01$) between TOC and OC; $\text{TOC} = 0.77 \times \text{OC} \%$ ($r^2 = 0.95$). These results are supported by previous studies that have found that OC determined by LOI overestimates the TOC concentration within sediments (Rockne et al., 2002; Fredrickson et al., 2004; Simpson et al., 2006). There were no significant relationships between the concentrations of TOC and BC, total-PAH concentrations, or TPHs, nor between, OC and BC (Table 1).

3.3. Toxicity and bioaccumulation of PAH by *Tellina deltoidalis*

The relationship between total-PAH concentrations and bivalve survival was generally poor (Table 2). Although the 14-day exposure period may not be sufficient for the bivalves to reach steady state body concentrations (Meador et al., 1995; Kraaij et al., 2001; Thorsen et al., 2004; Moore et al., 2005), greater exposure periods would probably have caused even greater bivalve mortality. For the sediments that caused bivalve mortality over the 14-d exposure period, reduced bioaccumulation of PAHs would be expected as the organisms would probably not feed as greatly in the more toxic sediments.

Survival of the bivalves was 90-100% in the sediments HR 1, 2, 6 and DC 7, 8, 10; however, survival was reduced to 10-40% in sediments HR 3, 4, 5. Although the bivalves burrowed into the DC 9 sediment, this sediment was very oily with high TPH concentrations and no bivalves survived the 14-day exposure. For the HR 2, 4 and 5 sediments, despite similar total-PAH concentrations (1060, 1240, 1070 mg/kg, respectively) and TPH (19700, 10400, 14100 mg/kg) concentrations, bivalve survival was quite different (100, 15, 30%, respectively). These differences could not be accounted for by considerations of organic carbon concentrations (24, 9.9, 24%) or sediment particle size (31, 59, 66% $< 63 \mu\text{m}$).

Consistent with the concentrations of PAHs in the HR and DC sediments, the bivalves accumulated greater concentrations of PAHs from the HR sediments than from the DC sediments (Table 2). However, the greatest bioaccumulation of PAHs by bivalves was measured for the HR 1 sediment, despite this sediment having lower PAH concentrations than the other HR sediments. This may, in part, be due to the low TOC (3.4%) when compared to the other HR sediments (9.9-34%), as lower TOC concentrations should contribute to weaker binding of PAHs and increased bioavailability relative to sediments with greater TOC concentrations.

Of the total PAHs accumulated by the bivalves, the high-MW PAHs comprised $80 \pm 8\%$ (mean \pm SD, %) of total PAHs in bivalve tissues. Fluoranthene and pyrene comprised $24 \pm 19\%$ and $27 \pm 7\%$ of total-PAHs in bivalve tissues, respectively. This was consistent with the greater concentrations of these PAHs in the sediments, and with a previous study that measured greater concentrations of fluoranthene and pyrene than other PAHs in rock oysters (*Saccostrea glomerata*)

collected from the same region of the Hunter River (Lincoln-Smith and Cooper, 2004). No accumulation of low-MW PAHs was detected in the bivalves from the DC sediment exposures. For the HR sediments, all the low-MW PAHs were accumulated by the bivalves (mean \pm SD, % of low-MW PAHs in bivalve tissues): 27 \pm 17% naphthalene, 13 \pm 8% acenaphthylene, 13 \pm 10% acenaphthene, 7 \pm 5% fluorene, 26 \pm 18% phenanthrene, and 14 \pm 8% anthracene.

The relationships between PAHs in the sediments and PAH bioaccumulation in the bivalves is shown in Figure 1. Sediment HR 1 had lower TOC, was more sandy than most of the other HR sediments, and appeared as an ‘outlier’ to the general relationship of PAH bioaccumulation increasing with increasing sediment PAHs concentrations. For this reason, the regression line shown in Figure 1 does not include the HR 1 data. The low survival of the bivalves observed in the HR 4 sediments decreased the representativeness of the PAH bioaccumulation data. The relationships between PAH concentrations the sediment and bivalves were not improved by normalising the sediment PAH concentrations to the organic carbon concentration (ANZECC/ARMCANZ, 2000; Simpson et al., 2005).

Biota-sediment accumulation factors (BSAFs) for the PAHs were calculated as the lipid-normalised PAH concentration in the bivalves divided by the TOC-normalised PAH concentration in the sediments (Moore et al., 2005):

$$\text{BSAF (kg OC/kg lipid)} = (C_o/f_l)/(C_s/f_{OC}) \quad (1)$$

where C_o is the concentration of contaminant in the organism (both wet and dry weight commonly used), C_s is the contaminant concentration in the sediment (generally dry weight), f_l is the lipid fraction of the tissue, and f_{OC} is the organic carbon fraction of the sediment.

The average percentage lipid content of the bivalves was 1.47 \pm 0.22% and the moisture content was 88 \pm 2%. Where sufficient data were available, BSAFs for sediments are shown in Table 3. The DC sediments had lower BSAFs than the HR sediments. BSAF values were highest for the HR 6 sediment (0.80) which had the lowest PAH concentrations of the HR sediments. There were higher BSAF values for high-MW PAHs than for low-MW PAHs. The BSAF values were similar in range to those of other studies (Meador, 2003; Moore et al., 2005), however no other data exist for other benthic organisms from Australia.

There were no significant correlations ($p > 0.05$) found between BSAF values and concentrations of PAHs in sediment, nor between log K_{ow} (from Meador et al., 1995) and BSAF values. Similar observations were made in other studies of PAH-contaminated sediments and bioaccumulation in sediment ingesting organisms (Thorsen et al., 2004; Moermond et al., 2005). The BSAFs indicated that the sediments with the highest relative bioaccumulation potential were HR 1 and HR 6, which had the lowest total-PAH concentrations for the HR sediments. There was a significant positive relationship between TOC and BSAF ($r^2 = 0.60$), that was improved ($r^2 = 0.76$) when HR 1 (the sandier sediment) was omitted. The reason for this relationship remains elusive. There was a negative

relationship ($r^2 = 0.53$) was between BC (combustion-derived black carbon) and BSAF. The lower BSAFs of the DC sediments compared to the HR sediments may relate to the greater BC concentrations of the DC sediments. Many studies have suggested that high BC concentrations reduce the bioavailability of PAHs (Kraaij et al., 2002; Cornelissen et al., 2005; Moermond et al., 2005; Sundelin et al., 2004).

3.4. XAD-2 and GFM extractable PAHs

The concentrations of PAHs extracted from the test sediments using the 6-h XAD-2 resin are summarized in Table 4. As a percentage of PAHs in the sediments, the XAD-2 method generally extracted a greater percentage of low-MW PAHs than high-MW PAHs. There was a significant relationship ($p < 0.01$) between the low-MW PAHs extracted by XAD-2 and those in the sediment ($r^2 = 0.85$) (Figure 2), however this relationship was not evident for high-MW PAHs or total-PAHs. Greater concentrations of PAHs were extracted by the XAD-2 method from the HR sediments than from the DC sediments. For the HR sediments, the XAD-2 method extracted (mean \pm SD, % of the sediment PAH concentration) $18 \pm 11\%$, $14 \pm 10\%$, and $23 \pm 11\%$ of the total, high-MW and low-MW PAHs, respectively. Fluoranthene and pyrene comprised $42 \pm 6\%$ and $44 \pm 4\%$, respectively, of the high-MW PAHs extracted by the XAD-2 method. This was consistent with the relative amounts of these PAHs measured in the sediments and the PAHs accumulated by the bivalves. Naphthalene comprised $28 \pm 24\%$ of the low-MW PAHs extracted by the XAD-2 method; however, despite corrections for the naphthalene contamination of the XAD-2 material, the accuracy of the naphthalene data should be considered with suspicion.

The GFM also extracted a greater concentration of PAHs from the HR sediments than from the DC sediments (Table 4). For the HR sediments, the GFM method extracted (mean \pm SD, % of the sediment PAH concentrations) $1.6 \pm 1.6\%$, $0.7 \pm 0.9\%$, and $3.7 \pm 4.7\%$ of the total, high-MW and low-MW PAHs, respectively. The low concentrations of PAHs extracted by the GFM were consistent with previous studies that have shown that the amount of PAHs extracted by gut fluids of the polychaetes (*Arenicola* sp.) from sediments with high TOC concentrations (such as the HR and DC sediments) was low (Weston and Mayer, 1998; Voparil and Mayer, 2000). The low-MW PAHs comprised (mean \pm SD) $60 \pm 30\%$ of the total PAHs extracted by the GFM method. For each HR sediment, all six of the low-MW PAHs were measured in the GFM extracts, with naphthalene comprising $50 \pm 16\%$ of the low-MW PAHs extracted (71% for sediment HR 3). Fluoranthene and pyrene comprised greater than 80% of the high-MW PAHs extracted, with benz(a)anthracene and chrysene being the only other high-MW PAHs detected (sediments HR 1 and HR 2 only). Comparisons for the DC sediments were not possible due to the low concentrations of PAHs extracted.

3.5. Comparison between PAH bioaccumulation and PAHs extracted by XAD-2 and GFM methods

Ten Hulscher et al. (2003) found that the total PAHs in sediments determined by a 6-h Tenax extraction significantly correlated with PAH bioaccumulation by earthworms in soils from four different locations ($r^2 = 0.76-0.92$). Relationships were not as significant in the present study. The relationship between PAHs extracted by XAD-2 and PAH accumulation in bivalve tissues is shown in Figure 3. Assuming that the XAD-2 method measures PAHs present in the dissolved phases, including those that rapidly desorb from particles, then these results indicate that the bivalves accumulate PAHs both from the dissolved phase and via ingestion of sediment particles.

The HR 4 sediment had the highest XAD-2 extractable PAH concentrations, indicating that the PAHs in this sediment would be more bioavailable and more likely to be bioaccumulated by the bivalves than PAHs in the other sediments (Table 4). However, in the bioaccumulation assay, few of the bivalves survived in the HR 4 sediments and the PAH concentration measured in the bivalves were low possibly due to the bivalves not feeding because of the contamination. In sediments that cause toxicity or change organism behaviour, surrogate methods such as XAD-2 may be more useful for assessing potential bioaccumulation than direct bioaccumulation bioassays.

Although the GFM extractable PAH concentrations were low, there were reasonable relationships ($p < 0.05$) between pyrene and fluoranthene concentrations in the bivalves and the GFM-extractable pyrene ($r^2 = 0.76$) and fluoranthene ($r^2 = 0.60$). Studies by Voparil and Maher (2000) on the solubilisation of PAHs in the gut fluids of polychaetes have shown that larger (high-MW) PAHs tend to displace smaller (low-MW) PAHs from intestinal micelles resulting in a decreased solubility of low-MW PAHs during the passage of sediments through guts. This was reported to result in a greater uptake of the high-MW PAHs as the micelles are assimilated into the tissues, and the displaced low-MW PAHs are excreted. Those studies also found that pyrene and fluoranthene increase in concentration in real gut fluid with increased extraction time, while the other PAH concentrations remained constant (Voparil and Mayer, 2000). Assuming the gut fluids of bivalve have similar chemistry to polychaete gut fluids, this may contribute to pyrene and fluoranthene comprising the larger fractions of PAHs accumulated in the bivalve tissues and also being the major high-MW PAHs extracted by the GFM method.

In both HR 3 and HR 4 sediments, reduced survival of the bivalves was observed, which decreased the representativeness of the PAH bioaccumulation data. Sediment HR 3 had the greatest GFM-extractable total-PAH concentration, of which 67% was naphthalene. However, this sediment did not have an unusually high concentration of naphthalene compared to the other sediments (naphthalene concentrations were 52, 130, 280, 160 mg/kg in sediments HR 2, HR 3, HR 4, HR 5). The high GFM extractability of naphthalene for the HR 3 sediment was not consistent with the bivalve bioaccumulation, XAD-2 extraction or sediment PAH analyses. Sediment HR 4 had the highest

sediment PAH concentrations and the greatest XAD-2 extractable PAHs, and had the second highest GFM extractable PAH concentration. It is likely that the high concentrations of organic matter, including TPHs and other uncharacterised hydrocarbon fractions (e.g. tars), in the sediments inhibit the extraction of both high- and low-MW PAHs by the GFM and also influence micelle formation and uptake by the bivalve. Extraction of highly contaminated sediments has been shown to lower micelle action, and therefore lower PAH extraction, presumably due to the sorption of the surfactant into the sediment particles (Voparil and Mayer, 2000).

The XAD-2 and GFM extraction results were in contrast to our BSAF results (Table 3) which indicated that the high-MW PAHs were the most bioavailable (as a fraction of total sediment PAHs). The ability of *T. deltoidalis* to metabolise PAHs is unknown; however, molluscs generally have been found to possess a weak ability to metabolise PAHs (Meador et al., 1995; Meador, 2003). It is also suspected that high-MW PAHs are metabolised faster than low-MW PAHs, and this would result in lower BSAFs for the high-MW PAHs (Meador, 2003). Further studies are required to determine which factors most affect the extractability of PAHs from sediments by XAD-2 and GFM techniques and how these measurements relate to BSAFs.

4. Conclusions

The bivalve, *Tellina deltoidalis*, proved to be a useful organism for determining bioaccumulation of PAHs from sediments and there were significant positive relationships between PAH bioaccumulation by the bivalves and sediment PAH concentration. The relationships between PAH concentrations the sediment and bivalves were not improved by normalising the sediment PAH concentrations to the organic carbon concentration. The average percentage lipid content of the bivalves was $1.47 \pm 0.22\%$ and BSAFs for total-PAHs ranged from 0.06 to 0.80 (kg OC/kg lipid), and were similar in range to those of other studies.

Bioaccumulation bioassays usually take weeks to perform (e.g. 10-42 days), making them an expensive component of sediment quality assessments. Consequently, the application of surrogate methods that rapidly determine the bioavailable PAHs in sediments are becoming increasingly popular. Comparisons of the PAHs accumulated by *T. deltoidalis*, the sediment extractable-PAHs determined using a 6-h XAD-2 resin desorption method and a 4-h gut fluid mimic (GFM) sediment extraction method indicated that these methods required further development before they can be applied routinely as surrogate methods for assessing the bioavailability of PAHs in sediments.

The XAD-2 and GFM methods both extracted varying amounts of PAHs from the sediments. Low concentrations of PAHs were extracted by the GFM method (0.2-3.6% of total-PAHs in sediments) and may have been due to the high concentrations of organic carbon and/or oil. The GFM results were inadequate for generalising the bioavailability of the PAHs in the sediments. The XAD-2 method extracted greater amounts of PAHs (3-34% of total-PAHs in sediments), however the total-

PAH concentrations in the sediments provided a better, or equally good, prediction of PAH bioaccumulation by *T. deltoidalis*.

It was anticipated that these surrogate methods could be used in conjunction with, or substitute for, more expensive organism bioaccumulation assays as part of weight-of-evidence assessments. The present study indicates that the XAD-2 and GFM methods require further refinement and future research should be directed towards lowering detection limits for both XAD-2 resin and GFM type extractions and obtaining comparative data for a greater range of sediment types, contaminant classes and concentrations, and organisms of different feeding guilds and gut chemistries. Such methods may also improve our understanding of the different mechanisms of PAH exposure, e.g. bioaccumulation from dissolved (filter feeding, absorption through membranes) or particulate (sediment ingestion) sources.

Acknowledgements

This research was partially funded by a grant from the NSW Environmental Trust (Grant No. 22003/RD/G0002). The authors thank Joanne Blankman, Gary Low, and Shail Jahani (Environmental Forensics and Analytical Science, Department of Environment and Conservation, NSW, Australia) for providing V. Burston with laboratory facilities, training and expertise in analysis of organics. The authors are grateful to David Spadaro for assistance with experiments. We thank Graeme Batley, Gary Low and two anonymous reviewers for constructive comments on this manuscript.

References

- Ahrens, M.J., Hickey, C.W., 2003. Extractability of polycyclic aromatic hydrocarbons in sediments: a matter of association? *Aust. J. Chem.* 56, 193-199.
- ANZECC/ARMCANZ., 2000. Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australia and New Zealand Environment and Conservation Council/Agricultural and Resource Management Council of Australia and New Zealand
- Chapman, P.M., McDonald, B.G., Lawrence, G.S., 2002. Weight-of-evidence issues and frameworks for sediment quality (and other) assessments. *Human Ecol. Risk. Assess.* 8, 1489-1515.
- Cornelissen, G., Rigterink, H., Hulscher, D.E.M.T., Vrind, B.A., Van Noort, P.C.M., 2001. A simple Tenax extraction method to determine the availability of sediment-sorbed organic compounds. *Environ. Toxicol. Chem.* 20, 706-711.
- Cornelissen G., van Noort P.C.M., Grovers H.A.J., 1997. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: sediment extraction with Tenax and effects of contact time and solute hydrophobicity. *Environ. Toxicol. Chem.* 16, 1351-1357.
- Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T. O., Koelmans, A.A., van Noort, P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* 39, 6881-6895.
- Fredrickson, H., Furey, J., Talley, J.W., Richmond, M., 2004. Bioavailability of hydrophobic organic contaminants and quality of organic carbon. *Environ. Chem. Letters* 2, 77-81.
- Gustafsson, O., Haghesta, F., Chan, C., MacFarlane, J., Gschwend, P.M., 1997. Quantification of the dilute sedimentary soot phase: Implication for PAH speciation and bioavailability. *Environ. Sci. Technol.* 31, 203-209.
- King, C.K., Dowse, M.C., Simpson S.L., Jolley, D., 2004. An assessment of five Australian polychaetes and bivalves for use in whole sediment toxicity tests: toxicity and accumulation of copper and zinc from water and sediment. *Arch. Environ. Contam. Toxicol.* 47, 314-323.
- King C.K., Simpson S.L., Smith S.V., Stauber J.L., Batley G.E., 2005. Short term accumulation of Cd and Cu from water, sediment and algae by the amphipod *Melita plumulosa* and the bivalve *Tellina deltoidalis*. *Mar. Ecol. Prog. Ser.* 287: 177-188.
- Kraaij R., Ciarelli, S., Tolls J., Kater B.J., Belfroid A.C., 2001. Bioavailability of lab-contaminated and native polycyclic aromatic hydrocarbons to the amphipod *Corophium volutator* relates to chemical desorption. *Environ. Toxicol. Chem.* 20, 1716-1724.
- Kraaij R., Seinen W., Tolls J., Cornelissen G., Belfroid A.C., 2002. Direct evidence of sequestration in sediments affecting the bioavailability of hydrophobic organic chemicals to benthic deposit-feeders. *Environ. Sci. Technol.* 36, 3525-3529.
- Kukkonen, J.V.K., Landrum P.F., Mitra S., Gossiaux D.C., Gunnarsson J., Weston D., 2003. Sediment characteristics affecting desorption kinetics of select PAH and PCB congeners for seven laboratory spiked samples. *Environ. Sci. Technol.* 37, 4656-4663.

481 Lei, L., Suidan, M.T., Khodadoust, A.P., Tabak, H.H., 2004. Assessing the bioavailability of PAHs in
482 field-contaminated sediment using XAD-2 assisted desorption. *Environ. Sci. Technol.* 38, 1786-
483 1793.

484 Leppänen, H., Kukkonen, V.K., 2000. Effect of sediment-chemical contact time on availability of
485 sediment-associated pyrene and benzo[a]pyrene to oligochaete worms and semipermeable
486 membrane devices. *Aquatic Toxicol.* 49, 227-241.

487 Lincoln-Smith, M.P., Cooper, T.F. 2004. Combining the use of gradients and reference areas to study
488 bioaccumulation in wild oysters in the Hunter River estuary, New South Wales, Australia. *Mar.*
489 *Pollut. Bull.* 48, 873-883.

490 Luthy R.G., Aiken, G.R., Brusseau M.L., Cunnonham S.D., Gschwend P.M., Pignatello J.J., Reinhard
491 M., Traina S.J., Weber W.J., Westall J.C. 1997. Sequestration of hydrophobic organic
492 contaminants by geosorbents. *Environ. Sci. Technol.* 31, 3341-3347.

493 MacRae, J.D., Hall, K.J. 1998. Comparison of methods used to determine the availability of polycyclic
494 aromatic hydrocarbons in marine sediment. *Environ. Sci. Technol.* 32, 3809-3815.

495 Maruya, K.A., Risebrough, R.W., Home, A.J. 1997. The bioaccumulation of polynuclear aromatic
496 hydrocarbons by benthic invertebrates in an intertidal marsh. *Environ. Toxicol. Chem.* 16, 1087-
497 1097.

498 Meador, J.P., 2003. Bioaccumulation of PAHs in marine invertebrates, in PAHs: an Excotoxicological
499 Perspective. Douben, P.E. (Ed.), John Wiley and Sons, Florida, pp. 147-171.

500 Meador, J.P., Stein, J.E., Reichert, W.L., Varanasi, U. 1995. A review of bioaccumulation of
501 polycyclic aromatic hydrocarbons by marine organisms. *Rev. Environ. Contam. Toxicol.* 143, 79-
502 165.

503 Moermond, C.T.A., Zwolsman J.J.G., Koelmans, A.A. 2005. Black carbon and ecological factors
504 affect in situ biota to sediment accumulation factors for hydrophobic organic compounds in flood
505 plain lakes. *Environ. Sci. Technol.* 39, 3101-3109.

506 Moore, D.W., Baudo, R., Conder, J.M., Landrum, P.F., McFarland, V.A., Meador, J.P., Word, J.Q.,
507 2005. Bioaccumulation in the assessment of sediment quality: uncertainty and potential
508 application, in: *Use of Sediment Quality Guidelines and Related Tools for the Assessment of*
509 *Contaminated*, Wenning, R.J., Batley, G.E., Ingersoll, C.G., Moore, D.W., (Eds.), SETAC Press,
510 Pensacola, FL, USA.

511 Nakajima, F.A., Baun, A., Ledin, A., Mikkelsen, S.A., 2005. A novel method for evaluating
512 bioavailability of polycyclic aromatic hydrocarbons in sediments of an urban stream. *Water Sci.*
513 *Technol.* 51, 275-281.

514 Rockne, K.J., Shor, L.M., Young, L.Y., Taghon, G.L. and Kosson, D.S., 2002. Distributed
515 sequestration and release of PAHs in weathered sediment: the role of sediment structure and
516 organic carbon properties. *Environ. Sci. Technol.* 36, 2636-2644.

517 Simpson, S.L., Adams, M.S., Micevska, T., Stone, A., Maher, W., 2006. Establishing cause-effect
518 relationships in PAH and hydrocarbon contaminated sediments using the benthic marine alga,
519 *Entomoneis cf punctulata*. *Environ. Toxicol. Chem.* (submitted).

520 Simpson, S.L., Batley, G.E., Chariton, A.A., Stauber, J.L., King, C.K., Chapman, J.C., Hyne, R.V.,
521 Gale, S.A., Roach, A.C., Maher, W.A., 2005. *Handbook for Sediment Quality Assessment*. Centre
522 for Environmental Contaminants Research, Commonwealth Scientific and Industrial Research
523 Organisation (CSIRO), Bangor, New South Wales, Australia.

524 Stuer-Lauridsen, F., 2005. Review of passive accumulation devices for monitoring organic
525 micropollutants in the aquatic environment. *Environ. Pollut.* 136, 503-524.

526 Sundelin, B., Wiklund, A.K.E., Lithner, G., Gustafsson, O., 2004. Evaluation of the role of black
527 carbon in attenuating bioaccumulation of polycyclic aromatic hydrocarbons from field-
528 contaminated sediments. *Environ. Toxicol. Chem.* 23, 2611-2617.

529 Ten Hulscher, T.E.M., Postma, J., den Besten, P.J., Stroomberg, G.J., Belfroid, A., Wegener, J.W.,
 530 Faber, J.H., van der Pol, J.J.C., Hendriks, A.J., van Noort, P.C.M., 2003. Tenax extraction mimics
 531 benthic and terrestrial bioavailability of organic compounds. *Environ. Toxicol. Chem.* 22, 2258-
 532 2265.

533 Thorsen, W.A., Cope, W.G. Shea, D., 2004. Bioavailability of PAHs: effects of soot carbon and PAH
 534 source. *Environ. Sci. Technol.* 38, 2029-2037.

535 US EPA. 1996. Test methods for evaluating solid waste, physical/chemical methods, SW-846. United
 536 States Environmental Protection Agency; Office of Solid Waste; Economic, Methods, and Risk
 537 Analysis Division, Washington, D.C.

538 Van der Wal L., Jager, T., Flleuren R.H.L.J, Barendregt A., Sinnige T.L., Van Gestel, C.A.M.,
 539 Hermens J.L.M., 2004. Solid-phase microextraction to predict bioavailability and accumulation of
 540 organic micropollutants in terrestrial organisms after exposure to a field-contaminated soil.
 541 *Environ. Sci. Technol.* 38, 4842-4848.

542 Van den Heuvel, H and van Noort, P., 2005. Sorbate size-dependent maximum capacities for
 543 adsorption of organic compounds in the slowly and very slowly desorbing domains of a sediment.
 544 *Environ. Toxicol. Chem.* 24, 1918-1923.

545 Vinturella, A.E., Burgess, R.M., Coull, B.A., Thompson, K.M., Shine, J.P., 2004. The use of passive
 546 samplers to mimic uptake of PAHs by benthic polychaetes. *Environ. Sci. Technol.* 38, 1154-1160.

547 Voparil, I.M., Mayer, L.M., 2000. Dissolution of sedimentary polycyclic aromatic hydrocarbons into
 548 the lugworm's (*Arenicola marina*) digestive fluids. *Environ. Sci. Technol.*, 34, 1221-1228.

549 Voparil I.M., Mayer, L.M., 2004. Commercially available chemicals that mimic a deposit feeder's
 550 (*Arenicola marina*) digestive solubilization of lipids. *Environ. Sci. Technol.* 38, 4334-4339.

551 Voparil, J.M., Burgess, R.M., Mayer, L.M., Tien, R., Cantwell, M.G., Ryba, S.A., 2004. Digestive
 552 bioavailability of a deposit feeder (*Arenicola marina*) of polycyclic aromatic hydrocarbons
 553 associated with anthropogenic particles. *Environ. Toxicol. Chem.* 23, 2618-2626.

554 Weston, D.P., Mayer, L.M., 1998. In vitro digestive fluid extraction as a measure of the bioavailability
 555 of sediment-associated polycyclic aromatic hydrocarbons: sources of variation and implications for
 556 partitioning models. *Environ. Toxicol. Chem.* 17, 820-829.

Table 1. Sediment properties and concentrations of major organics contaminants for Hunter River (HR) and Duck Creek (DC) sediments.

Sediment	Concentrations, mg/kg (dry weight) ^a				Properties, %			
	Total PAHs	high-MW PAHs	low-MW PAHs	TPHs ^b	TOC	<63 μm ^c	OC ^d	BC ^d
HR 1	173	144	28.5	3900	3.9	29	5.8	4.5
HR 2	1060	807	253	19700	24	31	25	5.3
HR 3	817	402	415	6900	11	66	14	6.5
HR 4	1240	443	793	10400	9.9	59	13	7.1
HR 5	1070	566	504	14100	24	66	33	7.8
HR 6	75.6	54.5	21.1	2320	34	42	43	3.3
DC 7	80.2	73.0	7.2	1730	2.0	83	6.2	6.7
DC 8	<1.6	<1.0	<0.6	915	1.9	86	6.3	6.6
DC 9	49.8	33.1	16.7	19000	3.0	60	8.5	6.6
DC 10	22.2	20.0	2.2	2340	2.2	90	7.1	10

^a For concentrations of individual PAHs below the detection limits (DLs), the concentrations used were half the DL concentration of 0.10 mg/kg. ^b TPHs = the sum of C6 to C36 chain hydrocarbons. ^c Percent of sediment particles less than 63 μm in size. ^d OC = organic carbon (LOI 375 °C) and BC = black carbon (LOI 1050 °C)

Table 2. The 14-day survival of *Tellina deltoidalis* exposed to Hunter River (HR) and Duck Creek (DC) sediments, and the subsequent concentration of bioaccumulated PAHs

Sediment	Survival, %	Bivalve tissue PAH concentrations, mean \pm SD, mg/kg (dry weight) ^a		
	Mean \pm SD	Total	high-MW	low-MW
HR 1	100	20.7 \pm 5.8	19.0 \pm 6.0	1.7 \pm 0.2
HR 2	100	16.8 \pm 4.8	14.0 \pm 3.8	2.8 \pm 1.0
HR 3 ^b	15 \pm 15	15.5	12.0	3.5
HR 4 ^b	15 \pm 5	10.0	8.0	2.0
HR 5	30	18.8 \pm 5.2	15.1 \pm 3.6	3.7 \pm 1.6
HR 6	100	2.6 \pm 0.5	2.1 \pm 0.3	0.5 \pm 0.2
DC 7	100	3.6 \pm 0.9	3.3 \pm 0.9	<0.3
DC 8	100	0.9 \pm 0.5	0.6 \pm 0.3	<0.3
DC 9 ^b	0	-	-	-
DC 10	95 \pm 5	1.3 \pm 0.5	0.95 \pm 0.3	<0.3

^a The bivalves lipid content was 1.47 \pm 0.22% and the moisture content was 88 \pm 2%.

^b Low survival resulted in insufficient tissue for analysis in one or both tests.

Table 3. Biota-sediment accumulation factors (BSAF) for *Tellina deltoidalis* exposed to Hunter River (HR) and Duck Creek (DC) sediments

Sediment ^a	BSAFs for PAH (kg OC/kg lipid)		
	Total	high-MW	low-MW
HR 1	0.32	0.35	0.16
HR 2	0.26	0.28	0.18
HR 3	0.14	0.22	0.06
HR 4	0.05	0.12	0.02
HR 5	0.29	0.44	0.12
HR 6	0.80	0.89	0.55
DC 7	0.06	0.06	<0.06
DC 10	0.09	0.07	<0.2
Range	0.05-0.8	0.06-0.89	0.02-0.55

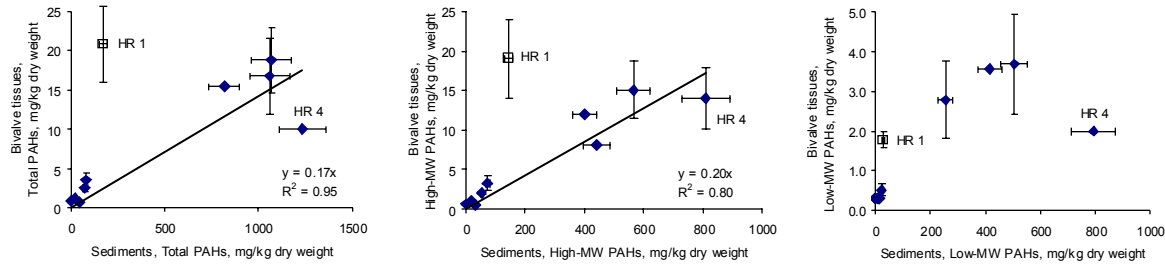
^a BSAFs were not calculated for DC 8 and DC 9 due to low PAH concentrations and no bivalve survival, respectively.

Table 4. Concentrations of PAHs from Hunter River (HR) and Duck Creek (DC) sediments extractable with the 6-h XAD-2 and 4-h gut fluid mimic (GFM) extractions

Sediment	XAD-2 PAHs, mg/kg (dry weight) ^a			GFM PAHs, mg/kg (dry weight) ^a		
	Total PAHs	high-MW PAHs	low-MW PAHs	Total PAHs	high-MW PAHs	low-MW PAHs
HR 1	27 ± 9	19 ± 5	8.5 ± 4	6.5	3.2	3.2
HR 2	54 ± 4	34 ± 2	20 ± 2	3.9	1.9	2.0
HR 3	140 ± 12	35 ± 5	105 ± 7	22.4	1.4	21.0
HR 4	425 ± 45	132 ± 4	293 ± 41	9.3	1.6	7.7
HR 5	159 ± 17	77 ± 6	82 ± 11	2.3	1.9	0.5
HR 6	<2.4	<1.5	<0.9	<0.5	<0.3	<0.2
DC 7	5.1 ± 2	5.0 ± 2	<0.9	<0.5	<0.3	<0.2
DC 8	<2.4	<1.5	<0.9	<0.5	<0.3	<0.2
DC 9	38 ± 4	7.9 ± 2	30 ± 2	<0.5	<0.3	<0.2
DC 10	<2.4	<1.5	<0.9	<0.5	<0.3	<0.2

^a For concentrations of individual PAHs below the detection limits (DLs), the concentrations used were half the DL concentration (0.15 and 0.03 mg/kg for the XAD-2 and GFM methods, respectively) and these concentrations were included in the total-, high-MW and low-MW sums.

606



607

608

609

610

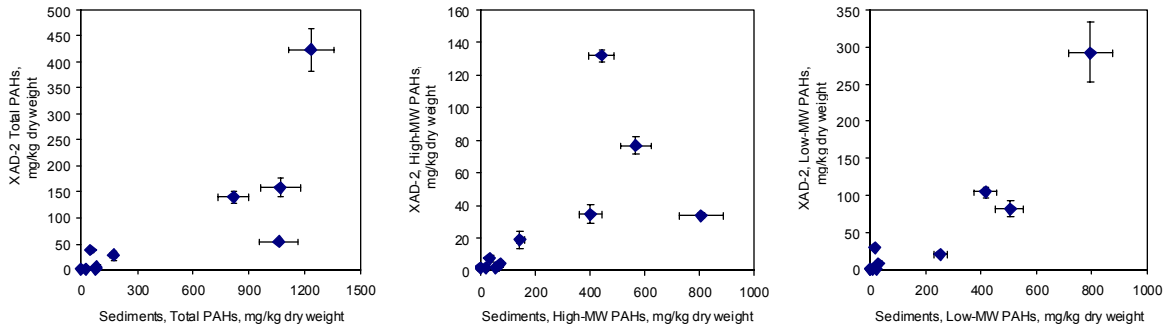
611

612

613

Figure 1 Relationships between concentrations of PAHs in sediments and PAHs accumulated in bivalve tissues. The regression line does not include the HR 1 data due to its very low TOC compared to the other sediments.

614



615

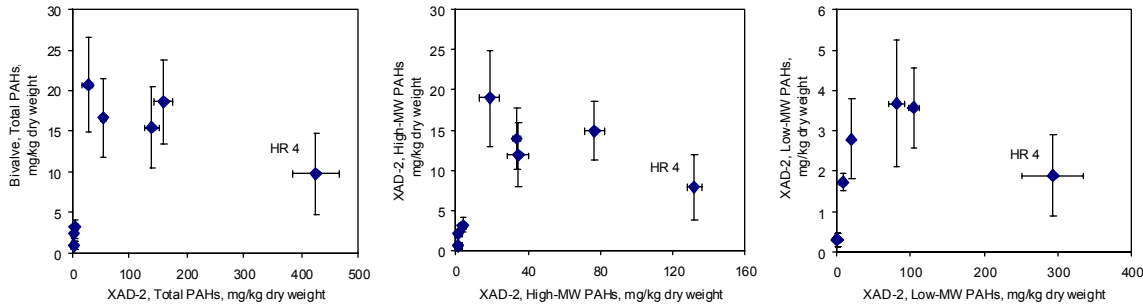
616

617

618

Figure 2 Relationships between concentrations of PAHs in sediments and PAHs extracted from sediments by XAD-2 resin

619



620

621

Figure 3 Relationships between concentrations of PAHs extracted from sediments by XAD-2 resin and PAHs accumulation in bivalve tissues