



ELSEVIER

Aquatic Toxicology 42 (1998) 229–242

**AQUATIC
TOXICOLOGY**

Effect of particle-xenobiotic contact time on bioavailability of sediment-associated benzo(a)pyrene to benthic amphipod, *Diporeia* spp

Jussi V.K. Kukkonen ^{a,*}, Peter F. Landrum ^b

^a *Laboratory of Aquatic Toxicology and Ecology, Department of Biology, University of Joensuu, P.O. Box 111, FIN-80101 Joensuu, Finland*

^b *NOAA/Great Lakes Environmental Research Laboratory, Ann Arbor MI 48105, USA*

Received 30 January 1997; received in revised form 1 July 1997; accepted 15 July 1997

Abstract

A sample of Lake Michigan sediment was dosed with [¹⁴C]benzo(a)pyrene ([¹⁴C]BaP) and stored in the dark at 4°C. Sets of experiments exposed *Diporeia* spp. for 28 days to this dosed sediment after 1 week, 6 and 13 months storage. Just prior to the exposures, the sediment was dosed again with [³H]benzo(a)pyrene ([³H]BaP). The accumulation of [¹⁴C]BaP with and without [³H]BaP was also examined after 13 months contact time to see whether the dosing with [³H]BaP affected the bioavailability. After 1 week contact time, the uptake clearance (K_s , g sed. g⁻¹ h⁻¹) for [¹⁴C]BaP was about 38% lower than the K_s for [³H]BaP. After 6 months and 13 contact time the K_s for [¹⁴C]BaP was 46% and 42% lower, respectively, than the K_s for [³H]BaP suggesting that contact time between the compound and sediment particles may affect the bioavailability of BaP. The K_s for [¹⁴C]BaP with and without [³H]BaP was the same. The log K_{oc} of BaP varied from 5.25 to 6.18 at different time points but there was no large difference between [³H]BaP and [¹⁴C]BaP. The particle size distribution of [¹⁴C]BaP did not change during the 13 months storage and it was similar to the distribution of [³H]BaP. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Sediments; Bioavailability; Organic contaminants; Benzo(a)pyrene; Contact time; Sorption

* Corresponding author. Tel.: +358 13 2513575. fax: +358 13 2513590; e-mail: Jussi.Kukkonen@joensuu.fi

1. Introduction

Neutral hydrophobic compounds, such as polycyclic aromatic hydrocarbons (PAHs), sorb to the organic matrix of particles and tend to accumulate in sediments. Sorptive partitioning of such compounds between aqueous and sediment phases is often incompletely reversible on time scales relative to exposure times used in biological assays. It has been assumed that sorption occurred rapidly and therefore that chemical equilibrium was attained in a very short time (Di Toro et al., 1991; Pignatello and Xing, 1996). However, slow adsorption and desorption processes leading to non-equilibrium behaviour has been observed for several types of compounds, including PAHs, polychlorinated biphenyls (PCBs), halogenated benzenes, and halogenated aliphatic hydrocarbons (Haddock et al., 1983; Coates and Elzerman, 1986; Wu and Gschwend, 1986; Steinberg et al., 1987; Pignatello, 1990; Jepsen et al., 1995; Borglin et al., 1996; Tye et al., 1996). This means that the chemical equilibrium may not be a good approximation in many experimental or environmental situations.

Laboratory dosed sediment samples have been widely used to determine and to estimate the environmental fate, bioavailability and possible biological effects of sediment-associated pollutants (Dewitt et al., 1990; Heim et al., 1994; Kukkonen and Landrum, 1994). The time gap between dosing sediment and performing the bioassay varies normally from hours to a few months maximum. However, the bioavailability of some sediment-associated compounds has been observed to decrease with increased contact time between the sediment and the xenobiotic (Varanasi et al., 1985; Landrum, 1989; Landrum et al., 1992a). For example, PAH compounds such as fluorene, phenanthrene, and pyrene were more available to organisms (as determined by uptake clearance) in dosed sediments aged less than 1 week than in that dosed and aged 60–150 days (Landrum et al., 1992a; Harkey et al., 1994). Even though reductions in accumulation have been observed, the potential impact of this process has not generally been recognized and taken account in testing procedures.

The bioavailability of some PAH compounds has been studied also in sediment cores taken from field. These results are somewhat confusing. Ferraro et al., 1990 reported a significant increase in calculated accumulation factors (AFs) for benzo(a)pyrene as a measure of bioavailability in surficial sediments (0–2 cm layer; i.e. recently contaminated sediment) versus material taken at 4–8 cm depth from the same sediment core and little change in AF was seen for pyrene, and chrysene. On the other hand, Harkey et al. (1995) reported that the highest bioavailability of PAHs was measured either at 4–8 cm or at 12–16 cm depths but not at the surface layer (0–4 cm depth). These results could be explained by compositional differences of natural organic matter associated with particles among the sediment depths but, certainly, more experimental data is needed to accurately explain the effect of contact time on the bioavailability of contaminants in the sediments.

To investigate further the effect of sediment aging on bioavailability of benzo(a)pyrene (BaP) under laboratory conditions, the benthic amphipod *Diporeia* spp. was exposed to BaP dosed sediment after different contact times. Our objectives

were to determine the uptake kinetics of aged [^{14}C]BaP in bioaccumulation assay over a period of 4 weeks and to compare it to uptake kinetics of freshly dosed [^3H]BaP added to the same sediment just 2 days before the start of the bioassay.

2. Materials and methods

2.1. Collection of organisms, sediment, and water

Diporeia spp. were collected from Lake Michigan off Grand Haven, Michigan (43°01.2'N, 86°17.6'W) at a depth of 24–28 m with a Ponar grab sampler. *Diporeia* collected from this site have low background concentrations of PAH; individual PAH congeners ranged from 0.2 to 2 mg g $^{-1}$ (Eadie et al., 1982). *Diporeia* were screened from the sediment, placed in clean lake water, and kept cold with ice during transport to the laboratory. The *Diporeia* were held in aquaria containing 3–4 cm of sediment, collected from the same site as the organisms, and 7 to 10 cm of lake water at 4°C. The lake water used throughout the study was Lake Michigan surface water, collected about 1 m below the surface and stored at 4°C.

Lake Michigan sediment for the assay was obtained by Ponar grab approximately 8 km off Grand Haven, MI (43°02.0'N, 86°21.9'W) at 45-m depth. This sediment has somewhat higher background concentrations of PAH than the site from which the *Diporeia* were collected. Individual components ranged from 40 to 200 ng g $^{-1}$ (Eadie et al., 1982). The sediment organic carbon content (0.45 ± 0.04%, n = 12) is higher than that from the site of *Diporeia* collection; thus, the sediment would have a more consistent food supply for longer exposures. The sediment was sieved at 1 mm to remove animals and large debris and held at 4°C until use.

2.2. Sediment dosing and storage

The radiolabelled benzo(a)pyrene stocks were obtained as follows: [^3H]benzo(a)pyrene ([^3H]BaP, 69 Ci mmol $^{-1}$, Amersham) and [^{14}C]benzo(a)pyrene ([^{14}C]BaP, 16.2 mCi mmol $^{-1}$, Sigma, St. Louis, MO). The radiopurity of both stocks was determined using thinlayer chromatography with hexane:benzene (4:1, v:v) as the solvent and liquid scintillation spectrometry (Landrum, 1982) and were greater than 98% pure prior to use.

Wet sediment (3300 g) and lake water in a 1:1 ratio was dosed with [^{14}C]BaP in acetone. The suspensions were stirred for 4 h at room temperature. The suspensions were allowed to settle overnight at 4°C; the overlying water was then decanted, and fresh lake water added and mixed with the sediment. The sediment was allowed to settle for 24 h and the overlying water was again decanted. Lake water (2 cm) was then placed over the sediment and allowed to stand at 4°C.

2.3. Sediment exposures

At the beginning of each exposure (1 week, 6 and 13 months after the [^{14}C]BaP dosing), the overlying water was removed, the sediment was stirred to visual homogeneity, and a subsample (about 800 g) was removed. Lake water (2 cm) was placed over the remaining sediment and it was placed back at 4°C. The subsample was dosed with [^3H]BaP using the same procedure as described above for dosing with [^{14}C]BaP. After the [^3H]BaP dosing, sediment was allowed to stand only for one day and after that the overlying water was decanted, the sediment was stirred, and 40 g were added to each of 400-ml beakers. Lake water (300 ml) was added carefully to each beaker to minimize sediment disturbance, and the sediment was allowed to settle at 4°C overnight. After settling, *Diporeia* (12 juveniles, 4–8 mg wet weight) were added to each beaker. Triplicate beakers were sampled at 1(2), 3(4), 7, 14, and 28 days. The total number of beakers was 15.

At each sampling period, the overlying water was sampled (two times 2 ml) for the radioactivity and decanted, organisms were removed and sediment samples for contaminant concentration and dry-to-wet weight measurements were taken, from each beaker. Six organisms per beaker were blotted dry, weighed as pairs, and [^3H] and [^{14}C] activity was measured. An additional three organisms per beaker were taken for lipid analysis and remaining three organisms were taken for dry-to-wet weight and other analysis (data not presented in this paper). The remaining sediments were centrifuged (5000 rpm, 25 min.) and freely dissolved concentration of BaP was analysed after Landrum et al., 1984; Eadie et al., 1990.

2.4. Analyses

Radioactivity determination for ^3H and ^{14}C was performed on a Wallac LKB 1217 liquid scintillation counter. After subtracting background, samples were corrected for quench and counting efficiency using the external standards ratio method. Wet sediment samples (approximately 100 mg) were added directly to scintillation cocktail (12 ml, Research Products International 3a70B) and sonicated with a Tekmar Sonic Disrupter for 2 min each. Additional samples were taken at the same time for wet-to-dry weight determination. The samples were allowed to stand for 24 h and ^3H - and ^{14}C -activity was determined. Organisms were added directly to scintillation cocktail, sonicated for 1 min, allowed to stand for 24 h, and analysed for ^3H - and ^{14}C -activity. The lipid contents of five organisms (out of nine reserved for this analysis) were measured at the beginning and end of each exposure by a microgravimetric method (Gardner et al., 1985).

Sediment dry weight was determined by drying aliquots at 90°C to constant weight. The sediment organic carbon content was measured, after removing carbonates with HCl, on a Perkin Elmer 2400 CHN analyser. The carbonate was removed by adding 2 ml, 1 N HCl per 100 mg dry sediment. The mixture was shaken for 24 h and dried at 90°C.

Particle size distribution of sediment mass and BaP were determined by a modified sedimentation technique (Royse, 1970; Siebert, 1977; Kukkonen and

Landrum, 1996). Approximately 40 g wet sediment was wet sieved using filtered (Gelman Sciences, glass fibre, type A/E) lake water through 420-, 105-, and 63-mm standard sieves. Materials remaining on each sieve were collected. Triplicate samples were taken for liquid scintillation counting; the remainder were dried to constant weight at 90°C for dry-weight analyses. Material passing through the 63-mm sieve was mixed at room temperature with filtered lake water (total volume 1 l) in a 1-l graduated cylinder. Samples (25 ml) from sediment suspension were taken at specific depths and times after mixing based on calculations using Stoke's law with 2.6 g ml^{-1} as the specific gravity of the particles (Royse, 1970). Three 2-ml aliquots from each sample were analysed by liquid scintillation counting. The remaining portion of the sample (19 ml) was dried to constant weight at 90°C for dry weight. This procedure is more closely described and discussed by Kukkonen and Landrum, 1996.

Three sediment samples (5–8 g wet weight) for the [^{14}C]BaP purity check were taken after 6 and 13 months storage and extracted twice with ethylacetate acetone (4:1, v:v) and twice with benzene. The extracts were combined, dried over Na_2SO_4 , and rotary-evaporated to a few millilitres. The rest of the solvent was transferred into a test tube and further evaporated to about 100 ml under a gentle stream of nitrogen for thin-layer chromatography (TLC) analysis. Remaining solvent was introduced onto silica TLC plate (E. Merck, 250 mm coating), some cold BaP was added over the sample, and the plate was developed with hexane:benzene (4:1, v:v) solvent. After the run, the pure BaP spot was marked under UV-light and the plate was analysed for radioactivity by scraping $2 \times 1 \text{ cm}$ segments of the silica gel and counting them in 12 ml of LSC cocktail.

The kinetics of BaP accumulation were determined by fitting the data to a first-order rate-constant model:

$$C_a = \frac{K_s \cdot C_s \cdot (1 - e^{-k_e t})}{K_e} \quad (1)$$

where K_s is the uptake clearance coefficient ($\text{g dry sediment g}^{-1} \text{ wet organism h}^{-1}$), C_s is the concentration in the sediment (mmol g^{-1}), t is time (h), K_e is the elimination rate constant (h^{-1}), and C_a is the concentration in the organism (mmol g^{-1}) (Landrum, 1989). Uptake clearance is defined as the amount of the source compartment scavenged of contaminant per mass of organism per time (Landrum et al., 1992b).

3. Results

Lake Michigan sediment (45 m station) is dominated by the particles in size range from 420 μm down to 43 μm . These particles make up 65–70% of the total sediment dry weight and the distribution remained the same during the 13 months storage period (Fig. 1A). This particle size distribution is similar to earlier reported particle size distributions for sediment from the same location (Kukkonen and Landrum, 1994, 1996). BaP is mostly (50–60%) bound by the 63–20 μm particles

(Fig. 1B,C). Within our measurement capability, the distribution of [^{14}C]BaP in the sediment does not change during the 13 months storage period (Fig. 1B) and it is also similar to the distribution of [^3H]BaP after different spikings (Fig. 1C). Similar BaP distributions were obtained earlier in the other spiking experiments with the sediment from the same location (Kukkonen and Landrum, 1995).

The radioactive purity of [^{14}C]BaP remained high throughout the storage period. Both after 6 and 13 months storage, $94.0 \pm 1.2\%$ ($n = 6$) of the extracted activity was found in the same spot on TLC plate as cold BaP. Thus, no large degradation occurred during the storage period. However, the measured [^{14}C]BaP content of the sediment decreased during the storage. After 1 week storage, the activity was 24690 (± 1817) DPM/g dry sediment (98.8% of the nominal concentration, $n = 45$), after

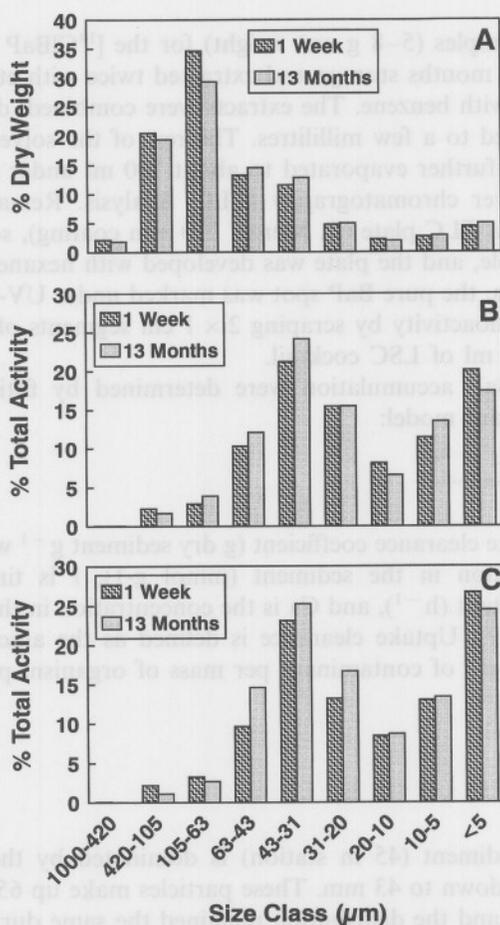


Fig. 1. Particle size distribution (A) [^{14}C]benzo(a)pyrene (B) and [^3H]benzo(a)pyrene (C) distribution in Lake Michigan sediment 1 week and 13 months after the dosing the sediment with [^{14}C]BaP. [^3H]BaP is freshly dosed in both cases. Values shown represent the mean of two replicates.

Table 1
Sediment organic carbon (OC,% of dry weight) content and total lipids (% of dry weight) in *Diporeia* during exposures

Storage	Sampling (days)	OC (%)	Lipid (%)
1 week	0	0.45 ± 0.03	24.8 ± 12.3
	28	0.45 ± 0.03	17.3 ± 3.9
6 months	0	0.46 ± 0.05	31.4 ± 5.1
	28	0.47 ± 0.05	33.5 ± 9.7
13 months	0	0.39 ± 0.05	16.2 ± 12.1
	28	0.41 ± 0.02	11.7 ± 6.8

mean ± S.D., $n = 4$ for OC and $n = 5$ for lipid.

6 months the measured activity was 20869 (± 1677) DPM g^{-1} dry sediment (83.4% of the nominal, $n = 45$) and after 13 months 19940 (± 1051) DPM g^{-1} dry sediment (79.7% of the nominal, $n = 45$). Recoveries for [3H]BaP were 103, 95 and 102% of the nominal concentration for the 1 week, 6 and 13 months experiments, respectively.

The sediment organic carbon content did not show any significant change during the storage period (Table 1). The measured logarithmic value of organic carbon normalized partition coefficients (K_{oc}) ranged from 5.5 to 6.2 and 5.3 to 5.8 for [^{14}C]BaP and [3H]BaP, respectively. The K_{oc} of [^{14}C]BaP was always slightly higher than the value of [3H]BaP (Table 2). The slight increase in the value during every exposure period (from day 1 to 28) might be due to the effect of the organisms. The organisms readily accumulate BaP from the freely dissolved pool in pore water and when this concentration becomes lower the K_{oc} value is increased.

Table 2
The measured sorption coefficients ($\log K_{oc}$) of [^{14}C]BaP and [3H]BaP during the different exposures and storage times. The K_{oc} values were calculated using the measured organic carbon normalized BaP concentrations in the sediment and the measured freely dissolved BaP concentration in the pore water.

Storage	Sampling time (days)	[^{14}C]BaP $\log K_{oc}$	[3H]BaP $\log K_{oc}$
1 week	1	5.53	5.47
	3	5.64	5.48
	7	5.68	5.59
	28	6.18	5.79
6 months	2	5.60	5.25
	4	5.59	5.26
	7	5.71	5.52
	14	5.89	5.61
	28	5.67	5.67
13 months	4	5.66	5.44
	15	5.65	5.45
	28	5.70	5.50

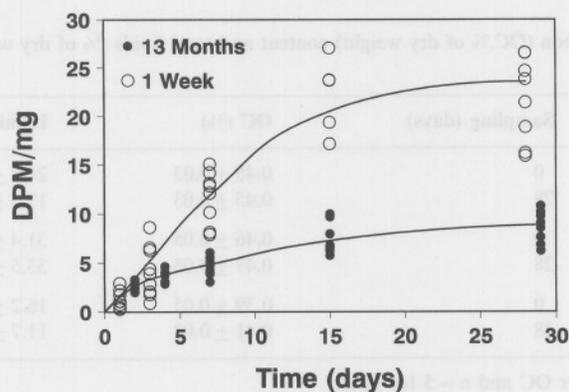


Fig. 2. [^{14}C]benzo(a)pyrene activity in *Diporeia* during the 28 days exposure to sediment stored 1 week and 13 months after the dosing.

After 13 months storage the accumulation of [^{14}C]BaP by *Diporeia* was lower than in the beginning, if just comparing the accumulated body residues (Fig. 2). The calculated uptake clearance (K_s) of [^{14}C]BaP show a slight time dependent decrease compared to the K_s of [^3H]BaP (Table 3). After 1 week contact time the K_s of [^{14}C]BaP is 38% lower than the K_s of [^3H]BaP. After 6 months contact time the difference is 46% and remains over 40% after 13 months contact time. It is noteworthy that the uptake clearance of [^{14}C]BaP is the same with and without [^3H]BaP. This indicates that the spiking the [^{14}C]BaP spiked sediment sample a second time did not change the bioavailability of [^{14}C]BaP (Table 3).

The calculated elimination rate constants (K_e) varied from one exposure to another (Table 3). One reason for this variation was changes in the lipid content of the organisms and a strong inverse relationship between lipid content and K_e was noticed (Fig. 3). For [^3H]BaP the regression is $K_e = -2.5(\pm 0.1) \times 10^{-4} \times$

Table 3

Calculated sediment clearance coefficients ($K_s \pm \text{S.E.}$, $\text{g dry sed g}^{-1} \text{h}^{-1}$) and elimination rate constants ($K_e \pm \text{S.E.}$, h^{-1}) for [^{14}C]BaP and [^3H]BaP after the different lengths of storage

Storage		[^{14}C]BaP	[^3H]BaP	[^{14}C]K _s /[^3H]K _s
1 week	K _s	0.0037 (0.0006)	0.0060 (0.0007)	0.62
	K _e	0.0032 (0.0008)	0.0050 (0.0007)	
6 months	K _s	0.0021 (0.0002)	0.0039 (0.0003)	0.54
		0.0011 (0.0003)	0.0020 (0.0004)	
13 months	K _s	0.0023 (0.0002)	0.0040 (0.0004)	0.58
	K _e	0.0052 (0.0007)	0.0066 (0.0009)	
13 months	K _s ^a	0.0025 (0.0003)		
	K _e ^a	0.0060 (0.0009)		

^a [^{14}C]BaP alone after 13 months storage without a new spiking with [^3H]BaP.

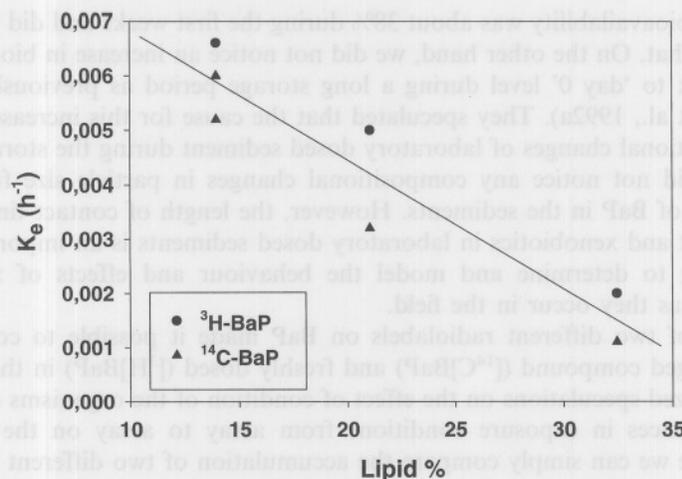


Fig. 3. Elimination rate of benzo(a)pyrene was inversely related to the percent lipids on a dry weight basis. See text for the regression equations.

%Lipid + 0.0102 (± 0.0002) ($r^2 = 0.998$, $P < 0.05$, $n = 3$) and for [^{14}C]BaP the regression is $K_e = -2.5 (\pm 0.4) \times 10^{-4} \times \% \text{Lipid} + 0.0089 (\pm 0.0008)$ ($r^2 = 0.954$, $P < 0.05$, $n = 4$). Combining the data we get regression $K_e = -2.4 (\pm 0.4) \times 10^{-4} \times \% \text{Lipid} + 0.0091 (\pm 0.0009)$ ($r^2 = 0.883$, $P < 0.01$, $n = 7$).

The calculated bioaccumulation factors (BAFs; the lipid normalized body burden divided by organic carbon normalized sediment concentration) after 28 days exposure are presented in Table 4. BAFs varied from 0.171 to 0.123 and from 0.130 to 0.084 for [^3H]BaP and [^{14}C]BaP, respectively.

4. Discussion

We expected the bioavailability of BaP to *Diporeia* to decrease with increasing sediment-BaP contact time as shown for lower molecular weight PAHs (fluorene, phenanthrene) (Landrum et al., 1992a). To a certain extent this happened, but the

Table 4

The organism lipid and sediment organic carbon normalized bioaccumulation factors (BAF, g OC g lipid $^{-1}$) for [^3H]BaP and [^{14}C]BaP

Storage	[^{14}C]BaP	[^3H]BaP	[^{14}C]BAF/[^3H]BAF
1 week	0.130	0.171	0.76
6 months	0.093	0.123	0.76
13 months	0.085	0.134	0.63
13 months	0.084 ^a		

^a [^{14}C]BaP alone after 13 months storage without a new spiking with [^3H]BaP.

decrease in bioavailability was about 38% during the first weeks and did not change much after that. On the other hand, we did not notice an increase in bioavailability of BaP back to 'day 0' level during a long storage period as previously reported (Landrum et al., 1992a). They speculated that the cause for this increase would be the compositional changes of laboratory dosed sediment during the storage. In this study, we did not notice any compositional changes in particle size fractions or distribution of BaP in the sediments. However, the length of contact time between the sediment and xenobiotics in laboratory dosed sediments is an important factor when trying to determine and model the behaviour and effects of xenobiotics particularly as they occur in the field.

The use of two different radiolabels on BaP made it possible to compare the uptake of aged compound ($[^{14}\text{C}]\text{BaP}$) and freshly dosed ($[^3\text{H}]\text{BaP}$) in the bioassay. This minimized speculations on the effect of condition of the organisms or effect of slight differences in exposure conditions from assay to assay on the calculated results. Here we can simply compare the accumulation of two different labels with different contact times on sediment particles. However, the second spiking of the sediment after a given contact time might affect the distribution of the compound already present in the sediment. To examine possibility, we performed the bioavailability assay after 13 months contact time with and without the second spiking. The uptake clearance values show that the new mixing of the sediment and adding of $[^3\text{H}]\text{BaP}$ does not affect the bioavailability of $[^{14}\text{C}]\text{BaP}$. Thus, when contaminated sediments are taken from the field, mixing the samples, in preparation for bioassay and possibly spiking them with some model compounds should not affect the distribution of the highly lipophilic non-polar compounds.

We did not notice any compositional changes in the sediment during the storage period, but we did not look closely at the organic matrix and possible changes may have occurred. One phenomenon we could not explain was the 20% decrease in $[^{14}\text{C}]\text{BaP}$ activity (= concentration) in the sediment during the storage. This is similar to results found in other experiments where laboratory dosed sediments were used (Landrum, 1989; Swartz et al., 1990; Landrum et al., 1991, 1992a). If this loss was due to degradation, we should see some degradation products on TLC plates but the radiopurity remained high. One possible explanation for the declining concentration is that the desorption of certain fraction of BaP from the organic matrix of sediment particles is so slow that the extraction methods used do not extract this fraction (Pignatello, 1990, 1991) and this fraction increases over time. This is supported by findings that contact time affects the desorption rate; the longer the contact time the slower the desorption rate (Borglin et al., 1996). This decreasing desorption rate may well represent decreasing extractability of the contaminant from sediment. If this is the case, the actual decrease in bioavailability of total BaP is likely higher than shown, because the uptake rate calculations of $[^{14}\text{C}]\text{BaP}$ were based on the measured (extractable) sediment concentrations not the total concentration represented by the beginning nominal concentration.

The measured K_{oc} values were similar for both ^{14}C - and ^3H -labelled BaP (Table 2) and these values are close to the estimated value ($K_{oc} = 5.57$) obtained by using Karickhoff's (Karickhoff, 1981) empirical equation 16, if a $\log K_{ow}$ of 5.98 is used

for BaP (Miller et al., 1985). No effect of contact time was observed for the K_{oc} values of [^{14}C]BaP as previously reported for some hydrophobic non-polar compounds (Landrum et al., 1992a; Brannon et al., 1995). On the other hand, this experimental set-up was not specifically designed to address this question, but merely to show the partitioning of different radiolabelled forms of BaP during the *Diporeia* exposures. However, the data for the first day of exposure shows the same trend as bioavailability data: the most of binding and decrease in bioavailability takes place during the first week. An increase in the K_{oc} values during every exposure (from day 1 to 28) was noticed. This might be due to the effect of the organisms. The organisms readily accumulate BaP from the freely dissolved pool in pore water and when BaP concentration in pore water becomes lower the K_{oc} value increases. This would occur only if the desorption rate off particles is slow compared to the uptake by organisms. This has been suggested previously (Landrum and Robbins, 1990).

Lipids are important in the storage of organic xenobiotics in organisms. As shown here, the elimination rate constant of BaP is inversely proportional to organisms lipid content (Fig. 3). This relationship is similar to that reported by Landrum, 1988 for *Diporeia*. On the other hand, lipid content of *Diporeia* does not directly affect the uptake rate of BaP. But, affecting the elimination rate it influences the overall tissue concentration that organisms accumulate. Organisms were close to the steady-state after 28 days exposure period (Fig. 2). The magnitude of the K_e values and the observed decrease with increasing lipid content are both about double that previously reported (Landrum, 1988). There are significant differences in the method of exposure and the models used for determination of the two data sets. The data in this work were estimated from a non-linear two compartment model. On the other hand, Landrum, 1988 measured the elimination rate and fit it to a first order decay model. The greater magnitude and greater variation with lipid content observed in this data set suggest that factors other than elimination are affecting the determination of the apparent elimination constant. Since *Diporeia* has not been found to biotransform PAH, the difference between the data sets suggests some feature such as reductions in bioavailability during the course of the experiment is modifying the uptake thus increasing the apparent elimination. This has been previously suggested for sediment accumulations (Landrum, 1989; Landrum and Robbins, 1990) as a kinetic limitation to desorption. In addition to the differences in elimination, this study also shows increases in the K_{oc} values over the course of the exposures. This change in K_{oc} values helps confirm the hypothesis of changing bioavailability and kinetic limitation to bioavailability in these experiments.

We can also compare the accumulated lipid normalized body burden after 28 days to the measured organic carbon normalized sediment concentration and calculate the bioaccumulation factors, BAFs (Table 4). These BAFs are referred as a bioaccumulation ratio (BSF) by Di Toro et al., 1991. According to the theory, the BAF should be a constant value close to one and independent of both particle and organism properties. In this study, BAFs varied from 0.171 to 0.123 and from 0.130 to 0.084 for [^3H]BaP and [^{14}C]BaP, respectively. Further, the BAFs for [^{14}C]BaP

decreased with increasing contact time. The equilibrium partition approach would predict an order of magnitude higher body residues for BaP in this experimental set-up. This discrepancy suggests that an equilibrium partitioning approach for evaluating bioavailability would be unsuccessful for strongly sorbed contaminants.

Acknowledgements

This work, performed at Great Lakes Environmental Research Laboratory (NOAA), Ann Arbor, MI, was supported, in part, through an interagency agreement, No. DW13935650-010, between the National Oceanic and Atmospheric Administration and the U.S. Environmental Protection Agency as well as by scholarships from the Academy of Finland/Research Council for Environmental Sciences and the Maj and Tor Nessling Foundation (Finland) to Dr Jussi Kukkonen. Although the information in this document was funded in part by the U.S. Environmental Protection Agency, it may not necessarily reflect the views of the agency; no official endorsement should be inferred. Mention of trade-names or commercial products does not constitute endorsement or recommendations for use. GLERL contribution number 1036.

References

- Borglin, S., Wilke, A., Jepsen, R., Lick, W., 1996. Parameters affecting the desorption of hydrophobic organic chemicals from suspended sediments. *Environ. Toxicol. Chem.* 15, 2254–2262.
- Brannon, J.M., Pennington, J.C., McFarland, V.A., Hayes, C., 1995. The effect of sediment contact time on K_{oc} of nonpolar organic contaminants. *Chemosphere* 31, 3465–3473.
- Coates, J.T., Elzerman, A.W., 1986. Desorption kinetics for selected PCB congeners from river sediments. *J. Contam. Hydrol.* 1, 191–210.
- Dewitt, T.H., Ozretich, R.J., Swartz, R.C., Lamberson, J.O., Schults, D.W., Ditsworth, G.R., Jones, J.K.P., Hoselton, L., Smith, L.M., Murphy, E.M., Zachara, J.M., Smith, S.C., 1990. The influence of organic matter quality on the toxicity and partitioning of the sediment-associated fluoranthene. *Environ. Toxicol. Chem.* 24, 1507–1516.
- Di Toro, D.M., Zarba, C.S., Hansen, D.J., Berry, W.J., Swartz, R.C., Cowan, C.E., Pavlou, S.P., Allen, H.E., Thomas, N.A., Paquin, P.R., 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals by using equilibrium partitioning. *Environ. Toxicol. Chem.* 10, 1541–1583.
- Eadie, B.J., Landrum, P.F., Faust, W.R., 1982. Polycyclic aromatic hydrocarbons in sediments, pore water and the amphipod *Pontoporeia hoyi* from Lake Michigan. *Chemosphere* 11, 847–858.
- Eadie, B.J., Morehead, N.R., Landrum, P.F., 1990. Three-phase partitioning of hydrophobic organic compounds in Great Lakes waters. *Chemosphere* 20, 161–178.
- Ferraro, S.P., Lee, H. II., Ozretich, R.J., Specht, D.T., 1990. Predicting bioaccumulation potential: A test of a fugacity-based model. *Arch. Environ. Contam. Toxicol.* 19, 386–394.
- Gardner, W.S., Frez, W.A., Chichocki, E.A., Parish, C.C., 1985. Micromethod for lipid analysis in aquatic invertebrates. *Limnol. Oceanogr.* 30, 1099–1105.
- Haddock, J.D., Landrum, P.F., Giesy, J.P., 1983. Factors affecting the extraction efficiency of anthracene from sediments. *Anal. Chem.* 55, 1197–1200.
- Harkey, G.A., Van Hoof, P.L., Landrum, P.F., 1995. Bioavailability of polycyclic aromatic hydrocarbons from a historically contaminated sediment core. *Environ. Toxicol. Chem.* 14, 1551–1560.

- Harkey, G.A., Landrum, P.F., Klaine, S.J., 1994. Comparison of whole sediment, elutriate and pore-water exposures for use in assessing sediment-associated organic contaminants in bioassays. *Environ. Toxicol. Chem.* 13, 1315–1329.
- Heim, K., Schuphan, I., Schmidt, B., 1994. Behaviour of [¹⁴C]-4-nitrophenol and [¹⁴C]-3,4-dichloroaniline in lab sediment-water systems. 1. Metabolic fate and partitioning of radioactivity. *Environ. Toxicol. Chem.* 13, 879–888.
- Jepsen, R., Borglin, S., Lick, W., Swackhamer, D., 1995. Parameters affecting the adsorption of hexachlorobenzene to natural sediments. *Environ. Toxicol. Chem.* 14, 1487–1497.
- Karickhoff, S.W., 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. *Chemosphere* 10, 833–846.
- Kukkonen, J., Landrum, P.F., 1994. Toxicokinetics and toxicity of sediment-associated pyrene to *Lumbriculus variegatus* (Oligochaeta). *Environ. Toxicol. Chem.* 13, 1457–1468.
- Kukkonen, J., Landrum, P.F., 1995. Effects of sediment-bound polydimethyl-siloxane on the bioavailability and distribution of benzo(a)pyrene in lake sediment to *Lumbriculus variegatus*. *Environ. Tox. Chem.* 14, 523–531.
- Kukkonen, J., Landrum, P.F., 1996. Distribution of organic carbon and organic xenobiotics among different particle size fractions in sediments. *Chemosphere* 32, 1063–1076.
- Landrum, P.F., 1982. Uptake, depuration and biotransformation of anthracene by the scud, *Pontoporeia hoyi*. *Chemosphere* 11, 1049–1057.
- Landrum, P.F., 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: role of physiological and environmental variables. *Aquat. Toxicol.* 12, 245–271.
- Landrum, P.F., 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod, *Pontoporeia hoyi*. *Environ. Sci. Technol.* 23, 588–595.
- Landrum, P.F., Nihart, S.R., Eadie, B.J., Gardner, W.S., 1984. Reverse-phase separation method for determining pollutant binding to Aldrich humic acid and dissolved organic carbon of natural waters. *Environ. Sci. Technol.* 18, 187–192.
- Landrum, P.F., Robbins, J.A., 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. In: Baudo, R., Giesy, J.P., Muntau, H., Sediments: Chemistry and Toxicity of In-Place Pollutants, Lewis Publishers, Chelsea, MI, pp. 237–263.
- Landrum, P.F., Eadie, B.J., Faust, W.R., 1991. Toxicity and toxicokinetics of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (sp.). *Environ. Toxicol. Chem.* 10, 35–46.
- Landrum, P.F., Eadie, B.J., Faust, W.R., 1992a. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. *Environ. Toxicol. Chem.* 11, 1197–1208.
- Landrum, P.F., Lee, H., Lydy, M.J., 1992b. Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environ. Toxicol. Chem.* 11, 1709–1725.
- Miller, M.M., Wasik, S.P., Huang, G., Shiu, W., Mackay, D., 1985. Relationships between octanol-water partition coefficient and aqueous solubility. *Environ. Sci. Technol.* 19, 522–529.
- Pignatello, J.J., 1990. Slowly reversible sorption of aliphatic halocarbons in soils. I. Formation of residual fractions. *Environ. Toxicol. Chem.* 9, 1107–1115.
- Pignatello, J.J., 1991. Desorption of tetrachloroethene and 1,2-dibromo-3-chloropropane from aquifer sediments. *Environ. Toxicol. Chem.* 10, 1399–1404.
- Pignatello, J.J., Xing, B., 1996. Mechanisms of slow sorption of organic chemicals to natural particles. *Environ. Sci. Technol.* 30, 1–11.
- Royse, C.F., 1970. An Introduction to Sediment Analysis. Arizona State University, Tempe AZ, p. 180.
- Siebert, P.C., 1977. Simple sedimentation methods, including the Andreason pipette and the Cahn sedimentation balance. In: Stockham, J.D., Fochtman, E.G. (Eds.), Particle Size Analysis. Ann Arbor Science, Ann Arbor MI, pp. 44–55.
- Steinberg, S.M., Pignatello, J.J., Sawhney, B.L., 1987. Persistence of 1,2-dibromoethane in soils: Entrapment in intraparticle micropores. *Environ. Sci. Technol.* 21, 1201–1208.
- Swartz, R.C., Schults, D.W., DeWitt, T.H., Ditsworth, G.R., Lamberson, J.O., 1990. Toxicity of fluoranthene in sediment to marine amphipods: A test of the equilibrium partitioning approach to sediment quality criteria. *Environ. Toxicol. Chem.* 9, 1071–1079.

- Tye, R., Jepsen, R., Lick, W., 1996. Effects of colloids, flocculation, particle size, and organic matter on the adsorption of hexachlorobenzene to sediments. *Environ. Toxicol. Chem.* 15, 643–651.
- Varanasi, U., Reichert, W.L., Stein, J.E., Brown, D.W., Sanborn, H.R., 1985. Bioavailability and biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment from an urban estuary. *Environ. Sci. Technol.* 19, 836–841.
- Wu, S., Gschwend, P.M., 1986. Sorption kinetics of hydrophobic organic compounds to natural sediments and soils. *Environ. Sci. Technol.* 20, 717–725.
- Kukkonen, J., Landrum, P.F., 1994. Toxicokinetics and toxicity of sediment-associated polycyclic aromatic hydrocarbons (PAHs). *Environ. Toxicol. Chem.* 13, 1457–1465.
- Kukkonen, J., Landrum, P.F., 1995. Effects of sediment-bound polycyclic aromatic hydrocarbons on the bioavailability and distribution of hexachlorobenzene in lake sediment. *Environ. Toxicol. Chem.* 14, 223–231.
- Kukkonen, J., Landrum, P.F., 1996. Distribution of organic carbon and organic xenobiotics among different particle size fractions in sediments. *Chemosphere* 32, 1663–1674.
- Landrum, P.F., 1992. Uptake, depuration and biotransformation of substances by the eel, *Anguilla anguilla*. *Chemosphere* 11, 1049–1057.
- Landrum, P.F., 1988. Toxicokinetics of organic xenobiotics in the amphipod *Daphnia magna*: role of physiological and environmental variables. *Aquat. Toxicol.* 13, 245–271.
- Landrum, P.F., 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Daphnia magna*. *Environ. Sci. Technol.* 23, 284–287.
- Landrum, P.F., Nriagu, J.S., Fisher, B.J., Gardner, W.S., 1984. Reverse-phase separation method for determining polycyclic aromatic hydrocarbon binding to aliphatic basic acid and desorbed organic carbon of natural waters. *Environ. Sci. Technol.* 18, 187–192.
- Landrum, P.F., Hobbins, J.A., 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. In: Hobbins, J.A., Goss, J.P., Munster, H., Sediment Chemistry and Toxicity of In-Pore Polycyclic Aromatic Hydrocarbons. *Chemosphere*, vol. 19, pp. 237–261.
- Landrum, P.F., Fisher, B.J., Fisher, W.R., 1991. Toxicity and toxicokinetics of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Daphnia magna*. *Environ. Toxicol. Chem.* 10, 33–44.
- Landrum, P.F., Fisher, B.J., Fisher, W.R., 1992a. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *Daphnia magna* with sediment type. *Environ. Toxicol. Chem.* 11, 1107–1108.
- Landrum, P.F., Lee, H., Fisher, W.R., 1992b. Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environ. Toxicol. Chem.* 11, 1709–1712.
- Miller, M.H., Wang, S.R., Huang, G., Shen, W., Mackay, D., 1993. Relationships between octanol-water partition coefficient and aqueous solubility. *Environ. Sci. Technol.* 27, 522–529.
- Pignatelli, B., 1990. Slowly reversible sorption of aliphatic hydrocarbons in soils. I. Formation of residual fractions. *Environ. Toxicol. Chem.* 9, 1167–1175.
- Pignatelli, B., 1991. Desorption of naphthalene and 1,2-dichloro-3-chloropropane from natural sediments. *Environ. Toxicol. Chem.* 10, 1399–1404.
- Pignatelli, B., Xing, B., 1996. Mechanisms of slow sorption of organic chemicals to natural particles. *Environ. Sci. Technol.* 30, 1–11.
- Royce, C.F., 1970. An introduction to sediment analysis. Arizona State University, Tempe, AZ, p. 180.
- Schick, F.C., 1977. Simple sedimentation methods including the Andreasen pipette and the Cohn sedimentation balance. In: Schick, F.C., Ed., *Methods for Soil Analysis*. Am. Agron. Soc., Am. Agron. Soc., pp. 44–52.
- Stroobant, E.M., Pignatelli, B., 1987. Retention of 1,2-dichlorobenzene in soils. *Environ. Sci. Technol.* 21, 1201–1206.
- Swartz, R.C., Swartz, D.W., Dewitt, T.H., Dineen, G.R., Lambert, L.G., 1990. Toxicity of fluoranthene in sediment to marine amphipods: A test of the equilibrium partitioning approach to sediment quality criteria. *Environ. Toxicol. Chem.* 9, 1071–1079.