Bioavailability of PAHs in Aluminum Smelter Affected Sediments: Evaluation through Assessment of Pore Water Concentrations and in Vivo Bioaccumulation

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Bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) from coal tar pitch polluted sediments was predicted by (1) a generic approach based on organic carbon–water partitioning and Gibbs linear free energy relationship (between K_{OW} and K_{OC}), and (2) measurements of freely dissolved concentrations of PAHs in the sediment pore water, using passive samplers and solid phase extraction. Results from these predictions were compared with those from in vivo bioaccumulation experiments using *Nereis diversicolor* (Polychaeta), *Hinia reticulata* (Gastropoda), and *Nuculoma tenuis* (Bivalvia). Measured sediment/water partition coefficients were higher than predicted by the generic approach. Furthermore, predicted biota-to-sediment accumulation factors (BSAFs) derived from measured pore water concentrations were more in agreement with the bioaccumulation observed for two of the three species. Discrepancies associated with the third species (*N. tenuis*) were likely a result of particles remaining in the intestine (as shown by microscopic evaluation). These results indicate the importance of conducting site-specific evaluations of pore water concentrations and/or bioaccumulation studies by direct measurements to accurately provide a basis for risk assessment and remediation plans. The importance of knowledge regarding specific characteristics of model organisms is emphasized.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a prioritized group of environmental contaminants in Norway and abroad. In Norway, point sources of PAHs have primarily been discharges from aluminum and ferromanganese smelters using the Söderberg anode in combination with seawater scrubbing of pot room gas or flue gas. Access to hydroelectric power made it possible to establish energy-consuming industry in Norway early in the 20th century. The invention of the Söderberg electrode (1917–1919) was a milestone for the metallurgical industry and the accelerating industrial development gave rise to several of the present local and regional environmental issues (1). In 2005, land based industry (mainly the aluminum industry) in Norway accounted for approximately 66% of the total national PAH discharge (2). Due to more recent legislation, smelters based on the Söderberg technology have been modernized or discontinued (3).

Aluminum is produced by electrolytic reduction in pots containing Al_2O_3 in an electrolyte of molten Na_3AlF_6. The carbon anode material is consumed during the process, thus requiring a continuous supply. With the Söderberg technology, the anode carbon is supplied as a pasty mix of petrol coke and coal tar pitch, baked to solid carbon in heat from the production process. PAH and volatile organic compounds evaporate, giving rise to the pollution problems associated with the discharge (1). The PAH composition in coal tar pitch is typical for a pyrogenic PAH mixture.

Although high concentrations of PAHs have been found in sediments in the vicinity of the smelters, observed environmental effects seem to be less than anticipated (1, 4). It was hypothesized that the underlying reason was that the PAHs from the Söderberg process were very strongly adsorbed to particles, rendering them less bioavailable than anticipated based on the then-prevailing theory of natural organic matter in soils and sediments being the main sorption phase for hydrophobic contaminants (5). These properties may be attributed to soot (black carbon) and soot-like materials in the sediments (6–9), and the effects of such geosorbents have been the subject of much attention and debate (9, 10). During the past two decades a growing number of observations indicate, however, that the (linear) one domain sorption model (describing a single partition coefficient for organic matter) is too simple and that at least a dual mode model is needed (involving nonlinear sorption, expressed through Freundlich coefficients 6, 7, 9). Black carbon has later been shown to contribute significantly to these alternative sorption patterns (9). Evidence for this strong sorption hypothesis was documented by Næs et al. (11) who quantified PAHs in effluent and recipient water outside a Söderberg aluminum smelter in Norway. Measurements showed that the partition coefficients were factors of 2–3 orders of magnitude higher than would be expected on the basis of organic carbon–water partitioning alone. This was further supported by a study where PAH partition coefficients (K_{OC}s) between sediment organic carbon and water were determined using 114 sediments (including outside Al smelters; 12, 13).

Contamination of sediments with PAHs (and other pollutants) requires feasible and reliable risk assessment. Tools/guidelines for such assessments have been developed in Norway (14) and Europe (15). To render the risk assessment of contaminated sediments feasible for a large number of actors, it is often of a generic nature. A common approach in these risk assessments is consequently comparing total contaminant concentrations in the sediments (normalized to the solid phase) with fixed environmental quality standards (e.g., based on ecotoxicological criteria) (14, 15). However, risk may be overestimated, as marginal values are deduced from generic sorption parameters, only considering sorption of contaminants to natural organic matter (16). Many studies, however, have shown that sediments at different locations have specific compositions of sorption domains within the particle phase with different binding strengths (17, 18). Therefore, measurements of bioavailable fractions or bioaccumulation may be cost-effective initiatives to ensure rational remediation plans and actions (16, 17). The Norwegian guidelines for risk assessment of contaminated sediments allow such site-specific evaluations (14), and the use of passive samplers and solid-phase extraction has been a favored approach (18, 19).

The objective of the present study was to predict bioaccumulation of PAHs observed in in vivo bioaccumulation

experiments using coal tar pitch polluted sediments, by two approaches:

1. A generic approach based on organic carbon−water partitioning and Gibbs linear free energy relationship (between K_{OW} and K_{OC}).
2. Measurements of freely dissolved concentrations of PAHs in the sediment pore water, using passive samplers and solid-phase extraction (SPE).

To address this objective, sediments were collected outside five Scandinavian aluminum plants. The passive sampling material used to measure freely dissolved concentrations in pore water was polyoxy methylene (POM) [8, 20]. In vivo bioaccumulation experiments were conducted using an established experimental setup [21] and the species *Nereis diversicolor* (Polychaeta) and *Hinia reticulata* (Gastropoda). In addition, *Nuculoma tenuis* (Bivalvia) was employed.

Materials and Methods

Chemicals. Solvents and other chemicals used are listed in the Supporting Information. Polyoxymethylene (POM; 0.5 mm thick sheets, density 1.41 g/cm^3) was obtained from Vink Plast AS, Oslo, Norway.

Sediments. Sediments were collected from four locations in Norway and one in Sweden, using a 0.1 m^2 Van Veen grab. The sites represent the primary seawater recipients for aluminum smelters using or having recently used the Söderberg technology. The sites are presented as Smelters A, B, C, D, and E. At all locations, sediments were sampled (approximately 10 L per site, representing the top 15 cm) from one representative site, apart from one fjord where three sites were sampled at increasing distance from the smelter (Smelter A). In addition, uncontaminated control sediment was collected at a fixed location in the outer Oslofjord (N 59° 27.6′, E 10° 37.4′), previously employed in bioaccumulation experiments and documented to have very low concentrations of organic pollutants [21]. Furthermore, a spiked sediment (spiked with selected PAHs) was prepared (based on the control sediment; see Supporting Information for further details). All sediments were stored at ∼4 °C after collection. Before the experiments, they were homogenized (60−90 s) using a mechanical stirrer. Aliquots were taken for chemical analyses, determination of particle/water-partition coefficients (K_{d}s) and bioaccumulation experiments.

Organisms. *Nereis diversicolor* and *Hinia reticulata* were collected at the same location as the control sediment as described earlier [21]. In addition, *Nuculoma tenuis* was collected at ∼100 m depth near Drøbak (N 59° 39.7′, E 10° 37.3′) by the use of the Van veen Grab and sieves (1 mm and 5 mm). After an acclimation period in the control sediment and the same water for use in the experiments of ≥14 days, the organisms were employed in the experiments.

These generally abundant species [22] intimately interact with the sediment, and are possible to obtain in sufficient numbers and to hold in aquaria for extended periods [21]. *N. diversicolor* and *H. reticulata* both prefer sandy or muddy sediment and tolerate low salinities. *H. reticulata* is primarily a scavenger, whereas *N. diversicolor* is omnivorous and one of the most studied marine invertebrates. It has often been used in bioaccumulation studies [23, 24]. *N. tenuis* is a primitive protobranch bivalve, fairly common in Norwegian waters. It is a selective subsurface deposit feeder, feeding by the use of ciliated tentacles that extend into the substrate. Particles are carried to the labial palps for sorting prior to ingestion.

Generic Approach. The generic organic carbon−water partition coefficients (K_{OC}s) were derived from K_{OW}s (octanol/water-partition coefficients), by the (Gibbs) free-energy relationship, following Karickhoff et al. [5]:

$$\text{Log } K_{oc} = \text{Log } K_{ow} - 0.21 \qquad (1)$$

The K_{OW}s applied were according to Mackay et al. [25]. Assuming that the partition coefficient between organism lipids and water equals K_{OW} ($K_{lipid} = K_{OW}$), the generic Biota to Sediment Accumulation Factors (BSAF$_{Predicted (Generic)}$) equal 1.61 (regardless of compound and K_{OW}; see Supporting Information for more details).

Equilibrium Pore Water Concentration Approach. Estimates of freely dissolved fractions of PAHs in the sediments were obtained with a solid-phase extraction (SPE) method using plastic polyoxymethylene (POM) described by Jonker and Koelmans [20], with some modifications by Cornelissen and Gustafsson [8]. Prior to the application of the method to the sediments, partition coefficients of PAHs between POM material and water (K_{POM}) were evaluated (see Supporting Information for details).

The K_{d}s for the sediments were determined as follows. For each of the sediments ∼2 g of sediment (wet) and ∼1 g of POM (both weighed accurately) were transferred to bottles (triplicates for each sediment) and 300 mL of distilled water was added (containing 25 mg/L sodium azide and 0.01 M calcium chloride; as described by Jonker and Koelmans [20]). In addition a bottle containing only POM and water was added (blank). Subsequently all bottles were placed on a shaker (100 rpm). After 30 days the POM was removed (using forceps) and carefully rinsed in distilled water. The POM was then transferred to a glass vial (Fisher Scientific, Memphis, TN), before 40 mL of dichloromethane and internal standards (200 ng of each of the deuterated PAH components, see Supporting Information for details) were added. Vials were sealed and placed on a shaker (250 rpm) for 7 days. The extract was decanted to another vial before the extract was evaporated to 200 µL prior to chemical analyses by gas chromatography and mass spectrometry (GC-MS; see below). The detection limit was 2 ng/g POM for each PAH compound (>3 times signal noise).

The PAH mass balance in the three-phase system— particles ⇌ water ⇌ POM— is expressed as described by Jonker and Koelmans [20] (see Supporting Information for more details). Predicted BSAFs (BSAF$_{Predicted (SPE)}$) were calculated as

$$\text{BSAF}_{\text{Predicted(SPE)}} = \frac{C_{\text{lipid}}}{C_{\text{OC}}} = \frac{K_{\text{lipid}} \cdot C_{\text{W}}}{\left(\dfrac{C_{\text{S}}}{f_{\text{OC}}}\right)} \qquad (2)$$

where C_{lipid} is the lipid normalized concentration in the organism, C_{OC} is the organic carbon normalized concentration in the sediment, C_{W} is the concentration in (pore) water, C_{S} is the concentration of the compound in the sediment (µg/kg dry wt.), f_{OC} is the fraction of organic carbon content in the sediment (dry/dry), and $K_{lipid} = K_{OW}$.

The in Vivo Exposure Approach. The experimental setup for the in vivo experiments was established a decade ago and described previously [21] (see Supporting Information for more details). Temperature and salinity in the primary header tank were 8.2−9.4 °C and 33.9−34.3 ‰, respectively. Three replicate aquaria were used for each test sediment. Each aquarium contained 20 individuals of *N. diversicolor*, 10 individuals of *H. reticulata*, and 9 individuals of *N. tenuis*. The duration of the accumulation period was 28 days, to ensure steady-state tissue residues [26]. Upon termination of the experiment, the test organisms were retrieved from the aquaria. Individuals of *N. diversicolor* were transferred to beakers filled with seawater in which they were held for 6−8 h to empty all remnants of sediments from the intestines before freezing (−20 °C) in glass containers. The soft parts of *H. reticulata* were separated from their hard shell using a nut-cracker. The soft parts were then rinsed in seawater and transferred to glass containers before storage at −20 °C until chemical analysis. Individuals of *N. tenuis* were rinsed

in seawater and frozen (−20 °C) in glass containers. Upon thawing (prior to analysis), the soft parts of *N. tenuis* were excised from the shells. All individuals of the same species from each aquarium (replicate) were pooled into one sample. To optimize the amount of biomass for analysis, the triplicate samples of *N. tenuis* had to be pooled.

Reproducibility of results, uncertainties concerning *N. tenuis* (see below), and metabolic capability in *N. diversicolor* were evaluated by conducting a second bioaccumulation experiment using sediment from Smelter E (in addition to the control sediment). Five replicate aquaria in each group were run with *N. diversicolor* (60 individuals in each), 3 replicates with *H. reticulata* (20 individuals in each) and 3 replicates with *N. tenuis* (80 individuals in each). Upon termination (28 d) of this experiment, subsamples of each *N. diversicolor* replicate were prepared (as described above) for analyses of PAHs and PAH metabolites. Replicates of *H. reticulata* were also prepared (as described above) for PAH analyses. One subsample of each *N. tenuis* replicate was prepared (as described above, but no pooling of replicates) for PAH analyses, while one subsample of each replicate was transferred to beakers filled with seawater in which they were held for 12 h in an attempt to empty remnants of sediments from their intestines prior to PAH analysis. Furthermore, subsamples from each replicate aquarium with *N. tenuis* exposed to the Smelter E sediment (both with and without attempted gut purging) were prepared for microscopic evaluation of intestinal content.

Biota to sediment accumulation factors were calculated for each species as

$$\mathrm{BSAF_{Species}} = (C_{\mathrm{Species}}/f_{\mathrm{lipid}})/(C_{\mathrm{S}}/f_{\mathrm{OC}}) \qquad (3)$$

where C_{Species} is the dry weight concentration in the organism, and f_{lipid} is the fraction of tissue dry weight that is lipid.

PAH Analyses. All sediment, biota, and POM extract samples were analyzed by GC/MS, as described by Ruus et al. (*21*) for PAH compounds at NIVA's laboratory, accredited by the Norwegian Accreditation as a testing laboratory according to the requirements of NS-EN ISO/IEC 17025 (2000). Analytical quality of the laboratory is also ensured by the participation in international calibration tests, including QUASIMEME twice a year (see Supporting Information for a detailed description).

Analyses of Lipid, Total Dry Matter, Organic Carbon, and Particle Size Fraction. Total dry matter in the sediments was analyzed gravimetrically. Sediment subsamples were freeze-dried, crushed, and acidified (1N HCl). Subsequently sediments were analyzed for total organic carbon (TOC) by catalytic combustion at 1800 °C in a Carlo Erba 1106 elemental analyzer (Carlo Erba SpA, Rodano, Italy). Aliquots of the homogenized biota material from each of the groups were used to determine the lipid content gravimetrically, after lipid extraction (cyclohexane and acetone). Proportion (weight percentage) of particles with size <63 μm was analyzed according to the methods described by Krumbein and Pettijohn (*27*).

Analyses of PAH Metabolites from N. diversicolor Tissues. 1-OH-pyrene and 3-OH-benzo(a)pyrene were analyzed in *N. diversicolor* homogenates by HPLC (*28*), as described in the Supporting Information.

Microscopy of N. tenuis. Individuals of *N. tenuis* fixed in formalin (37% buffered with Borax; sodium tetraborate decahydrate; Na$_2$B$_4$O$_7 \cdot$10H$_2$O and diluted to 6% with filtered (0.2 μm) seawater) were carefully dissected under an Olympus ZS61 Microscope (3.5–4.5×; Olympus Corp., Tokyo, Japan) and photographed with an Olympus Camedia C-8080 camera mounted to the microscope.

Data Treatment and Statistical Methods. The median of triplicates (organisms and POM extracts) were used in further calculations of BSAFs. Statistical analyses (linear regressions, principal component analysis, PCA, and nonparametric statistics) were performed using Statistica software (Ver 7.1; Statsoft, Tulsa, OK). A significance level of $\alpha = 0.05$ was chosen.

Results and Discussion

General Observations. Aluminum smelter-affected sediments contained a wide range of PAH concentrations (see Table S1, Supporting Information). The sum of all analyzed PAHs amounted to >300 mg/kg (dry wt) in two sediments (Smelter B and C), corresponding to ∼2 orders of magnitude higher concentrations than in sediments from Smelter A. Benzo(b,j)fluoranthene generally constituted the highest concentrations. Concentrations in the control sediment (first exposure and SPE experiment) ranged from <2 to 6.3 μg/kg. PAH compounds added to the spiked sediment ranged from 200 to 720 μg/kg. The Smelter B and C sediments also had the highest TOC contents (6.76 and 5.46% dry wt., respectively). The other smelter-affected sediments contained 1.19–1.59% TOC, whereas the TOC contents of the control and PAH-"spike" sediments were lower ($\leq$0.63%). The amount of particles of size <63 μm constituted 30% (Smelter D) to 96% (Smelter A3) dry wt (all sediments included). The range in sediment PAH concentrations was reflected in concentrations measured in POM and organisms (see Tables S2–S5, Figures S1 and S2 in Supporting Information), and concentrations were generally higher in *N. tenuis* than in *N. diversicolor* and *H. reticulata* (up to 2 orders of magnitude; see Tables S3–S5, Supporting Information).

TOC–Water Partitioning. Organic carbon/water partition coefficients (K_{OC}) were deduced from the pore water concentrations measured by the passive sampler material. Despite large variation in PAH content, all sediments displayed comparable K_{OC} values, varying within ∼1.5 order of magnitude (Figure 1). Particle/water partition coefficients (K_{d}) values were generally lower for the PAH-spike sediment, than for the Al smelter affected sediments (approximately an order of magnitude for phenanthrene, anthracene, fluoranthene, and pyrene; not shown). However, the TOC content was also much lower in this sediment, thus the K_{OC}s for the PAH-spike sediment did not deviate from those of the smelter-affected sediments (Figure 1). The observed K_{OC} values showed a significant linear relationship with K_{OW} (Figure 1; $P < 0.000001$; $R^2 = 0.66$). Furthermore, the K_{OC}s were on average 1–2 orders of magnitude higher than those from the Karickhoff et al. (*5*) free energy relationship (Figure 1; i.e., a higher logK_{OC}/logK_{OW} ratio). The largest discrepancies appeared for the PAHs with the lowest K_{OW} values (Figure 1).

It should be mentioned, however, that a difference of 0.01–0.39 log units was found between the K_{POM}s of POM samplers with thickness 55 and 500 μm (used here) (*29*). This difference was attributed to either a variation among different manufacturers or a lack of equilibrium in the 500 μm POM–water systems (despite earlier testing indicating equilibrium reached in 30 days). In the present study, POM material from the same manufacturer (Vink) used by Jonker and Koelmans (*20*) was utilized. A hypothetical situation of nonequilibrium would imply that pore water estimates were somewhat underestimated, but within a factor of 2.

Our observations of strong particle association are in accordance with results from other studies of aluminum smelter residues (*11–13, 30*), and are most likely attributed to the strong sorption behavior of coal tar pitch, although individual PAHs in coal tar pitch exhibit distinct sorption behavior (*31*). Furthermore, it is known that PAHs can be entrapped in soot and coal fractions during their formation, thus hindering them from partitioning (*17, 32*). Sediments from other locations heavily influenced by anthropogenic activities have also shown similar behavior (*33*), due to
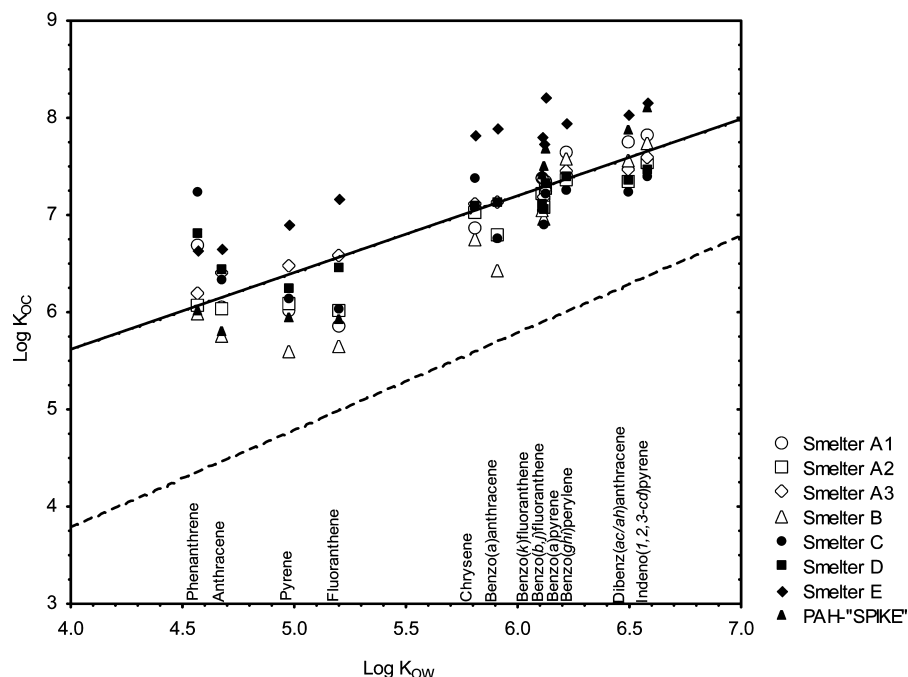
FIGURE 1. Logarithms (Log$_{10}$) of octanol/water partition coefficients (K_{OW}) versus the logarithms of organic carbon/water partition coefficients (K_{OC}) determined by the use of passive samplers (polyoxy methylene; POM) and solid phase extraction. PAH compounds are superimposed along K_{OW}-axis. Stapled line: Log K_{OC} = Log K_{OW} − 0.21 (*5*); solid line: linear regression ($P < 0.000001$, $R^2 = 0.66$).

relatively high black carbon (BC) content (*9*), but also other sorbents (*18*). In a study of Norwegian harbor sediments it was shown that total sorption was best described when other nonlinear sorbing carbonaceous geosorbents were accounted for, such as unburned coal and kerogen (*18*). Furthermore, in a recent study of organic contaminant binding behavior (*17*), the authors found that reported organic carbon/water partition coefficients varied over up to 3 orders of magnitude. It should also be mentioned that recent studies (e.g., *34*) have reported higher log K_{OC} values for organic carbon than previously observed, suggesting that the Karickhoff model (*5*) may underestimate sorption by natural organic matter.

Predicting in Vivo Bioaccumulation: The Generic Approach vs the Equilibrium Pore Water Concentration Approach. Assuming $K_{lipid} = K_{OW}$ and a (Gibbs) free-energy relationship between K_{OC} and K_{OW} following Karickhoff et al. (*5*), the generic Biota to Sediment Accumulation Factors (BSAF$_{Predicted (Generic)}$) equal 1.61, regardless of compound and K_{OW} (see above). This BSAF was a median factor 36 (range 1.3−1337) higher than the in vivo BSAFs measured for *N. diversicolor* (all PAHs and sediments included, except control where many concentrations were below LOD; Figure 2). The highest discrepancies were shown for the Smelter E sediment, in which the BSAF$_{Predicted (Generic)}$ seemed largely overestimated compared with BSAF$_{N. diversicolor}$. Corresponding median BSAF$_{Predicted (Generic)}$/BSAF$_{Species}$ ratios for *H. reticulata* and *N. tenuis* were 47 (range 1.2−419) and 1.3 (range 0.2−34), respectively (Figure 2). In other words, BSAF$_{Predicted (Generic)}$ seemed an overestimate also compared with BSAF$_{H. reticulata}$, but not compared to BSAF$_{N. tenuis}$, which agreed well.

When comparing the BSAFs predicted from pore water concentrations estimated by POM-SPE with the in vivo BSAFs the following became evident: The BSAF$_{Predicted (SPE)}$ was a median factor 1.3 (range 0.1−14) higher than the in vivo BSAF$_{N. diversicolor}$ (all PAHs and sediments included, except control where many concentrations were below LOD), i.e., good agreement (Figure 2). There was some evidence that the discrepancy between BSAF$_{Predicted (SPE)}$ and BSAF$_{N. diversicolor}$ increased with molecular weight (and K_{OW}) of the PAHs (Figure 2). This is in accordance with Bathe et al. (*35*) who attributed this phenomenon to some steric hindrance of

biological membrane permeation by the larger molecules. Corresponding median BSAF$_{Predicted (SPE)}$/BSAF$_{Species}$ ratios for *H. reticulata* and *N. tenuis* were 1.4 (range 0.03−17) and 0.05 (range 0.01−0.4), respectively (Figure 2). In other words, BSAF$_{Predicted (SPE)}$ seemed to agree well also with BSAF$_{H.reticulata}$, but not with BSAF$_{N. tenuis}$, indicating apparent underestimation of bioaccumulation in *N. tenuis* by BSAF$_{Predicted (SPE)}$.

The observations that BSAFs predicted by SPE porewater estimates corresponded within 1 order of magnitude with in vivo BSAFs measured in *N. diversicolor* and *H. reticulata* are in accordance with earlier observations (*19*), including those by van der Heijden et al. (*16*), who compared four laboratory based methods in predicting in situ bioaccumulation in the oligochaete *Lumbriculus variegatus*. However, the divergences between the results for *N. tenuis* and these other two species gave reason for further scrutiny.

Pursuit of Interspecies Differences. Bioaccumulation is the net result of uptake (all exposure routes including dietary absorption, transport across respiratory surfaces, and dermal absorption) and elimination routes and rates. The observed interspecies differences in concentrations could theoretically be a result of differences in uptake and/or elimination. Ingestion of sediment is shown as an important mechanism for PAH accumulation (*36*) and an additional assessment factor of 10 is for instance therefore applied in risk assessment of very hydrophobic substances according to the EU technical guidance (*15*). Deposit feeders have gut fluids that exhibit enzyme activities and contents of organic colloids (e.g., proteins) with high surfactant properties, and Voparil et al. (*37*) showed that desorption extent of PAHs in the gut of the polychaete *Arenicola marina* was higher than predicted from equilibrium partitioning theory. However, both *N. tenuis* and *N. diversicolor* ingest sediment, indicating that sediment ingestion per se is a less likely explanation for the interspecies differences. However, as metabolism of certain PAHs is shown in *N. diversicolor* (*38, 39*), the interspecies differences could theoretically be attributed to dissimilar PAH biotransformation capabilities. This possibility was further explored (see below).

PAH concentrations measured in *N. tenuis* were up to 2 orders of magnitude (a factor >300) higher than those

a. $BSAF_{Predicted (Generic)}:BSAF_{N. diversicolor}$

b. $BSAF_{Predicted (SPE)}:BSAF_{N. diversicolor}$

c. $BSAF_{Predicted (Generic)}:BSAF_{H. reticulata}$

d. $BSAF_{Predicted (SPE)}:BSAF_{H. reticulata}$

e. $BSAF_{Predicted (Generic)}:BSAF_{N. tenuis}$

f. $BSAF_{Predicted (SPE)}:BSAF_{N. tenuis}$

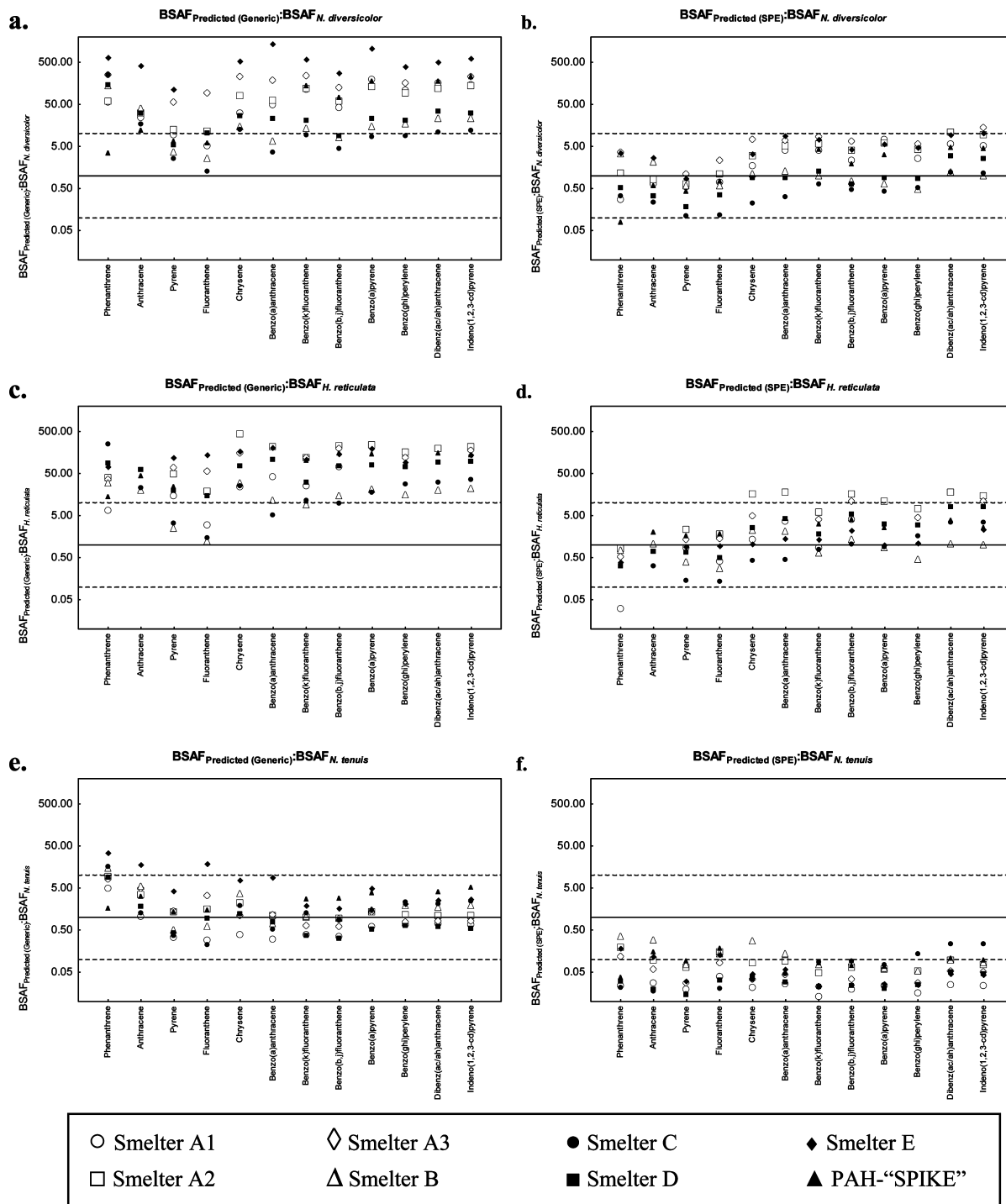| ○ Smelter A1 | ◇ Smelter A3 | ● Smelter C | ◆ Smelter E |
| □ Smelter A2 | △ Smelter B | ■ Smelter D | ▲ PAH-"SPIKE" |

FIGURE 2. Ratio between predicted biota-to-sediment accumulation factors (BSAFs) and observed BSAFs in sediment exposed organisms. Left figures (a, c, and e): BSAFs predicted from the generic approach based on organic carbon–water partitioning and linear free energy relationship between K_{OW} and K_{OC}; Log K_{OC} = Log K_{OW} − 0.21 (5); right figures (b, d, and f): BSAFs predicted from measurements of freely dissolved concentrations of PAHs in the sediment pore water, using passive samplers (polyoxy methylene; POM) and solid phase extraction. Upper figures (a and b): *Nereis diversicolor*; middle figures (c and d): *Hinia reticulata*; bottom figures (e and f): *Nuculoma tenuis*. Solid line: 1:1 relationship ($BSAF_{Predicted}/BSAF_{Observed}$ = 1). Stapled lines: One order of magnitude below and above the 1:1 relationship, respectively.

measured in *N. diversicolor* and *H. reticulata* (see Tables S3−S5, Supporting Information). Furthermore, only 0.35− 0.61 g of mussel soft tissue was obtained per sample for analysis. Thus, the risk of contamination of the biological tissue samples by contaminated particles is higher than for the other species (for which 1.5−6 g of tissue was obtained per individual sample for analysis), suggesting yet another explanation for the deviating *N. tenuis* results (sediment remnants; see below). Assuming that the PAHs in the sediment are mainly associated with organic material, only a small fraction of sediment organic material in a *N. tenuis* sample would increase its concentration substantially.

Different PAH profiles/compositional patterns (relative concentrations expressed as percentages of ΣPAH_{12}) were observed in the different sample matrices (see Figures S3 and S4, Supporting Information). The profile in *N. tenuis* was similar to the sediment PAH profile, while the profiles in *N. diversicolor* and *H. reticulata* showed resemblance with

the profile in pore water (estimated by SPE; see Figures S3 and S4, Supporting Information). A Principal Component Analysis (PCA) performed on the relative concentrations segregated sediment and *N. tenuis* from pore water along PC 1 (explaining ~52% of the variance), with *N. diversicolor* and *H. reticulata* situated between these. Fluoranthene, pyrene, anthracene, and phenanthrene generally constituted higher percentages of ΣPAH_{12} in pore water, *N. diversicolor*, and *H. reticulata*, than in sediment and *N. tenuis*. The higher molecular weight PAHs (with higher K_dS), such as benzo(b, j)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, and benzo(ghi)perylene, were of higher importance in sediment and *N. tenuis* (as shown by the loadings on PC 1; see Figure S4, Supporting Information). This could indicate that PAH-contaminated deposit material in the stomach and intestine of *N. tenuis* might have been analyzed as part of the organism. To evaluate the reproducibility of the results, uncertainties concerning *N. tenuis*, and metabolic capability in *N. diversicolor*, a second bioaccumulation experiment was conducted using the sediment from Smelter E (in addition to the control sediment).

Sediment Remnants. The interspecies differences in concentrations (and PAH profiles) appeared also in the second exposure experiment and attempts to empty all remnants of sediments/organic matter from the intestine of *N. tenuis* did not yield reduced concentrations (see Table S5, Supporting Information). Furthermore, microscopic evaluation of *N. tenuis* intestinal content revealed significant amounts of ingested material remnants (see Figure S5, Supporting Information).

Metabolic Capability. Analyses of pyrene and benzo(a)pyrene metabolites (1-OH-pyrene and 3-OH-benzo(a)pyrene; after deconjugation of possible phase II metabolites) in *N. diversicolor* showed significantly elevated concentrations of 1-OH-pyrene in polychaetes exposed to the Smelter E sediment, compared with the control ($p < 0.006$; Mann−Whitney U), while this could not be shown for 3-OH-benzo(a)pyrene (see Figure S6 and Table S6; Supporting Information). Metabolites of pyrene have previously been shown in *N. diversicolor* (*38, 39*). Interestingly, pyrene was also one of the compounds that accumulated to the highest concentrations in *N. diversicolor* and *H. reticulata*, as could be expected from correspondingly high proportions in pore water (see Figures S3 and S4, Supporting Information). More recalcitrant PAHs (constituting lower percentages of ΣPAH_{12} in the pore water) accumulated to a lesser extent in *N. diversicolor* and *H. reticulata*, but to a higher extent in *N. tenuis* (see Figures S3 and S4, Supporting Information). If the observed differences in PAH accumulation patterns between *N. diversicolor* and *N. tenuis* could be explained mainly by higher metabolic capability in *N. diversicolor*, higher concentrations of high molecular weight PAH (such as benzo(a)pyrene) degradation products in the exposed worms were expected. The metabolite analyses thus showed no indication of metabolism being responsible for the differences in PAH accumulation patterns between *N. diversicolor* and *N. tenuis*.

Combined, the PAH compositional patterns in the different matrices/organisms, the sediment remnants in the intestine of *N. tenuis*, and the results of the metabolite analyses may therefore indicate that in the present study the metabolic capability of the organisms was not very influential on the PAH accumulation that was governed by the bioavailable fraction of compounds in sediment pore water, and that particulate matter in the *N. tenuis* samples may have caused the deviating pattern observed in this species.

Relevance for Risk Assessment. This study showed that the dissolved concentrations of PAHs in sediment pore waters were lower than predicted from the generic approach based on organic carbon−water partitioning and Gibbs linear free energy relationship (between K_{OW} and K_{OC}). Our results therefore corroborate earlier findings that show the importance of conducting site-specific evaluations of pore water concentrations and/or bioaccumulation studies by direct measurements to accurately provide a basis for cost-effective risk assessment and remediation plans, rather than to infer this basis from total sediment concentrations (*12, 16, 17, 31*). The increasing number of reports emphasizing this suggest that the generic prediction approach is questionable per se as it is increasingly known that sorption of certain PAHs is highly influenced by the type of organic matter constituents. Our conclusion was further supported by in vivo bioaccumulation in *N. diversicolor* and *H. reticulata*, that could be fairly accurately predicted from the pore water concentration estimates. These findings are also in accordance with earlier observations (*16, 19*). However, *N. tenuis* apparently accumulated higher amounts of PAHs than could be predicted from the pore water concentration estimates, but this was most likely attributed to remnants of particulate matter in the samples. This demonstrates the importance of knowledge regarding specific characteristics of model organisms, possibly affecting bioaccumulation assessments.

Supporting Information Available

Detailed method descriptions (chemicals; preparation of sediment; BSAF calculations; experimental setup; K_{POM} evaluation; analyses of PAHs and metabolites), result tables (concentrations of PAHs in sediments, POM, and organisms; concentrations of PAH metabolites in *N. diversicolor*), and figures (concentrations of PAHs in sediments vs organisms and in POM vs organisms; PAH compositional patterns, including PCA analysis of such; photo of sediment remnants in *N. tenuis*; PAH metabolite chromatograms). This information is available free of charge via the Internet at http://pubs.acs.org.

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