Vol. 246: 163–172, 2003 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published January 16

Bioavailability of pyrene to the deposit-feeding polychaete *Arenicola marina*: importance of sediment versus water uptake routes

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ABSTRACT: The bioavailability of the PAH (polycyclic aromatic hydrocarbon) model compound pyrene to the deposit-feeding polychaete *Arenicola marina* was studied at pyrene concentrations ranging from 0 to 10 ppm. By manipulating the sediment organic content, different distributions of pyrene between particle-associated pyrene, pyrene bound to dissolved organic matter (DOM) and freely dissolved pyrene were obtained at the same pyrene concentration in bulk sediment. The results showed that organic matter influenced the partitioning of pyrene in the sediment matrix. The concentration of dissolved pyrene in porewater and overlying water was higher in sediment with a high organic content, probably due to an increased DOM concentration. In contrast, the concentration of freely dissolved pyrene was, as expected, higher in sediment with low organic content. Bioaccumulation of pyrene correlated very well with the amount of pyrene passing through the gut, indicating that particle-associated pyrene is bioavailable and that ingestion is an important uptake route. Body burden was correlated neither with total dissolved pyrene nor with freely dissolved pyrene, leading one to reject the hypothesis that pyrene uptake is due to simple diffusion processes from water to sediment. Furthermore, no relation between bioaccumulation and dissolved pyrene passing through the gills via irrigation was observed. Bioconcentration factors relative to sediment and water declined with increasing external pyrene concentrations, enhancing the likelihood of sediment toxicity being underestimated when using these factors in risk assessment.

KEY WORDS: Bioaccumulation · PAH · Infauna · Bioturbation · Partitioning · Sediment

INTRODUCTION

Prediction of the bioavailability of non-polar organic contaminants in aquatic sediments and the exposure of in- and epifaunal biota to these compounds is difficult due to the various chemical, physical and biological factors affecting the partitioning, bioavailability and possible uptake routes of organic compounds by epi- and infauna.

Sediment geochemistry (especially content and quality of organic matter, clay content, ageing and size distribution) influences both the partitioning between particle-bound and dissolved contaminant and bioavailability (Evans et al. 1990, Brannon et al. 1993, Harkey et al. 1995). The life strategies of exposed organisms may determine the relative importance of various uptake routes as well as the actual exposure on a micro-scale since biota interact with the local geochemical environment, hence influencing contaminant fate and therefore their own exposure (Meador et al. 1995, Forbes et al. 1998). The importance of the life history and physiology of the organisms is, however, often neglected in the assessment of sediment toxicity.

In the equilibrium partitioning theory (EPT) used in risk assessment, it is assumed that bioaccumulation—and hence sediment toxicity—is proportional to the concentration of freely dissolved contaminant in water and that the route of uptake has no impact on the final (equilibrium) tissue contaminant concentration (Di Toro et al. 1991). Accordingly, any reduction in the

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freely dissolved contaminant concentration (e.g. increased organic content) should reduce bioaccumulation and sediment toxicity.

Several studies support the EPT and the observation that organic matter apparently causes a decrease in the bioavailability of non-polar contaminants (Carlberg et al. 1986, Swindoll & Applehans 1987, Servos & Muir 1989, Di Toro et al. 1991). These studies and also many sediment-toxicity studies, however, mainly employ pelagic organisms that ingest little or no sediment. Infaunal deposit-feeding species ingest large amounts of sediment, and exposure may be more dependent on feeding rate and assimilation efficiency than on water concentration. Both feeding rate and assimilation of organic contaminants are affected by environmental factors such as food quality and sediment type as well as the contaminant concentration in the sediment (Taghorn & Jumars 1984, Klump et al. 1987, Mayer et al. 1996). If bioaccumulation in infauna is controlled mainly by ingestion, irrigation and other physiological processes, the use of EPT in risk assessment may be misleading. Selective feeding (e.g. avoidance of contaminated particles and/or a decrease in feeding rate or assimilation efficiency caused by a toxic effect) may decrease bioaccumulation, and a proportionality between water and biota contaminant concentration would thus not be expected, contrary to what would be predicted from the EPT. Consequently, both the bioconcentration factor (BCF; defined as C_a/C_w, where C_a and C_w are the contaminant concentrations in the organism and water, respectively) and the bioaccumulation factor (BAF; defined as C_a/C_s, where C_a and C_s are the concentrations in the organism and sediment, respectively) would decrease with increasing external contaminant concentrations, and they would, as Franke (1996) pointed out, be quite misleading and not reflect sediment toxicity.

The polychaete *Arenicola marina*, the common lugworm, is an intense bioturbator, ingesting sediment at a rate several times its own body weight per day (Riisgård & Banta 1998). Hence, the potential bioavailability of particle-bound contaminants is of great importance for these animals. The lugworm is a dominant member of near-shore communities (Reise 1985), and it prefers sandy sediment with a low organic content. It is a conveyor-belt feeder living in J-shaped tubes at a depth of about 25 cm below the sediment surface; it meets its oxygen demands by ventilating (irrigating) its burrow and feeding gallery with overlying water at rates between 10 and 100 ml h^{-1} (Riisgård et al. 1996).

The objective of this study was to examine the bioavailability and possible uptake routes of pyrene to *Arenicola marina*; we used the fact that the partitioning of pyrene between a particle-associated pool and a pool dissolved in porewater and overlying water can be changed by manipulating the organic carbon content in the sediment (Karickhoff et al. 1979, Weston 1990). Theoretically, it is possible to establish experimental systems with similar pyrene concentrations in the bulk sediment but different water concentrations. If bioaccumulation in this species is predictable from the concentration of dissolved pyrene, tissue concentrations are expected to differ in relation to sediment organic content. If, however, bioaccumulation depends on sediment ingestion, such a relationship would not be expected.

MATERIALS AND METHODS

Experimental set-up. Sediment and lugworms were collected at a pristine, near-coastal sandflat heavily inhabited by lugworms in Roskilde Fjord, Denmark. Sediment was sieved (1 mm) *in situ* to remove stones and macrofauna; it was frozen (–20°C) for 2 wk to kill meioinfauna. The salinity at the site at the time of collection was 15‰.

Sediment with a low content of organic matter (0.2% dry weight [dry wt]) was prepared by washing the natural sediment in 15‰ seawater from the sampling site; sediment with an organic content of 4% dry wt was prepared by mixing washed sediment and industrial cellulose fibres with a maximum length of 350 µm (Whatman CF11). Cellulose was chosen as a carbon source instead of, for example, natural muddy sediment to avoid differences in particle size and distribution, bacterial community, food sources for *Arenicola marina*, etc., between sediments with high and low contents of organic matter. Sediment organic content was measured as loss on ignition by combusting sediment for 6 h at 550°C. The sediment mixtures were designated LOC (low organic content, 0.2% dry wt) and HOC (high organic content, 4% dry wt) sediments.

Sediments with nominal pyrene concentrations of 0, 10, 100, 1000 and 10 000 µg kg^{-1} wet weight (wet wt) were prepared using both LOC and HOC sediments. Each pyrene concentration was prepared separately using the following procedure: Pyrene was dissolved in methanol and mixed with 500 ml seawater. The pyrene-methanol-seawater solution was mixed by hand with approximately 1 l of dried sediment. The contaminated sediment was then mixed with 21 l of wet sediment and left 2 to 3 d before being used in the microcosms.

A total of 48 polypropylene tubes (14.5 cm diameter, 18 cm height) were filled with approximately 2000 ml of contaminated sediment and 400 ml of Millipore-filtered (0.2 µm) seawater diluted with deionised water, to give a final salinity of 15‰. This water was

used throughout the experiment and will be referred to as seawater. Each microcosm was fitted with aeration stones, and microcosms were stored for 2 d at experimental conditions (15°C) and kept dark to avoid photodegradation of pyrene. Lugworms collected at the site of sediment collection were incubated overnight in aerated seawater in order to empty their guts before being weighed. Worms with a mean ± SD of 3.6 ± 0.5 g body weight were used.

At the start of the experiment, samples of sediment, interstitial water and surface water were taken for pyrene analysis, and 1 worm was added to each tube. All worms burrowed within 15 min. The experimental period was 14 d, which, according to, for example, Kure (1997) and Christensen et al. (2002), is sufficient time to ensure a steady-state tissue concentration in the lugworms. During the experimental period, overlying water was changed twice and the surface of the sediment was levelled 4 times for feces collection. At the end of the experiment, worms were removed from the cores and sediment sub-samples were taken from each tube after the sediment had been gently mixed.

Feeding rate. Feeding behaviour of each individual was followed during the test by collecting fecal casts 6 to 8 h after the sediment surface had been levelled. Fecal pellets were dried (20°C) on filter paper for 2 d and weighed. Since the experimental lugworms feed on 96% (HOC sediment) or 99.8% (LOC sediment) inorganic sand particles, excreted material is very close to ingested material on a weight basis, and egestion rate is assumed to be a reasonable estimate for feeding rate.

Irrigation measurements. Irrigation rates for *Arenicola marina* were measured once a week using ^{109}Cd as a tracer. ^{109}Cd was chosen instead of, for example, Br since it is easier and less time consuming to measure ^{109}Cd than to Br and because it is possible to measure irrigation repeatedly during an experimental period with ^{109}Cd but not with Br.

When overlying water was removed, 400 ml of fresh seawater was added. Carrier-free ^{109}Cd was added as a tracer to the overlying water at 2000 CPM (counts per min) ml^{-1}. Samples (2 ml) of overlying water were taken at regular time intervals for 24 h, and the ^{109}Cd activity was quantified by γ-counting in a Packard Cobra™ II auto-gamma counter.

Since Cd is particle reactive and the Cd flux can be considered almost completely unidirectional from water to sediment during the time-scales used for irrigation measurements (Petersen et al. 1998, Rasmussen et al. 2000), it is possible to develop a model which can be used to calculate irrigation rates from *Arenicola marina*.

In microcosms without worms the observed decrease in overlying water Cd concentration is due to both dilution and sorption, which is limited to diffusion processes over the surface of the sediment core. Dilution in this case apparently follows first-order kinetics (Rasmussen et al. 1998, this paper), and it can be described by

$$\frac{dC}{dt} = -kC \quad (1)$$

where k is an empirically determined removal rate (h^{-1}) due to diffusion and sorption of Cd at the sediment surface and C is the Cd concentration in overlying water.

In microcosms with worms, dilution is also due to injection of surface water into the feeding funnel, and sorption may take place at all sites in contact with surface water injected by the worm. The worm injects overlying water into the sediment at a rate defined as $P(t)$ (ml h^{-1}), and since the volume of overlying water, V_0 (ml), is constant, the water pumped into the sediment must return from the sediment to the surface water. As all Cd from injected water is assumed to remain in the sediment, the changes in Cd concentration in overlying water in systems with *Arenicola marina* can then be described as

$$\frac{dC}{dt} = -\frac{P(t)}{V_0}C - kC \quad (2)$$

The solution to this differential equation is

$$C(t) = C_0 \exp\left[-\left(\frac{P(t)}{V_0} + k\right)t\right] \quad (3)$$

where C_0 is the Cd concentration in overlying water at $t = 0$. By ln-transforming data from cores with *Arenicola marina* and making linear regression, the coefficient $K = P(t)/V_0 + k$ equals the slope of the linear regression. Since V_0 is easy to measure or calculate and k can be determined from ln-transformed data from cores without *A. marina*, the irrigation rate can be calculated using

$$P(t) = (K - k)V_0 \quad (4)$$

Pyrene analysis. Pyrene concentrations in bulk sediment, interstitial water, surface water and tissue were measured at the beginning and end of the experimental period by gas chromatography/mass spectrometry (GC/MS). GC/MS was performed in SIM mode on an HP 5890 GC with an HP 5971A MS detector and an HP-5 column. In all extractions anthracene was used as an internal standard. Pyrene was extracted from sediment by sonication with dichloromethane (DCM) (Kure 1997): 10 g of dry sediment was extracted with 6 ml DCM, whirl-mixed for 1 min and sonicated for 10 min. The supernatant was transferred to test tubes after centrifugation at 1100 × g, and the extraction was repeated a total of 3 times. The combined supernatants (approx. 18 ml) were evaporated in atmospheric air.

Samples were redissolved in 2 ml of DCM and transferred to GC vials. The recovery was >91% (data not shown).

Porewater was collected by centrifuging (120 × *g*, 10 min) wet sediment in double centrifuge tubes with a Whatman GF/C filter in between (Thomsen & Kristensen 1997). The total concentration of dissolved pyrene (i.e pyrene bound to dissolved organic matter [DOM], pyrene bound to light pellets and freely dissolved pyrene) was liquid extracted with DCM. The samples (30 ml of overlying water or 15 ml of porewater) were shaken with 1 × 10 + 2 × 5 ml of DCM. The organic phase was dried with Na_2SO_4 and evaporated in air to approximately 1 ml. The procedure for extraction of freely dissolved pyrene from the water was performed according to Larsen (1996) and Kure (1997). Briefly, 30 ml of water was centrifuged (23 000 × *g* for 30 min) to precipitate light pellets. The upper 10 to 15 ml (water without light pellets) was carefully removed to a measuring cylinder. Freely dissolved pyrene was then separated from DOM-bound pyrene by solid-phase extraction using a nonpolar C-18 cartridge (500 mg in a 6 ml glass reservoir; Chromabond) that selectively retains only the freely dissolved PAH fraction. The cartridge was preconditioned with 5 ml of methanol and 10 ml of Millipore filtered water. Ten ml of centrifuged pore- or overlying water was then vacuumed directly through the C-18 cartridge at a speed of 4 ml min^{-1}. The retained pyrene was released from the cartridge by passage of DCM.

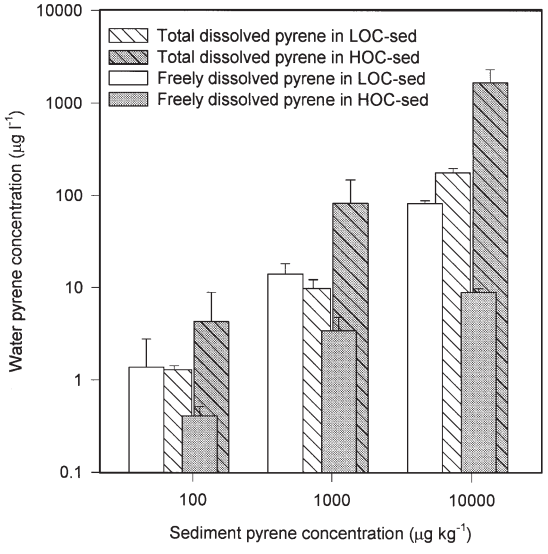

Fig. 1. Pyrene partitioning in the sediment matrix. Measured concentrations of freely dissolved pyrene and total dissolved pyrene in HOC and LOC (high [4% dry wt] and low [0.2% dry wt] organic content) sediment relative to sediment pyrene concentration (n = 4, ±SD). No significant difference between pyrene concentrations in porewater and overlying water was detected ($p > 0.21$). In sediment with 0 and 10 ppb pyrene, water pyrene concentrations were below the detection limit

After emptying of the gut in clean seawater for 8 to 10 h, worms were transferred to test tubes, they were mashed with a glass spatula and pyrene was extracted from tissue as described for sediment.

Statistical analysis. Growth rates, feces production and irrigation rates as well as pyrene concentration in tissue, sediment and water fractions among treatment groups were analysed by 2-way (pyrene concentration and organic content) ANOVA after assuring that the ANOVA assumptions (normal distribution of residuals and homogeneous error) were fulfilled. For pyrene tissue concentrations errors were not homogeneous and data were thus log-transformed before the ANOVA analysis. ANOVA main effects were further analysed using a post hoc Honestly Significantly Different (HSD) test. Possible correlations between various routes of uptake and bioaccumulation were investigated using Pearson product-moment correlation analysis. All statistical analyses were based on a 5% significance level.

RESULTS

Pyrene in the sediment-water system

Bulk-sediment pyrene concentrations were 70 to 102% of the nominal concentrations. Pyrene concentrations measured at the beginning and end of the experiment were not significantly different, and no difference between sediment pyrene concentration in LOC and HOC sediment was detected ($p \geq 0.1$).

The concentration of total dissolved pyrene (i.e. pyrene bound to light pellets, DOM-bound + freely dissolved pyrene) was, as expected, lowest at 100 ppb sediment pyrene concentration and highest at sediment pyrene concentrations of 10 000 ppb (Fig. 1). A significant difference was detected between HOC and LOC sediments ($p \leq 0.035$), with up to 10 times higher concentrations of dissolved pyrene in HOC sediment than in LOC sediment. In LOC sediment, the concentrations of dissolved and particle-associated pyrene were almost proportional, with the dissolved concentration being 1 to 2% of sediment concentrations, whereas the concentrations of dissolved pyrene in HOC sediments were between 3 and 17% of the particle-associated pyrene concentrations.

Also, the concentration of freely dissolved pyrene increased with increasing sediment pyrene concentration ($p \leq 0.002$), and a significant difference between LOC and HOC sediments was detected ($p \leq 0.0002$). In contrast to total pyrene, the concentrations of freely dissolved pyrene in LOC sediment were higher than in HOC sediment (Fig. 1).

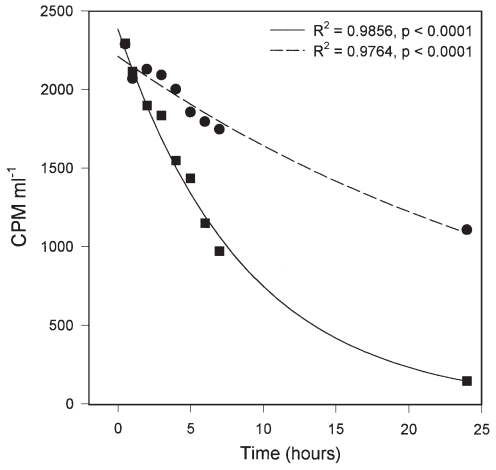

Fig. 2. Measured removal kinetics of ^{109}Cd from overlying water in cores with (■) and without (●) *Arenicola marina*. Lines represent model predictions (see 'Materials and methods')

Calculated K_p values (K_p indicates the partitioning between particle-associated pyrene and freely dissolved pyrene in water; Karickhoff et al. 1979) were 87 ± 22 and 508 ± 340 l kg^{-1} in LOC and HOC sediment, respectively. In LOC sediment freely dissolved pyrene accounted for 50 to 103% of the total dissolved pyrene pool, whereas freely dissolved pyrene only accounted for 0.5 to 13% of the total dissolved pyrene pool in HOC sediment.

Arenicola marina

All worms survived throughout the experiment except worms held in the highest pyrene concentration (10 ppm). The mortality was 2/4 in 10 ppm LOC sediment and 3/4 in 10 ppm HOC sediment.

The relative growth rate calculated as (wet $wt_{Day\ 14}$ − wet $wt_{Day\ 0}$)/wet $wt_{Day\ 0}$ was affected by pyrene exposure, there being significantly higher growth rates at low pyrene concentrations than at the highest concentration ($p < 0.04$). In the control group and the 10 ppb group, worms increased their body weight approximately 10% during the experimental period corresponding to an actual growth rate of 0.03 g d^{-1}, whereas negative mean growth rates of up to 30% were detected at higher pyrene concentration (data not shown). No significant difference in growth rate was observed between worms held in HOC and LOC sediments ($p = 0.4$).

Irrigation rates

The removal of Cd from overlying water seemed to result in exponentially decreasing water concentrations in systems both with and without *Arenicola marina*, indicating that sediment binding sites were not saturated and that the model developed above describes the removal processes (Fig. 2). The model was used to calculate irrigation rates as described above, and the results are shown in Fig. 3. In 2 cores, however, an apparently steady-state water concentration much higher than 0 seemed to appear within 15 to 20 h, indicating that desorption of Cd from particles to the overlying water was not negligible. In these situations only the linear part of the ln-transformed data (obtained during the first 7 h of the measuring period) was used to calculate irrigation rates.

Irrigation rates were significantly affected by the sediment pyrene concentration ($p \leq 0.01$), with the 0 and 10 ppb groups having up to 4 times higher pumping rates than worms exposed to 10 000 ppb (Fig. 3). No significant difference was detected between irrigation rates in HOC and LOC sediments, and results are shown as an average of both organic concentrations in Fig. 3.

Irrigation was linearly correlated with the concentration of total dissolved pyrene ($p = 0.01$), but with a high variability not explained by pyrene concentrations ($R^2 = 0.1$). Irrigation was not significantly correlated with the concentration of freely dissolved pyrene ($p = 0.4$, $R^2 = 0.01$).

Feeding rates

Feeding rates were significantly affected both by the sediment concentration of pyrene ($p < 0.001$) and

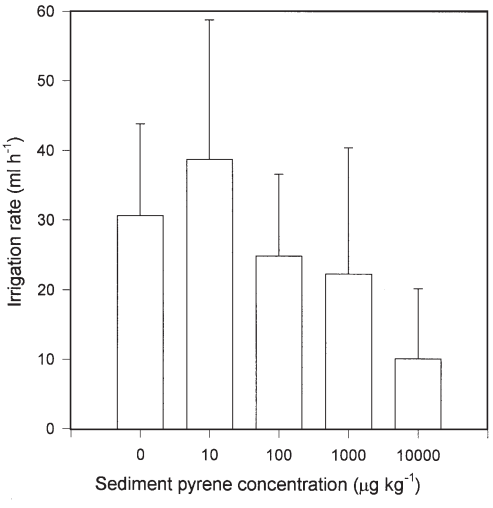

Fig. 3. Irrigation rates—average of data from LOC and HOC sediments versus sediment pyrene concentration (n = 6, ±SD). Irrigation rates were measured using Cd removal from overlying water and model calculations (see 'Materials and methods')

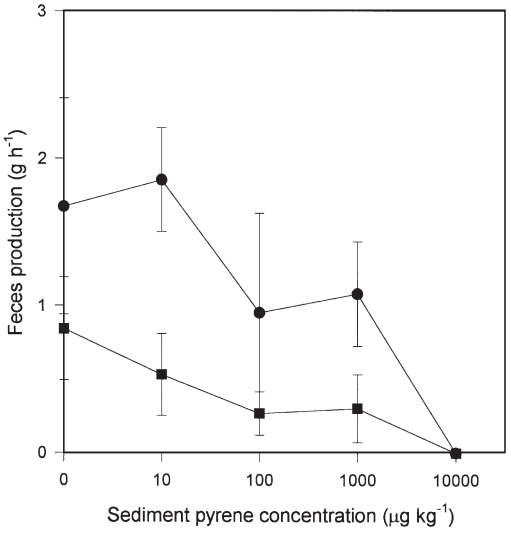

Fig. 4. Feeding rates for worms held in LOC (●) or HOC (■) sediments measured as defecation rate (n = 5, ±SD)

by the sediment organic content ($p < 0.001$) (Fig. 4). In general, feeding rates tended to decrease with increasing sediment pyrene concentrations, with the control group having a significantly higher feeding rate than worms in sediment with 10 ppm pyrene. For worms held in sediment with 10 ppm pyrene, feces production was observed but not quantified (i.e. no production in the 6 to 7 h measuring period). Feces production in LOC sediment was 2.3 to 3.8 times higher than in HOC sediment and the measured feces production both in HOC and LOC sediment was within the range of reported values for *Arenicola marina* (Cadeé 1976).

Accumulation of pyrene by *Arenicola marina*

Tissue pyrene concentrations in *Arenicola marina* were significantly different in worms from sediments with 10, 100 or 1000 ppb pyrene ($p \leq 0.002$), with the group exposed to 10 ppb pyrene having the lowest tissue concentrations and the group exposed to 1000 ppb pyrene having the highest tissue concentrations (Table 1). No difference was observed between tissue concentrations in worms held in sediment with 1000 or 10 000 ppb pyrene.

Bioaccumulation in *Arenicola marina* was also significantly affected by the organic content ($p = 0.01$). Worms held in LOC sediment reached body burdens that were approximately 1.5 times higher than those in worms held in HOC sediment.

Assuming steady state after 14 d of exposure, tissue concentrations measured in *Arenicola marina* were used to calculate bioaccumulation factors from sediment (BAF) and water (BCF). Worms in all treatments reached pyrene concentrations higher than pyrene concentrations in sediment, resulting in $BAF_{Day\ 14}$ values around 40 and 25, in LOC and HOC sediments respectively at the 3 lowest sediment concentrations. The $BAF_{Day\ 14}$ was only about 2 for the group exposed to 10 000 ppb pyrene (Fig. 5).

Table 1. Measured tissue concentrations in *Arenicola marina*. All concentrations are in ppb, and numbers in brackets indicate the standard deviation. Significant differences between LOC and HOC sediment ($p < 0.01$) and between different sediment pyrene concentrations ($p < 0.002$) were found

Sediment concentration	Tissue concentration (worms in LOC sediment)	Tissue concentration (worms in HOC sediment)
10	440 (100)[a]	240 (80)[a]
100	3370 (1170)[a]	2570 (1040)[a]
1000	37820 (10000)[a]	26600 (10800)[a]
10000	24640 (3700)[b]	19800[c]

[a]n = 5, [b]n = 2, [c]n = 1

BCF values relative to total pyrene and freely dissolved pyrene in pore water (BCF_{pw} and BCF_{fpw}, respectively) were much greater than the BAF values, and both decreased with increasing external pyrene concentrations (Fig. 6).

In this experiment there was no significant correlation between tissue concentration and the concentration of total dissolved pyrene ($p = 0.4$) and between tissue concentration and freely dissolved pyrene ($p = 0.06$) (Fig. 7).

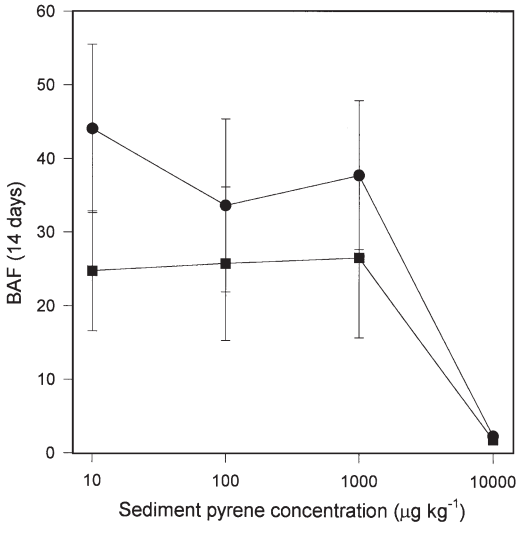

Fig. 5. Bioaccumulation factors (BAF: tissue/sediment ratio) for *Arenicola marina* held for 14 d in LOC (●) or HOC (■) sediments versus sediment pyrene concentration (n = 5, ±SD)

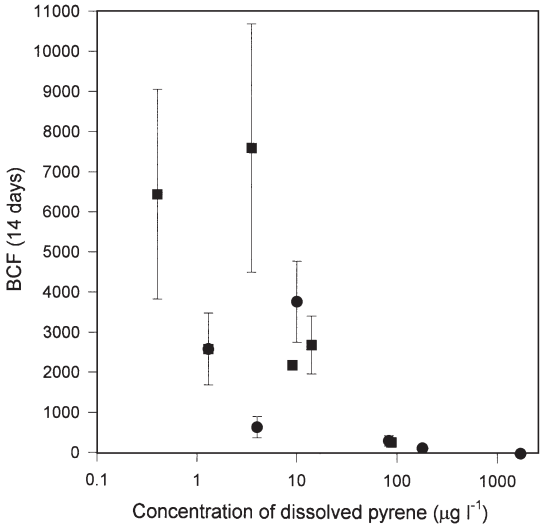

Fig. 6. Bioconcentration factors (BCF) for *Arenicola marina* held for 14 d versus total pyrene concentration in porewater (●) and versus the concentration of freely dissolved pyrene (■). BCF values for worms held in 10 ppb sediment were not calculated since porewater concentrations were below the detection limit (n = 5, ±SD)

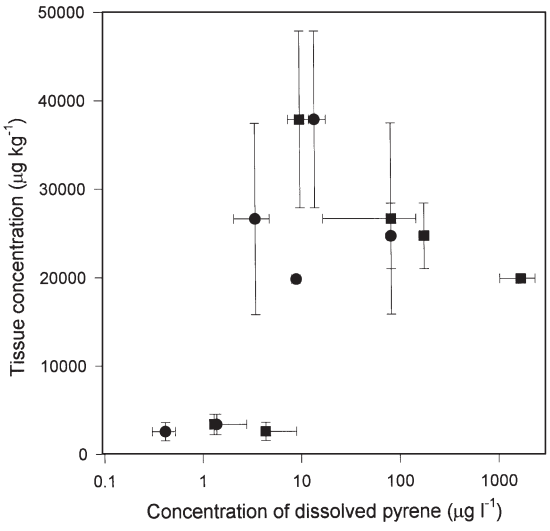

Fig. 7. Tissue pyrene concentration in *Arenicola marina* versus the concentration of total dissolved pyrene (■) or freely dissolved pyrene (●) (n = 5, ±SD for tissue pyrene concentration; n = 4, ±SD for [total or freely] dissolved pyrene). The correlation between tissue pyrene concentration and the concentration of dissolved pyrene (both total and freely dissolved) was not significant

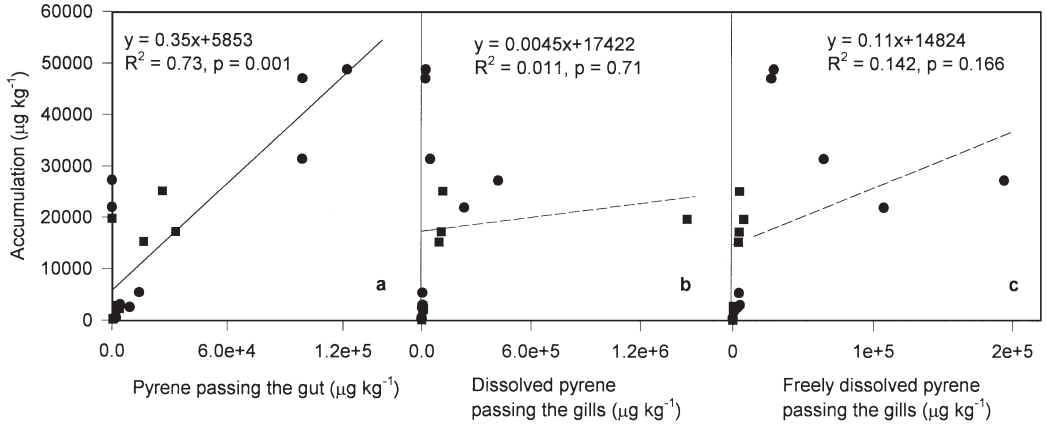

Fig. 8. Correlation between bioaccumulation and the amount of (a) pyrene passing the gut calculated as [feeding rate (g g^{-1} d^{-1}) × sediment concentration (µg g^{-1}) × 14 (d)] and between bioaccumulation and the amount of (b) total and (c) freely dissolved pyrene passing the gills calculated as [irrigation rate (ml g^{-1} d^{-1}) × concentration of dissolved pyrene in overlying water (µg ml^{-1}) × 14 (d)]. Symbols denote calculations based on data from LOC (●) and HOC (■) sediments

A significant correlation was, however, observed between pyrene ingested during the experiment and pyrene accumulation in *Arenicola marina* ($p < 0.001$, $R^2 = 0.73$, Fig. 8a). No correlation was observed between bioaccumulation and dissolved pyrene passing the gills ($p = 0.71$, $R^2 = 0.011$, Fig. 8b) or bioaccumulation and freely dissolved pyrene passing the gills ($p = 0.166$, $R^2 = 0.142$, Fig. 8c).

DISCUSSION

Pyrene distribution in the sediment matrix

According to the EPT, the concentration of (freely) dissolved PAH in porewater (C_w) is proportional to the sediment PAH concentration (C_{sed}) and inversely proportional to the fraction of organic carbon content in

the sediment (f_{oc}). Accordingly, the porewater concentration can be calculated using the empirical relation (Van der Kooij et al. 1991)

$$C_w = \frac{C_{sed}}{0.6K_{ow}f_{oc}} \quad (5)$$

hence $C_{sed}/C_w = K_p = 0.6K_{ow}f_{oc}$, where K_p is the partitioning coefficient between particle associated pyrene and freely dissolved pyrene in water and K_{ow} the octanol-water partitioning coefficient. Unfortunately, total organic carbon (TOC) was not measured in the sediment used in this experiment; however, TOC in sediment taken from the same location at a different time was measured to be 0.12%, equivalent to a f_{oc} value of 0.0012 (Hansen unpubl.). K_p was calculated from this value and a K_{ow} for pyrene of $10^{5.05}$ (Ellington & Stancil 1988) to be 80.79. This value is close to the measured K_p value of 87 ± 22 in LOC sediment. If we assume that the TOC value is also representative for our experiment, the result indicates the validity of EPT even at this low TOC value, which is below levels normally used in EPT work (>0.2%, DiToro et al. 1991). The good agreement between the predicted and measured K_p value (and hence water pyrene concentrations) could, however, be a coincidence.

As expected, manipulation of the sediment organic content affected the partitioning of pyrene between particles, porewater and overlying water, so the same total pyrene concentration in bulk sediment yielded different pyrene concentrations in the water phases. Addition of cellulose reduced the concentration of freely dissolved pyrene due to an increased sorption to particulate organic matter and DOM. Since C_w is inversely proportional to f_{oc} (Eq. 5), the concentration in HOC sediment should be 20 times lower than in LOC sediment. However, LOC sediment only contained up to 9.2 times more freely dissolved pyrene. Several factors affect the binding capacity of PAHs in sediments, including the quality of organic matter (DeWitt et al. 1992), particle size (Evans et al. 1990) and ageing (Harkey et al. 1995), and the relatively high water pyrene concentration in HOC sediment indicates that cellulose has a lower binding capacity for PAHs than 'average' organic matter, which seems reasonable considering the high concentration of hydroxyl groups in cellulose.

Even though the concentration of freely dissolved pyrene was reduced, the total amount of dissolved pyrene was higher in sediment with cellulose, most likely due to an increased DOM concentration in interstitial and overlying water. An increased content of total organic matter might increase the apparent water solubility of PAHs and other hydrophobic compounds if the organic matter is water soluble, e.g. cellulose or humic acids (Chiou et al. 1986).

Bioaccumulation and bioavailability

Previous studies have shown that both organic matter quantity and quality apparently affect bioavailability of hydrophobic compounds (Weston 1990, Landrum et al. 1996, Gunnarsson et al. 1999). The often observed decrease in bioaccumulation with increasing organic content is normally explained by a reduced amount of (freely) dissolved contaminant (Landrum et al. 1985, Swartz et al. 1990, Park & Erstfeld 1999). In this study the higher pyrene concentrations found in worms from LOC compared to HOC sediment can, however, not be explained by differences in the concentration of freely dissolved pyrene, since no statistically significant correlation between porewater pyrene concentration (both total and freely dissolved) and tissue concentration was observed (Fig. 7).The higher tissue concentrations in worms in LOC sediment than in HOC sediment observed in the present study did, however, not show a statistically significant correlation with porewater pyrene concentration (neither total nor freely dissolved; Fig. 7). If pyrene uptake was controlled by diffusion of freely dissolved pyrene from porewater, bioconcentration would be proportional to the concentration of freely dissolved pyrene; hence, BCF_{fpw} values would be equal among exposure concentrations. In this study both BCF_{pw} and BCF_{fpw} decreased with increasing porewater concentration (Fig. 6). Also, in both HOC and LOC sediments, tissue pyrene concentrations were the same or lower at 10 ppm than at 1 ppm pyrene, indicating that attainment of equilibrium is not governed by diffusion alone.

Since tissue concentration in *Arenicola marina* is not correlated with porewater pyrene concentration, the lower tissue concentration found in worms held in HOC sediment is probably not caused by decreased bioavailability. Besides changing the partitioning of PAHs in the sediment matrix, organic matter also changes the rate of processes offering potential uptake routes (sediment ingestion, assimilation and irrigation) for infauna (Taghorn & Jumars 1984, Klump et al. 1987). Several studies indicate that sediment ingestion is an important route for bioaccumulation of highly hydrophobic contaminants (Weston 1990, Meador et al. 1995, Leppänen & Kukkonen 1998), whereas smaller and more water-soluble PAHs probably are taken up from porewater.

In the present study, a significant correlation was found between pyrene ingested through feeding and pyrene tissue concentration. This indicates that ingestion is a major route of uptake and that this route of uptake is capable of explaining approximately 70% of the variation in observed tissue pyrene concentration (Fig. 8a). Furthermore, the calculated assimilation efficiency (i.e. the slope of the linear regression) was esti-

mated to be 35%, which is within the range of reported assimilation-efficiency values for deposit-feeding macrofauna (e.g. Gordon 1978, Kukkonen & Landrum 1995). It is therefore likely that the observed difference between tissue concentrations in LOC and HOC sediments is caused by differences in feeding rate and not changed bioavailability. Feeding rates in deposit feeders are affected by food availability and quality (Taghon & Jumars 1984, Lopez & Levinton 1987) often resulting in an increased feeding rate in sediments with low organic content (low nutritional value) in order to meet energetic requirements. In this experiment cellulose (a poor nutritional source) was used to enrich the sediment. A possible explanation for the observed low feeding rate in HOC sediment (Fig. 4) could be that cellulose was utilized as a carbon source by bacteria, and the resulting biomass was in turn used by *Arenicola marina*. There was no significant difference in growth rate between worms held in LOC and HOC sediments, even though worms in LOC sediment ingested 3 times more sediment, supporting the hypothesis that cellulose or food induced by cellulose (bacteria) is a carbon source for *A. marina.*

Of course, any other uptake route related to feeding activity could explain the observed correlation between feeding and accumulation. Surprisingly, no correlation between irrigation and feeding rate was observed ($p = 0.11$), but rather a decoupling at the lowest pyrene concentrations. Thus, since the calculated amount of pyrene passing the gills was not related to accumulation (Fig. 8b,c), uptake from water is probably not a dominant uptake route. A functional relation between feeding and accumulation results in a strong correlation between feeding rate and BAF. In sediment-toxicity assessment, low BAF values generally signal low risk. However, if feeding rate in general is affected by sediment toxicity (among other factors), then increasing sediment toxicity would result in decreasing BAF values, and during long-term exposure, a toxin-induced decreased feeding rate is likely to affect individual fitness and thereby conceivably population stability.

In this study, tissue pyrene measurements were based on total worm homogenates. A significant contribution to total tissue pyrene could conceivably be adhesion to the surface of the worms where toxic damage most likely is low. The observed relationships between external pyrene concentration, ventilation, ingestion and accumulation indicate, however, that this is unlikely to be the case.

If ingestion is a significant uptake route for hydrophobic contaminants, as indicated in this experiment, the reliability of toxicity predictions based on the EPT might be doubted. Often ingestion and other physiological processes are affected by toxic contaminants, resulting in decreasing BAF or BCF values with increasing sediment contaminant concentration; hence, the actual sediment toxicity will be underestimated. Differences in feeding behaviour including sediment avoidance at high external contaminant concentrations might explain why the EPT, in some cases, fails to predict bioaccumulation as well as sediment toxicity (e.g. Harkey et al. 1994, Kukkonen & Landrum 1994, Kure 1997). In general, there is a need for incorporation of infauna (feeding) behaviour in riskassessment models for sediment-associated contaminants.

Acknowledgements. This work is part of the BIOPRO Centre activities supported by the Danish Environmental Research Program.

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: October 23, 2000; Accepted: October 15, 2002
Proofs received from author(s): December 19, 2002