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BIOACCUMULATION OF POLYCYCLIC AROMATIC COMPOUNDS: 1. BIOCONCENTRATION IN TWO MARINE SPECIES AND IN SEMIPERMEABLE MEMBRANE DEVICES DURING CHRONIC EXPOSURE TO DISPERSED CRUDE OIL

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Abstract—Assessing the fate in marine biota of hydrocarbons derived from oil particles that are discharged during exploration and production is of relevant environmental concern. However, a rather complex experimental setup is required to carry out such investigations. In this study, a sophisticated tool, the continuous-flow system (CFS), was used to mimic dispersed oil exposure to marine biota. Polycyclic aromatic hydrocarbon (PAH) uptake was studied in two species, the blue mussel *Mytilus edulis* and juvenile of the turbot *Scophthalmus maximus*, and in semipermeable membrane devices (SPMD) exposed to crude oil dispersed in a flow-through system. After an exposure period of 8 to 21 d, elimination in organisms and devices was analyzed for 9 to 10 d following transfer to PAH-free seawater. Principal component analysis (PCA) revealed different PAH patterns. In mussel and SPMD, the PAH profiles were very close to that analyzed in seawater. Slight differences were, however, indicated for large molecules with log K_{ow} above six. Nonachievement of steady-state concentration and bioavailability of PAH in oil droplets may account for these differences. The PAH composition in fish revealed only congeners with two to three aromatic rings. A combination of bioavailability and efficient metabolism of the larger PAH molecules may explain this pattern. The CFS made possible a better understanding of some critical factors governing bioconcentration in marine biota from dispersed oil. Yet the results illustrate that uptake of PAH from exposure to oil particles is complex and that different species may bioconcentrate different molecules depending on factors like life style and metabolic capability to degrade the potential harmful substances. Hence, risk assessment of the actual impact of discharges to marine biota should consider these essential biological and ecological factors.

Keywords—Produced water discharges Polycylic aromatic hydrocarbons Bioconcentration Marine biota Semi-permeable membrane devices

INTRODUCTION

Discharges from offshore oil and gas production contribute to a significant input of oil in the North Sea. Produced water is the major wastewater stream. Environmental authorities have expressed concern about these discharges considering an increasing volume expected during the next few years and the lack of knowledge regarding effects of several potential toxic compounds to biota. Moreover, as industries are moving their activities toward environmentally sensitive marine areas, there recently has been an even larger focus upon these discharges.

The current regulation, which is handled within the Oslo-Paris Commission, stated that the oil content of produced water should not exceed 40 mg/L at the discharge point. This value is based on the fraction of dispersed hydrocarbons commonly accepted as having low environmental impacts below that concentration [1]. Since a rapid dilution is occurring in the water column immediately after discharge, the acute environmental risk seems to be restricted to the vicinity of offshore installations. Yet the possible long-term effects of chronic, low environmental concentration to marine biota are poorly described, and questions are raised whether the current limitation should be decreased. In fish, e.g., hydrocarbon concentrations as low as ≈1 mg/L were reported to induce change in enzyme levels and activity [2,3]. Such disruptions in the biochemical or physiological status of an organism can have more deleterious consequences like carcinogenesis in the liver or mutagenesis in the genetic material. Eventually, this may impair the reproductive success at the scale of a population. More investigations with regard to possible long-term effects to marine food chains are required.

The substances at focus in the produced water are the polycyclic aromatic hydrocarbons (PAHs) because of their persistence, the potential for accumulation, and toxicity in marine organisms and ultimately in the humans that consume them. Consequently, an environmental problem addressed by industries is to estimate the potential of PAH to bioconcentrate in the biological tissues and generate toxic effects upon chronic, low-level exposure. One of the challenges is to find appropriate body burden/toxicity relationships since PAH body burdens may increase to toxic levels due to direct or indirect (dietary pathways) exposure [4–6].

Bioconcentration is defined as the accumulation of waterborne substances alone. At any time during an exposure, it is the net result between uptake and elimination of the substances. Uptake is essentially governed by the bioavailability of PAH in the surrounding environment, which is related to hydrophobicity. The octanol/water partition coefficient (K_{ow}) is often used to express hydrophobicity. The dissolved fraction is mainly accumulated in the lipid phase of marine organisms by passive diffusion across, notably, the tiny gill epithelial membrane until an equilibrium status is reached. The solubility of

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Table 1. The two continuous flow system (CFS) experiments performed in 1997 (BAL150 = blended Arabian light topped at 150°C; SPMD = semi-permeable membrane devices)

CFS runs	Total duration (days)		Species	Oil tested (oil dispersed)	Range (mg/L)
	Exposure	Elimination			
1	21	9	Juvenile turbot (*Scophthalmus maximus*)	BAL150	0.06–2
2	8	10	Adult mussel (*Mytilus edulis*) and SPMD	BAL150	1.0

PAH decreases as K_{ow} increases. Hence, the low molecular weight, two- to three-ring PAHs are relatively more soluble than the heavier molecular weight compounds that have a strong tendency to bind onto particulate or dissolved organic material [7–9]. However, depending on the life styles of organisms, accumulation of heavier PAHs is possible by ingestion of contaminated particles from the water column [10–12].

Elimination can occur by simple thermodynamically driven diffusion or excretion after biotransformation (metabolization) of parent compounds. The PAH biotransformation is a degradation process controlled by enzymatic systems, which result in the production of polar metabolites. Consequently, organisms that metabolize PAHs extensively will decrease their body burden. Although biotransformation occurs in many aquatic organisms, it varies widely between the different species [13,14], but higher activities are revealed in species located at higher levels in the food web, like fish [15,16].

The main objective of these investigations was to analyze the composition and the levels of PAHs upon chronic exposure to low doses of dispersed oil. Chronic exposures were represented by continuous exposures to sublethal levels of dispersed oil for a period ranging from several days to several weeks. Experiments were carried out using juvenile turbot fish and blue mussels together with semipermeable membrane devices maintained in a flow-through system. This article describes the composition of PAHs in body tissues of organisms and in devices using multivariate analysis. The patterns reveal differences in the potential to bioconcentrate substances that are poorly water-soluble, like PAHs from oil droplets. The results are discussed in relation to bioavailability and metabolism.

METHODS

The data were collected during two successive laboratory experiments conducted in 1997. Blended Arabian light oil topped at 150°C (BAL150) was used during these two assays. The BAL150 is comparable with some North Sea crude oils and its composition reflects slightly weathered conditions. The topping process also reduces potential detrimental exposure of the most volatile components. The seawater used during these experiments was directly pumped from the fjord at 78 m and was sand filtered before use in the laboratory.

Semipermeable membrane devices and mussels

Semipermeable membrane devices, consisting of a thin film of a neutral lipid (triolein) enclosed in a thin, low-density polyethylene wall [17] were purchased from a supplier located in Sweden (ORIGO, HG, Umeå University).

Blue mussels (*Mytilus edulis*) were collected along the shore line at an uncontaminated site in the Stavanger fjord in the southwestern part of Norway. After sampling, they were placed for several days in a storage tank with running seawater. No food was provided to the mussels. Prior to transfer for the exposure experiment, the mussels were brushed to clean sessile organisms from the shell surface.

Fish

Juvenile turbot (*Scophthalmus maximus*) were used in this experiment. Juvenile fish were chosen to limit variability and to avoid differences related to sex of the animals. They were purchased from a fish farm (Tinfos Aqua A/S, Farsund, Norway). After shipping, they were immediately stored in tanks with running seawater regulated at a temperature of 19 ± 1°C. During storage prior to the experiments, the individuals were fed dry fish pellets used at the fish farm site.

Exposure experiment

The two experiments were conducted in a light- and temperature-controlled room. The exposure system was completely closed to avoid evaporation. It consisted of a series of 18-L chambers closed with conical lids. Each exposure chamber contained a central cylinder with a propeller stirred at a speed of approximately 250 rpm for homogenization of the test solution and circulation of the water. The chambers were connected to the continuous-flow system with test solution input at the bottom and output at the top, as described in Sanni et al. [18]. A flow rate of approximately 250 ml/min was maintained through the chambers, enough to meet the oxygen requirement. Oil was kept in a dark, sealed storage bottle and was directly pumped in the stream of seawater at a constant flow rate according to the exposure concentration. No solvent was added. Droplets were created with mechanically driven energy via a mixing valve (for more details of the continuous-flow system [CFS], see [18]). The mixture of oil and seawater was then supplied to the exposure chambers. The average droplet size was 10 μm in diameter (measurement performed with a Coulter© multisizer, Coulter, Toronto, ON, Canada). Air and seawater temperatures were adjusted for juvenile turbot (19 ± 1°C). The fjord seawater was used with no adjustment for temperature for mussels and semipermeable membrane devices (SPMDs) (7 ± 1°C). In both experiments, a day:night regime of 12 h:12 h was maintained. A sublethal concentration of oil was injected in the system during exposure periods. The durations, oil types, and oil concentrations used in the two assays are shown in Table 1.

CFS1 bioassay. In this first experiment, each exposure chamber contained 24 individuals of juvenile turbot (*Scophthalmus maximus*) with a size range of 5 to 15 g. A range of six concentrations (nominal values of 0.06, 0.12, 0.25, 0.5, 1, and 2 mg/L) of BAL150 was tested. The exposure period was 21 d, followed by an elimination period of 9 d. The fish were fed pellets once every day during the entire experiment. Excess food was removed by suction.

CFS2 bioassay. In the second experiment, approximately

100 mussels were used per exposure chamber, each at a nominal oil concentration of 1 mg/L. Additionally, five to six SPMDs were placed in chambers used in parallel with the chambers with mussels. They were exposed to the same concentration of oil. Each membrane was attached to an individual holder and twisted in an S-shaped form before transfer to the chamber. Surface contact of the different segments was avoided. The exposure period was 8 d, and the elimination period was 10 d. Mussels were split into a group continuously fed with a solution of the algae *Isochrysis galbana* (≈4,000 algae/ml) and a group with no food. This treatment was equal for SPMD.

At the end of the exposure period, each group of animals and devices was temporarily transferred into individual buckets filled with clean seawater. Then the exposure chamber was washed with hot water and animals and devices were relocated in their respective chambers. They were kept in clean running seawater during the elimination period with the same flow-through rate and the same feeding regime.

Sampling procedure

For comparison purposes of the samples, the data collected at day 8 (end of exposure for mussels and SPMD) and at the end of the elimination periods are presented. During the sampling of organisms, siphoning first reduced the volume of water in the exposure chamber. Then the lid was opened and the individuals were caught with a net (fish) or by hand (mussel). For SPMD, each sample was taken after siphoning out the water from the exposure chamber. Special care was made to avoid contact with fingers on the surface of the devices.

Sample preparation

Semipermeable membrane devices. The SPMDs were handled with nitrile gloves cleaned in a solution of dichloromethane (DCM) and cyclohexane (CYH) (1:1). The SPMDs were detached from the holders and the membranes wiped off with the DCM:CYH solution. The membranes were twisted in rolls and stored separately in rinsed metal boxes at −80°C until extraction and analysis.

One (or more) SPMD was placed in 250-ml Teflon® tubes after carefully trimming excess polyethylene from the ends of the bags without disrupting the heat seal defining the portion of the SPMD containing the lipid material. One hundred milliliters of cyclopentane (pesticide grade) was poured into the centrifuge tubes and a mixture of eight deuterated PAHs (quantitation internal standard [QIS]) was added as an internal standard and was used for quantification (Table 2). The Teflon tubes were tightly capped and placed on a shaker table for 24 h at 4°C, followed by decantation of the solvent into 250-ml Erlenmeyer flasks and storage at 4°C. The extraction was repeated with a reduced amount of solvent (50 ml) and the extracts combined. Anhydrous sodium sulfate (pesticide grade) was added in excess and allowed to rest for 30 min before filtering through a glass sinter filter (pore size 2) and concentrating to 1 ml by means of a TurboVap 500 (Zymark, Hopkinton, MA, USA).

Prior to gas chromatography/mass spectrometry (GC/MS) analysis, the concentrated extracts were purified by gel permeation chromatography (GPC). Two columns (Envirogel GPC Cleanup Column, 19 × 150 mm, and Envirogel GPC Cleanup Column, 19 × 300 mm, supplied by Waters, Milford, MA, USA) were serially connected, and the mobile phase used was 100% DCM at a flow rate of 1 ml/min. Detection was performed by use of ultraviolet at 254 nm. The retention time was determined prior to sample cleanup by separation of a calibration solution containing corn oil (20 mg/L), sulfur (40 mg/L), naphthalene (20 mg/L), and perylene (10 mg/L) dissolved in DCM. Purified extract was concentrated to 1 ml by use of the TurboVap concentrator. A mixture of three deuterated internal standards (RIS), used for recovery calculations, were added to the extract prior to GC/MS analysis.

Table 2. List of deuterated polycyclic aromatic hydrocarbons used as internal standards (QIS = quantitation internal standard; RIS = recovery internal standard)

Compound	Mass m/z
Naphthalene-d8, QIS	136.2
Phenanthrene-d10, QIS	188.2
Dibenzothiophene-d8, QIS	192.2
Fluoranthene-d10, QIS	212.2
Pyrene-d10, QIS	212.2
Chrysene-d12, QIS	240.2
Benzo[*a*]pyrene-d12, QIS	264.3
Dibenzo[*a*,*h*]anthracene-d14, QIS	292.3
1-Methylnaphthalene-d10, RIS	152.2
Anthracene-d10, RIS	188.2
Perylene-d12, RIS	264.3

Seawater. Silicon tubing was connected to the output of the exposure chambers and, for each sample, a volume of 2 L was collected in a prewashed and heat-sterilized (500°) amber Duran (Mainz, Germany) glass bottle filled with 16 ml of a 10% hydrochloric acid solution so that pH did not exceed two.

Samples were extracted within 2 d of sampling. An appropriate amount of QIS was added to the water bottles and stirred for 15 min on a magnetic stirrer. Cyclohexane (50 ml) was added to the water, stirred for another 30 min, and then poured into a separatory funnel. The water phase was drained back into the sampling flask and extracted two additional times. The combined organic extracts were dried and concentrated to 1 ml, as described for the SPMD. No further cleanup was required and the samples were transferred to vials for GC/MS analyses immediately after addition of RIS.

Mussel and fish tissue. After sampling, the fish was first held in two successive baths of clean seawater, the body surface was wiped for 3 to 5 s with paper toweling in order to remove extra mucus secretion, and weight and length were measured. One replicate of each exposure concentration was collected at each sampling interval except for the 2-mg/L exposure, where three replicates were sampled. The whole fish was then cut into small pieces with scissors rinsed successively in one bath of cyclohexane and two baths of extra pure distilled water. Scissors were fully cleaned between samples. The whole tissue was then transferred into a burned glass vial sealed with a Teflon lid and was stored at −80°C until analysis.

For the mussel, the shells of four individuals were opened, residual seawater trapped in each shell was removed, and the pooled soft tissues were then transferred into a burned glass pot and homogenized without any solvent addition by means of an Ultra-Turax homogenizer (IKA-Maschinen bau, Staufen, Germany). The same cleaning procedure was used for the rod and blades of the Ultra-Turax between mussel samples as was used for the scissors. The homogenate was then transferred into a burned glass vial sealed with a Teflon lid and was stored at −80°C until analysis.

Mussel and fish samples were then treated similarly. The

Table 3. List of all polycyclic aromatic hydrocarbons (PAHs) analyzed by GC-MS in seawater, sample tissues, and devices; mean concentrations of PAH present in BAL150 (blended Arabian light topped at 150°C) are indicated

Compound	Code	Mass m/z	Mean[ab] (μg/g)	SD (μg/g)	% of ΣPAH
Naphthalene	na	128.2	148	10.2	2.17
C1-naphthalenes	1na	142.2	609	47	8.93
Acenaphthene	ay	152.2	9.3	1.1	0.14
Acenaphtylene	ae	154.2	4.9	1.5	0.07
C2-naphthalenes	2na	156.2	1,418	142	20.78
Fluorene	f	166.2	42	4.9	0.62
C3-naphthalenes	3na	170.2	1,395	171	20.45
Anthracene	an	178.2	ND	—	—
Phenanthrene	ph	178.2	79	9.2	1.16
Dibenzothiophene	dbt	184.2	342	14	5.01
C1-phenanthrenes	1ph	192.2	241	18	3.53
C1-dibenzothiophenes	1dbt	198.2	827	63	12.12
Fluoranthene	fl	202.2	ND	—	—
Pyrene	py	202.2	4.9	1	0.07
C2-phenanthrenes	2ph	206.2	320	17	4.69
C2-dibenzothiophenes	2dbt	212.2	1,330	57	19.49
Chrysene	ch	228.2	6.9	1.3	0.10
Benzo[*a*]anthracene	baa	228.2	ND	—	—
C1-chrysenes	1ch	242.2	19	3.3	0.28
Benzo[*b*]fluoranthene	bbf	252.3	1.7	0.4	0.02
Benzo[*k*]fluoranthene	bkf	252.3	ND	—	—
Benzo[*a*]pyrene	bap	252.3	ND	—	—
C2-chrysenes	2ch	256.3	25	4.2	0.37
Indeno[*1,2,3,c,d*]pyrene	ip	276.3	ND	—	—
Benzo[*g,h,i*]perylene	bghip	276.3	ND	—	—
Dibenzo[*a,h*]anthracene	daha	278.3	ND	—	—
Benzo[*b+k*]fluoranthene	bbkf	—		—	—

[a] Based on four replicates.
[b] ND = not detected.

QIS was added to homogenized tissue (5–10 g) and boiled for 2 h in a 10% solution of potassium hydroxide in methanol to achieve saponification. The digest was filtered and extracted repeatedly three times with cyclohexane. The extracts were combined, dried, and concentrated to 1 ml, as described for SPMD. Cleanup of extracts was performed by solid-phase extraction using 3-ml tubes containing 0.5 g of normal phase packing (Supelclean LC-Si, Supelco, Bellefonte, PA, USA). The compounds of interest were eluted with a mixture of DCM: CYH (1:3). The purified extract was finally concentrated to 0.5 ml and stored in capped vials until GC/MS analysis after the RIS mixture was added.

GC/MS selected ion mode analysis of samples

Bioconcentration was studied for all U.S. Environmental Protection Agency-recommended PAHs listed ranging from the two aromatic (naphthalene) to the hexa aromatic (benzo[*ghi*]perylene). In addition, methylated forms of naphthalene (C1–C3), phenanthrene (C1, C2), and chrysene (C1, C2) were covered. Parental and C1 to C2 forms of dibenzothiophene were also added to the list of PAHs. In total, 27 compounds were analyzed (Table 3). The analysis of all samples was performed by gas chromatography (HP5890, Hewlett Packard, Avondale, PA, USA) connected to a mass spectrometer (Finnigan SSQ7000, Bremen, Germany) and PAHs were analyzed in selected ion mode. The GC was equipped with a CP-SIL 8CB fused silica column (Chrompack, 50 m × 0.25-mm i.d., film thickness 0.25 μm; Varian, Palo Alto, CA, USA). Injector and detector temperatures were both 300°C. The column was held at 50°C for 1 min, ramped at 25°C/min to 120°C, 3°C/min to 320°C, and held for 17 min at 320°C. Helium was used as the carrier gas, with a flow rate of 0.6 ml/min at 50°C.

Calculation and quality assurance

Calibration standards for the various PAHs were prepared in seven different concentrations. Response factor curves were calculated for the individual PAHs and were used for calculation of sample concentrations (this calculation was done by SI-QUAN, Kjell Urdal, SINTEF Petroleum Research, Oslo, Norway). The reproducibility of the response factors for three standards covering the concentration range was checked for each series of samples analyzed. Recovery of PAH was calculated by comparing the relation of RIS- and QIS-signal intensity in samples and calibration standards (defined to be 100%). One procedure blank and one control sample were extracted for every series of samples ($n = 12$). The controls used were either a certified reference material (SRM2974, National Technical Information Service, Springfield, VA, USA) or an unexposed sample spiked with an appropriate amount of a certified mixture of PAH (Dr. Eherenstorfer Reference Materials, Augsburg, Germany). The recovery of the different PAHs was estimated to be good in the range of 40 to 120%. This range of variation was obtained in former experiments based on more than 100 sample analyses. It was shown that the recovery of tissue residues was better for three to four aromatic-ring PAHs (>90%) than for two, five, and six aromatic-ring PAHs (40–50%).

The final level of contaminant was expressed on the basis of body wet weight or lipid weight. Total lipid extraction of 1.5 g (turbot) to 2.5 g (mussel) of tissue sample was made using a mixture of chloroform and methanol in the ratio 2:1 (method based on Folch et al. [19] following recommendations of Christie [20]). For turbot, the total lipid content was an average of 3% of the body wet weight, and in mussel, it was an average of 2% of body wet weight.

Statistical analysis

A Kruskal–Wallis nonparametric rank test was performed to determine whether mussels fed algae and SPMD fed algae had different PAH profiles than the nonfed samples. The relationships between bioconcentration factors and partition coefficients (K_{ow}) were evaluated by model I linear regression analysis. All analyses were tested for significance using a probability threshold of 0.05.

Principal component analysis was also performed as a pattern recognition method to compare the bioconcentration of PAH in SPMD, mussel, and fish. Generally, PCA is used to extract the various trends in a data set by assigning the variability to the principal components, therefore changing the original multidimensional space (*M*-space) to a reduced dimensional space (*R*-space) where the largest variability is expressed. Each component is orthogonal and therefore independent of the others. Our objective was to investigate similarities and differences between the various samples (SPMD, mussels, fish) using the PAH compounds analyzed as variables for the classification. Three PAHs (bkf, ip, and daha) were discarded from the analysis because they were only detected in one sample (mussel) while being completely absent from all other samples. Thus, the PCA was run on a matrix composed of 14 lines (i.e., four SMPD samples, two mussel samples, and eight fish samples) and 24 columns (PAHs). Because different oil concentrations were used in the two experiments, the levels of all compounds were first expressed by their per-

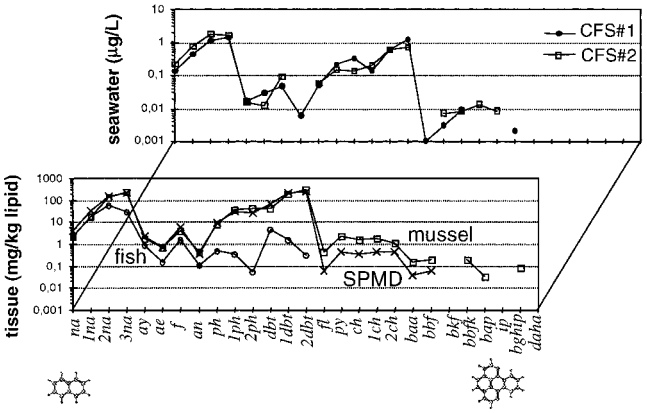

Fig. 1. Bioconcentration pattern of polycyclic aromatic hydrocarbons (PAHs) in semipermeable membrane devices (SPMDs), mussels, and fish after 8 d of chronic exposure (1 mg/L) to dispersed oil. The PAHs are ranked from two to five or six aromatic rings. The top graphic is the PAH profile in the seawater for the two continuous-flow (CFS) runs. Tissue units as mg PAH/kg lipid; seawater units as µg/L. Codes are na, naphthalene; 1na to 3na, mono- to trimethylnaphthalene; ay, acenaphthylene; ae, acenaphthene; f, fluorene; an, anthracene; ph, phenanthrene; 1ph and 2ph, mono- and dimethylphenanthrene; dbt, dibenzothiophene; 1dbt and 2dbt, mono- and dimethyldibenzothiophene; fl, fluoranthene; py, pyrene; ch, chrysene; 1ch and 2ch, mono- and dimethylchrysene; bbf, benzo[*b*]fluoranthene; baa, benz[*a*]anthracene; bap, benzo[*a*]pyrene; bbkf, benzo[*k*]fluoranthene; bghip, benzo[*ghi*]perylene.

centages relative to the ΣPAH in the matrix, then all data were $\log(x + 1)$ transformed to eliminate the effects of different scales that could dominate the analysis and give equal weight to all PAHs. The PCA result was visualized by a two-dimensional plot where the *x*-axis represents one component and the *y*-axis represents another component. A hierarchical clustering analysis of the variables (PAHs) was performed to help the interpretation. This analysis was run on the variable loading calculated from the first two components of the PCA. The distance between variables on the dendrogram plot was calculated by the centroid method (Euclidean distance).

All analyses were made using JMP software (*Windows,* Ver 3.1, SAS Institute, Cary, NC, USA).

RESULTS

General pattern

The bioconcentration patterns of PAHs in mussel and SPMD at the end of the exposure closely resembled that of the seawater (Fig. 1). Naphthalene and dibenzothiophene (parent and alkylated molecules) dominated among the PAH compounds, a pattern similar in that analyzed in BAL150. Together they represented as much as 90% of the PAHs. There was no significant change in PAH profile with feeding of mussels or SPMD ($p > 0.05$). Also, the PAH profiles between mussel and SPMD for both fed and nonfed samples were not significantly different ($p > 0.05$). The mean total levels of PAH were 972 and 959 mg/kg lipid, respectively, in mussels and SPMD (average of fed and nonfed samples). The fish PAH profile after 8 d of exposure presented a more altered composition in comparison with the seawater and was significantly different from those of mussel and SPMD ($p < 0.05$). There was a predominance of the small, two aromatic-ring molecules. Naphthalene congeners alone represented more than 90% of the PAH in fish tissue. The total amount of PAHs in the fish exposed to 1 mg/L was approximately eightfold less than in mussels (121 mg/kg lipid).

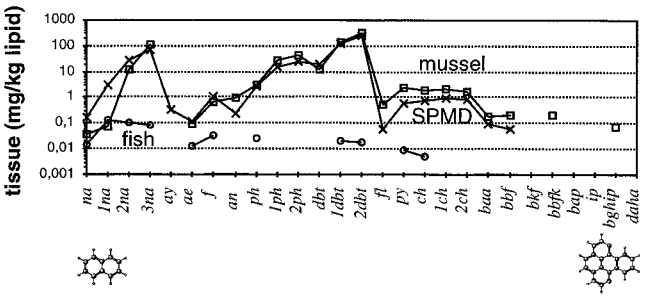

Fig. 2. Polycyclic aromatic hydrocarbon composition in semipermeable membrane devices (SPMDs), mussels, and fish at the end of the elimination period. Same codes as in Figure 1.

At the end of the elimination period, a relatively high amount of PAHs remained in the tissue of the mussels and in the SPMD. The total level in mussel was 63% of that at the end of the exposure period and 55% in the SPMD, indicating a relatively slow elimination process. The low molecular weight PAHs, mainly naphthalenes, were eliminated faster than the other molecules. In contrast with mussels, all PAHs in fish were eliminated rapidly and levels were back to the background concentrations within the elimination period (Fig. 2).

PCA analysis

Extended examination of the PAH patterns was done by mean of the PCA. Differences/similarities in PAH composition between SPMD, mussel, and fish were evaluated. It was possible to separate the different samples by their relative PAH composition as shown by the result of principal component 1 (PC1) and principal component 2 (PC2) object score plots (Fig. 3). Together these two components represented 89% of the total variation in the data set. Along PC1 (63%), fish were clearly separated from mussel samples. Mussels and SPMD were rather discriminated along PC2 (16%). Looking at the corresponding variable loading plot helps the interpretation of this separation among the samples. The variables that are located far from the origin along the α-axis and the β-axis are elements explaining the separation of the object samples along PC1 and PC2. Seven groups (G1–G7) were identified by the cluster analysis performed on the variable loading from the first two components. They have been represented on the variable loading two-dimensional plot (see Fig. 3). Fish samples are characterized by relatively small, more water-soluble PAHs, represented in the clusters G1 and G2. The separation with mussels samples is explained by the occurrence in their soft tissues of larger, less water-soluble PAHs (G4, G5, and G7). Note that alkylated phenanthrenes and alkylated dibenzothiophenes (G7) accounted significantly in separating fish from mussels. Indeed, in all fish samples, there was a decrease of bioconcentration levels with increasing alkylation of phenanthrene and dibenzothiophene. For the naphthalenes, however, the pattern in fish resembled more that of the seawater. The separation of SPMD and mussel samples along PC2 was explained by the presence of high relative levels of the larger PAHs (cluster group G4) in the mussels while those could not be detected in SPMD samples or were relatively low.

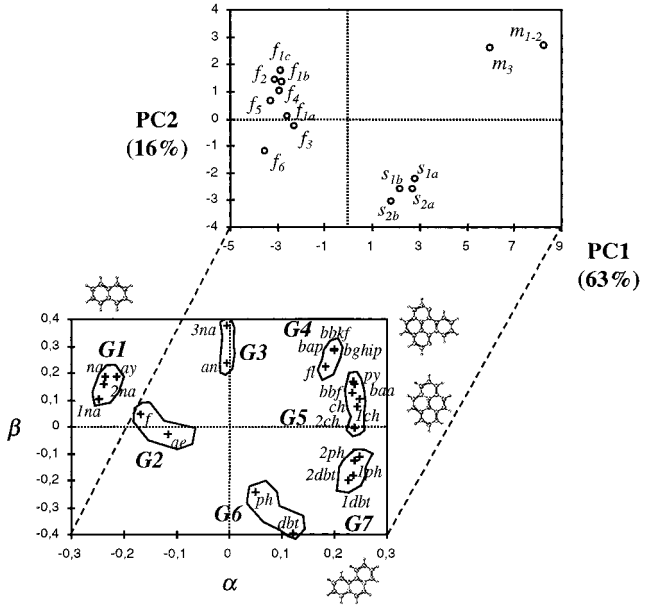

Fig. 3. The principal components analysis results. (**Top**) Principal component 1 (PC1) plotted against principal component 2 (PC2) for the 14 samples analyzed (s_{1a}, s_{1b}, s_{2a}, and s_{2b}, semipermeable membrane device samples; m_{1-2} and m_3, mussel samples; f_6 to $f_{1a,b,c}$, fish samples). (**Bottom**) Variable loading plot corresponding to the object score plot along PC1 and PC2. The seven groups (G1–G7) identified after the clustering analysis are surimposed on the graph. The PAH codes are as in Figure 1.

Relationships between bioconcentration factor and log K_{ow}

The bioconcentration factors (BCFs; ratio of the PAH residues in samples to the PAH levels in the seawater) were calculated based on lipid weight of the different samples. The values are listed in Table 4. Because large, five to six aromatic-ring PAHs were not detected by GC/MS from the water samples, BCFs for these aromatics were not computed.

Compared with SPMD and mussel, the range of BCF magnitudes in fish was relatively small. High BCF values were measured for alkylated dibenzothiophenes in both SPMD and mussel while the largest BCF value in fish was measured for acenaphthylene.

The BCF was plotted against hydrophobicity of PAH expressed by the octanol/water partition coefficients (K_{ow}). A significant linear relationship was found for mussel samples ($r^2 = 0.65$; $p < 0.0001$), indicating that lipid-based bioconcentration factors increase with hydrophobicity and that K_{ow} was a good descriptor of bioconcentration. However, BCFs tended to level off for PAHs with log K_{ow} above five, as suggested in Figure 4, where the data are fit to a two-quadratic polynomial curve.

No linear relationship was found for SPMD ($p > 0.05$). Rather, the polynomial fit was well adjusted ($p < 0.0002$) to a two-quadratic polynomial because lower values for PAHs with log K_{ow} above five were observed. The BCF seemed to be linearly related to log K_{ow} only in a limited range of PAHs with log $K_{ow} < 4.5$. In fish, declining values of BCFs were indicated for PAHs with log K_{ow} above four. Hence, log K_{ow} was not a good descriptor of the bioconcentration levels in fish.

Table 4. Lipid-based bioconcentration factors (BCF, the ratio of the concentration of substance in sample and concentration of substance in seawater) in sample tissues and devices (SPMD = semipermeable membrane device)

			BCF lipid weight ($\times 10^4$)	
Compound	log K_{ow}[a]	SPMD	*Mytilus edulis*	*Scophthalmus maximus*
Naphthalene	3.34	1.86	1.33	1.78
C1-naphthalenes	3.88	4.49	2.76	4.17
Acenaphthene	3.95	5.56	6.54	0.55
Acenaphtylene	4.1	15.84	14.46	5.33
C2-naphthalenes	4.37	8.95	8.30	5.22
Fluorene	4.21	6.12	5.09	3.31
C3-naphthalenes	4.86	12.84	15.98	2.18
Phenanthrene	4.57	16.55	14.66	1.03
Anthracene	4.58	—	—	1.72
Dibenzothiophene	4.38	32.19	21.15	3.21
C1-phenanthrenes	5.1	19.26	23.39	0.16
C1-dibenzothiophenes	—	37.29	33.43	0.26
Fluoranthene	5.1	—	—	0
Pyrene	5.1	6.53	33.43	0
C2-phenanthrenes	—	18.79	26.48	0.02
C2-dibenzothiophenes	—	35.28	37.66	0.03
Benzo[*a*]anthracene	5.67	—	—	0
Chrysene	5.71	4.36	20.76	0
C1-chrysenes	—	3.24	10.69	—
Benzo[*b*]fluoranthene	6.4	—	—	—
Benzo[*k*]fluoranthene	6.5	—	—	—
Benzo[*a*]pyrene	6.3	—	—	—
C2-chrysenes	—	5.35	9.75	—
Indeno[*1,2,3,c,d*]pyrene	6.92	—	—	—
Benzo[*g,h,i*]perylene	7	—	—	—
Dibenzo[*a,h*]anthracene	6.71	—	—	—
Benzo[*b+k*]fluoranthene	—	—	—	—

[a] [13].

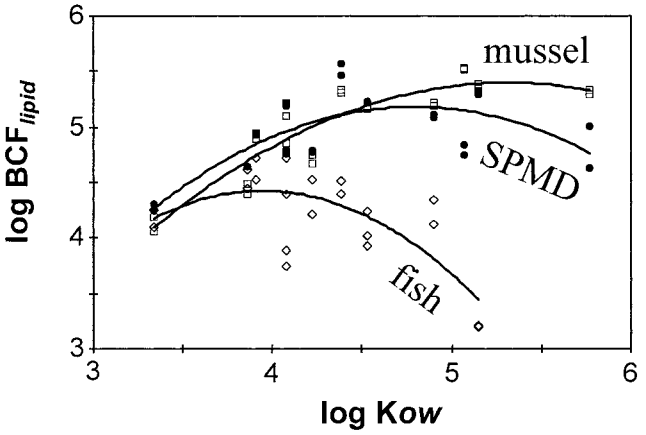

Fig. 4. Relationship between the bioconcentration factor (BCF_{lipid}) and the octanol/water partition coefficient (K_{ow}) plotted on a log-log scale, in semipermeable membrane devices (SPMDs), mussels, and fish. The fish samples correspond to the individuals exposed to 1 and 2 mg/L. Adjustment of data points by polynomial fit (degree two). All adjustments were significant at the threshold level of $p < 0.05$.

DISCUSSION

In this article, PAH profiles after chronic exposure to dispersed oil were analyzed for differences/similarities in the mussel *M. edulis* and the fish *S. maximus*. In addition, SPMDs were used as a mimic of PAH uptake by biota.

Bioavailability of PAHs, species difference in uptake pathways, and enzymatic degradation of PAHs appear to be the main criteria involved in the potential for accumulation of hydrocarbons in the two organisms and devices. A combination of these different parameters has been identified as essential to explain the final dose of contaminants in marine biota [7,11,12,14,21,22].

From a field experiment with freshwater isopods, van Hattum et al. [23] concluded that, for highly hydrophobic PAHs present in the water column, only a small fraction might actually be available for uptake by organisms even if these PAHs can be detected in the water samples. The complex equilibrium partitioning of hydrophobic molecules from oil droplets to the aqueous phase has been little described, and further investigations are demanded. However, it is technically challenging to distinguish between PAHs that stay in the dispersed phase and the truly dissolved PAHs. It is, however, likely that the heavy molecules, poorly soluble in water, are bound strongly in oil droplets in comparison with the lighter, more water-soluble molecules, which more rapidly reach equilibrium in the water phase. Hence, a reduction of the uptake of the larger PAHs could be due to their binding in oil droplets. However, the relative importance of that process is also dependent on the species considered [21]. Marine organisms like mussels and fish accumulate more easily PAHs dissolved in the water column, but the two organisms have different potentials for ingesting larger, less water-soluble PAHs bound to oil particles [24].

Blue mussels are suspension-feeder organisms that can retain a large quantity of particles down to a size of 1 to 2 μm on their gills, including oil particles of the size range created in this experiment. Like numerous other bivalves, they concentrate many xenobiotics in their tissues and have been used extensively for biomonitoring of pollutants [10,25,26]. The PAHs bound to oil droplets may be ingested and retained on the gill epithelium or assimilated in the tissue lipids after incorporation into intracellular vacuoles in the epithelium of the hepatopancreas. Since the binding affinity of PAHs toward oil droplets will increase with large, less water-soluble (high K_{ow}) compounds, a significant fraction of the four, five, and six aromatic-ring PAHs is likely to be measured in mussel tissue.

Fish concentrate lipophilic contaminants mainly by exchange across the gills according to oxygen requirements and gill ventilation rate of the animal, while dietary uptake is minor [24,27]. Since low molecular weight PAHs are relatively more water soluble, the predominance of two and three aromatic-ring PAHs in fish exposed to dispersed oil supports other findings reported in experimental studies and in the field [28–30]. Naphthalenes included in oil droplets seem to rapidly dissolve in the water phase and become more available for fish. Hence, parent naphthalene and its alkylated congeners represent a major proportion of the total PAHs in body tissue, as found by other authors [28,31,32]. Absorption of PAHs bound to oil particles is certainly possible through diffusion in the gut. However, this pathway seems to be relatively minor in fish. For example, in adult rainbow trout, it has been shown that PAHs are not accumulated when contained in the diet because of the combined effects of poor absorption efficiencies through the gut wall and rapid elimination rates [33,34].

The SPMDs were used as a mimic for biological membranes in biota. The SPMDs are time-integrated passive samplers that have been successfully deployed in situ to monitor the bioavailable hydrophobic contaminant [17]. They can be regarded as a substitute for an aquatic organism where uptake is only due to passive thermodynamically driven partitioning between the lipid and the water phases with no biotransformation of the sequestered PAH residues. The SPMDs can only sample truly dissolved PAHs. The polyethylene membranes do contain cavities, but their maximum diameter is of about 10Å [17]. These apertures are far too small in comparison with the mean average size of oil droplets created in this study. Consequently, only the more soluble PAHs may cross the tiny membrane of SPMDs. This seems to explain the separation of the mussel and the SPMD samples along PC2 of the multivariate numerical analysis. Large PAHs were not detected in SPMDs because they were sequestrated in oil droplets and hence were not bioavailable to the devices. In addition, it is possible that biofouling could have reduced the uptake of PAHs in devices during the exposure [25], but this possible reduction is difficult to quantify.

Elimination of PAHs in mussel was relatively slow in comparison with fish. Bivalves like *Mytilus* accumulate hydrocarbons rapidly, but the release is considerably slower, and significant fractions of residues persist even after a long period in hydrocarbon-free water. This is attributed to the sequestration of some hydrocarbons into stable lipid-rich tissue compartments with slow exchange to water. Naphthalene and methylnaphthalene are excreted more rapidly [35–38]. Despite their low rate of metabolism, it has been shown that mussels do biotransform xenobiotics [39,40]. Based on the relationship between log BCF and log K_{ow}, it may be concluded that PAH biotransformation in mussel was minor, if at all, supporting Stegeman's [40] conclusion that bivalve mollusks have only a low level of oxidative metabolism to PAHs. If an efficient metabolism had occurred, the body burden of the larger PAHs would have been reduced significantly after excretion of polar metabolites, hence reducing the BCF. Indeed, some indication of that could be given from the plateau reached for log K_{ow} above five in Figure 4, and PAH metabolizing enzymes were

possibly induced. We rather think, however, that this apparent plateau is due to the nonachievement of the steady-state concentration of the larger PAH compounds in the mussel tissue at the end of the exposure period. The time to reach steady state can be expressed as a linear function of log K_{ow} [5]. Hence, large molecules are expected to reach tissue equilibrium later than small molecules. For example, Moy and Walday [37] observed that mussels exposed to B*a*P in a closed continuous-flow system showed no steady state during a 14-d exposure period. Thus, in our CFS experiment, it is likely that mussels were still in their linear uptake phase for PAHs with log $K_{ow} \geq 5$ when they were transferred into clean seawater. The same observation is also relevant for SPMDs. Because of the high lipid amount in the membranes, it is likely that SPMDs did not reach steady-state equilibrium within the 8 d of the exposure period. Hence, it is not entirely clear whether the lack of large molecular weight compounds in SPMDs is related to the relatively short exposure period or to the sequestration of these compounds in oil droplets not bioavailable to the devices.

Unlike most invertebrates, fish have enzymatic systems that can detoxify efficiently xenobiotics like PAHs [15,29,41]. This detoxification process was shown to be a prerequisite to the occurrence of hepatic lesions [42–44]. A possible explanation of the different PAH patterns between *Mytilus* and turbot is that the latter have a higher and more extensive metabolic ability to degrade and eliminate PAHs. This degradation process has been shown to be more efficient for more hydrophobic PAHs [45,46]. The enzymatic activity (ethoxyresorufin-*O*-deethylase measurement) is induced rapidly in different organs when fish are exposed to oil. Upshall et al. [47] found a maximum induction between day 1 and day 4 after the start of exposure. Evidence of a detoxification process was also revealed in our CFS experiment by Camus et al. [48], who measured a maximum level of fluorescence of PAH metabolites in the bile of fish after 24 h of exposure to dispersed BAL150. It is very likely that metabolic degradation and excretion of PAHs may have increased the rate of elimination in fish, hence decreasing substantially the PAH body burden for molecules with log K_{ow} above four or five within 8 d. Clearly, it was obvious that chemical equilibrium partitioning was not sufficient to explain the PAH composition for fish, and it was not possible to predict BCFs from K_{ow} alone. Bioavailability of PAHs in oil droplets and extensive enzymatic degradation seem consequently the two fundamental factors governing the PAH composition in fish tissue [11].

The PCA revealed differences in the relative proportion of parental to alkylated naphthalene (na), phenanthrene (ph), and dibenzothiophene (dbt) in the samples. Unlike mussels and SPMDs, alkylated ph and dbt in fish displayed a decreasing accumulation with increasing substitution, an observation that supports other studies with flounder [27,31]. For naphthalene, the order of concentration observed was 2na $>$ 1na $\approx$ 3na $>$ na. It is possible that the enzymatic system can degrade and eliminate with better efficiency the congeners with alkylation than the parental molecule.

Our results suggest that the more hydrophobic PAHs in oil droplets are slowly released in the water phase. Although there are species differences, the potential for bioaccumulation of these compounds seems consequently to be reduced. Although a quantification of this process is challenging, this article calls for a greater understanding of the processes controlling the transfer of PAH compounds from the dispersed phase to the water phase and the rates at which these processes occur. To our knowledge, this study is among the first to give insight into a better understanding of the bioconcentration potential for hydrocarbons in oil particles in marine biota. The comparison of bioaccumulation patterns in organisms as different as mussels and fish leads us to conclude that it is essential to consider more than one category of animals to assess PAH levels in marine organisms. This study has shown that substantial species differences exist. Different species may experience different PAH levels in their tissues under the same environmental exposure. Bioavailability related to feeding habits or life styles varies for the different species. In addition, all the species do not have the same physiological ability to metabolize contaminants [4,13,49]. The PAHs are known to have potential genotoxic effects that are exerted after metabolization. Hence, the metabolites are the truly causative agents of the genotoxicity. Consequently, the fate of metabolites in body tissue is important to investigate. This supports the view of Feijtel et al. [5] that assessment of the ecological risk should consider the establishment of quantitative structure–activity relationships for PAH metabolites rather than for PAH parent compounds.

CONCLUSIONS

The current approach in risk assessment of substances discharged into the marine environment is to establish a relationship between the body residue at steady state and the predicted toxic response of low-level exposures. Such a relationship is challenging to obtain from field data because of different sources of contaminants and an unknown past history of the organisms, especially those freely moving in the water column. Laboratory systems, which facilitate controlled exposures like the CFS, represent one alternative even if there is also a necessary field validation to consider. The CFS was extremely useful in investigating the uptake and understanding the potential for accumulation of dispersed hydrocarbons in marine biota. The present study demonstrated that PAH body residue could vary considerably among species. A combination of different physical, chemical, and biological properties are used in determining body burden levels accumulated in the tissue of biota upon chronic exposure. Bioavailability of the substances in the water column is a critical factor because the measured BCFs were, in certain cases, lower than expected from hydrophobicity alone. Depending on bioavailability, the bioconcentration and, accordingly, the potential for biological effects may vary for the different species. Moreover, in organisms that do biotransform contaminants, the levels and consequent effects of metabolites should be investigated further to assess actual environmental risk for marine biota.

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