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BIOACCUMULATION OF POLYCYCLIC AROMATIC COMPOUNDS: 2. MODELING BIOACCUMULATION IN MARINE ORGANISMS CHRONICALLY EXPOSED TO DISPERSED OIL

THIERRY BAUSSANT,*† STEINAR SANNI,‡ ARNFINN SKADSHEIM,† GRETE JONSSON,† JAN FREDRIK BØRSETH,† and BERTRAND GAUDEBERT§

†RF—Rogaland Research, PO Box 2503, Ullandhaug, N-4091 Stavanger, Norway
‡Akvamiljø AS, Mekjarvik 12, N-4070 Randaberg, Norway
§Elf Petroleum Norge, PO Box 168, Dusavik, N-4001 Stavanger, Norway

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Abstract—Within the frame of a large environmental study, we report on a research program that investigated the potential for bioaccumulation and subsequent effect responses in several marine organisms exposed to chronic levels of dispersed crude oil. Body burden can be estimated from kinetic parameters (rate constants for uptake and elimination), and appropriate body burden–effect relationships may improve assessments of environmental risks or the potential for such outcomes following chronic discharges at sea. We conducted a series of experiments in a flow-through system to describe the bioaccumulation kinetics of polycyclic aromatic hydrocarbons (PAH) at low concentrations of dispersed crude oils. Mussels (*Mytilus edulis*) and juvenile turbot (*Scophthalmus maximus*) were exposed for periods ranging from 8 to 21 d. Postexposure, the organisms were kept for a period of 9 to 10 d in running seawater to study elimination processes. Rate constants of uptake (k_1) and elimination (k_2) of the PAHs during and following exposure were calculated using a first-order kinetic model that assumed a decrease of the substances in the environment over time. The estimated bioconcentration factor was calculated from the ratio of k_1/k_2. The kinetic parameters of two-, three-, and four-ring PAHs in mussel and fish are compared with estimates based on hydrophobicity alone, expressed by the octanol-water partition coefficient, K_{ow} (partitioning theory). A combination of reduced bioavailability of PAHs from oil droplets and degradation processes of PAHs in body tissues seems to explain discrepancies between kinetic rates based on K_{ow} and actual kinetic rates measured in fish. Mussels showed a pattern more in compliance with the partitioning theory.

Keywords—Chronic exposure Polycyclic aromatic hydrocarbons Kinetic rates Fish Mussels

INTRODUCTION

Produced water discharges at sea is a matter of concern to environmental authorities because some compounds like the polycyclic aromatic hydrocarbons (PAHs) are known to be toxic to biota. Consequently, research and development programs have been initiated by industry to improve our understanding and estimate the impact of discharges to marine biota [1,2]. One common approach to this problem is based on a proper assessment of the environmental hazard and risk. The current risk assessment models rely on the predicted environmental concentration–predicted no-effect concentration ratio. Standard laboratory acute toxicity tests are used to estimate the predicted no-effect concentration. However, a more realistic approach for environmental risk of chronic discharges is to estimate effects based on actual body burdens that stem from time varying exposure of organisms [3–5]. Internal body concentration rather than external water or sediment concentration is then used to estimate a critical body residue of substances at focus and above which toxic effects are expressed. Appropriate dose–effect relationships may allow for a more accurate prediction of the environmental risk and thus promote a more precise regulation and management of chemical discharges at sea. Biological effects of time-varying chronic levels of toxic substances like PAHs can be expressed at different levels. Relevant ecological impacts are those impairing growth or reproduction success or causing an accumulation of genetic damage that may be inherited in subsequent generations. A chain of events occurs from the molecular or cellular levels to the population level, but the links between these events are complex and are still poorly understood. In most cases, the chemicals must enter the body before they can exert their toxic effects. Hence, uptake rates and bioaccumulation levels of substances within the body tissues are of particular interest because they can be related to toxicity. Consequently, the relationships between toxicity and body burden can be used to predict impact [6], and new risk assessment models of continuous discharges to the marine environment are developed on this basis rather than the predicted environmental concentration–predicted no-effect concentration approach [5].

The body burden is defined as the internal time-varying exposure concentration of a contaminant in an organism. It is determined by the dynamic balance between uptake and elimination processes, which are influenced by several factors like temperature, ventilation rates, metabolism, and type of species and are compound dependent. Uptake is, to a large extent, a function of the lipophilic nature of the substance and the type of exposure. Elimination is the result of both passive, thermodynamically driven diffusion from the organisms to the external medium and enzymatic pathways (metabolization) that convert lipo (= petro)philic parent molecules to more polar and hence more water-soluble metabolites.

For risk assessment, it is important to estimate the body burden at the equilibrium that may elicit a toxic response [7]. A convenient way to express this is to estimate the biocon-

* To whom correspondence may be addressed (thierry.baussant@rf.no).

centration factor (BCF), which is defined as the ratio of substance concentration in the organism to the concentration of substance in the medium at equilibrium. The BCFs have frequently been found to correlate well with hydrophobicity expressed by the octanol/water partition coefficient (K_{ow}) and linear relationships have been established on log scales [8–11]. Since the partition coefficient of the larger PAHs is high, BCFs predicted on the basis of K_{ow} should consequently be higher than for smaller PAHs. However, a combination of reduced bioavailability and/or enzymatic degradation of contaminants (metabolism) make the measured BCFs lower than the BCFs estimated from K_{ow} [12–15].

Bioavailability may be seen as the potential for a substance to enter an organism. It is related to various physicochemical properties of the substances that influence rates of phase shifts and equilibrium levels, i.e., how compounds distribute between water, dissolved or suspended particulate matter, or dispersed oil droplets. Biological processes like biodegradation are also determining bioavailability of a substance to marine biota.

The environmental fate of PAHs is, to a large extent, dependent on the molecular size. The rate at which the PAH molecules leave the oil and enter the water decreases with increasing molecule size [16]. In addition, with increasing molecule size, PAHs are increasingly at an energetic nonequilibrium when entering the water. Within the series from benzene to pyrene [17], the free energy of the aqueous solvation was found to be virtually independent of the molar volume of the PAH. The PAHs were approximately equally hydrophobic. Zhang and Gobas [17] found that increasing K_{ow} and environmental partition coefficients with increasing molecular size reflected the dependence of chemical–chemical interactions on the molecular volume in the (subcooled) liquid phase, i.e., between the PAH molecules themselves, rather than the chemical–water interactions, as is usually assumed. In other words, increased K_{ow} with increased molecular size is due to an increase in lipophilicity, not to an increase in hydrophobicity. The (subcooled) liquid phase may, as a practical approximation and approach, be considered as the PAH in the oil phase. In an environmental context, this means that, with increasing molecular size, the rate at which a PAH leaves the water to partition into organisms increases, as does the strength of association with the organisms or other phases with organic matter or hydrocarbons.

A reduced bioavailability of single PAH compounds bound to particulate or dissolved organic material has been experimentally demonstrated by several authors [13,16–20]. A similar reduction seems to occur with complex mixtures of PAH compounds bound to oil droplets [21]. The small, less hydrophobic PAHs ($\log K_{ow} < 4$) dissolve relatively faster in the water, therefore being more readily bioavailable than larger, more hydrophobic PAHs ($\log K_{ow} > 4$) that remain longer in oil droplets and dissolve at a slower rate. Bioavailability is also density and species dependent since routes of uptake may vary for different types of organisms [22–24]. For example, on exposure to oil dispersions, relatively larger molecular PAHs are taken up as the density of unicellular algae is decreased. Moreover, as organism size increases (unicellular algae, crustacean *Artemia* nauplii, small fish), relatively smaller molecular PAHs constitute the body burden [25].

Biotransformation of parental nonpolar substances like PAHs into polar, hence water-soluble, metabolites involves enzymatic degradation processes. The presence and efficiency of these metabolic processes is also species dependent. Mussels can metabolize PAHs by enzymatic processes, but the efficiency of this degradation pathway seems to be less than in vertebrates [26]. In contrast with mussels, fish efficiently metabolize PAHs even after relatively short exposure periods and exhibit lower concentrations of parental molecules [27,28]. Enzymatic activity induced by substances like PAHs may apparently efficiently decrease the body burden of parental molecules in these organisms. This enzymatic degradation process seems to be even more efficient on larger PAHs [29].

The dissolution rate of the various PAH molecular forms from oil droplets to the surrounding seawater is difficult to assess and is, to date, poorly described. However, the water-dissolved state seems in turn to determine the rate at which these organic molecules are bioaccumulated in the body tissues of marine organisms exposed to oil particles [25,30,31].

The lack of models and data on hydrocarbon release rates from oil dispersions constitutes a challenge to environmental impact assessment of such discharges. Existing approaches rely on poorly controllable preparations of water-accommodated fractions of oil or on the use of dissolution from oil films, the water-soluble fraction approach [25]. Exposures with well-characterized dispersions containing oil droplet sizes typical of produced water discharges provide the most realistic simulation of such uptake. These dispersions will also, due to the increased surface-to-volume ratio of small oil particles compared with the other approaches cited above, provide real rate and phase dependence functions (relationships) on bioconcentration. Present environmental impact assessment models are accordingly weak on oil dispersions. Considering all hydrocarbons in dispersions as water dissolved constitutes an overestimate of the potential for uptake, while relying on water-accommodated fractions or water-soluble fractions constitute an underestimation of the same. Both outcomes may have subsequent consequences for licensing and regulation of industrial activity by the authorities. Also, focus may possibly be put on the wrong phase. For example, the conservative approach of treating dispersions as water dissolved will underestimate the retainment of larger molecular PAHs and other slowly dissolving molecules in the particulate phase. As bacteria quickly grow on new particulate matter at sea and molecules like large PAHs quickly associate with any new organic matter, it may also lead to an underestimation of the sedimentation processes and the potential for impact in the sediments.

We performed a series of experiments where mussels and fish were exposed to low levels of dispersed crude oil for periods of 8 and 21 d, respectively. The experiments were carried out in a continuous-flow system (CFS) in order to simulate chronic discharges of hydrocarbons and study their impact on marine organisms. Chronic levels of poorly miscible substances like crude oil may be supplied to marine biota for extensive period of times [32]. We have presented evidence of the combined effects of bioavailability and metabolism to explain the bioconcentration patterns of PAHs in mussels and fish exposed to dispersed oil [21]. The present study is aimed at estimating the kinetics of uptake and elimination in these two organisms. A kinetic model allowing decline of seawater concentration over time was used to calculate uptake rates in fish. The bioconcentration factor was estimated from the ratio of uptake and elimination rate constants. The results are discussed in relation to prediction based solely on partitioning of PAHs based on K_{ow}.

Table 1. List of polycyclic aromatic hydrocarbons identified in blended Arabian light topped at 150°C (BAL150) and North Sea crude oil based on U.S. Environmental Protection Agency recommendations; the compounds in italics were, however, not analyzed in the present article; values for log K_{ow} are taken from Meador et al. [40] and Neff and Burns [23] (ND = not detectable)

				BAL150		North Sea crude oil	
Compound	Code	log K_{ow}	Molecular weight	Mean (μg/g)	SD (μg/g)	Mean (μg/g)	SD (μg/g)
Naphthalene	na	3.34	128.2	148	10.2	1,169	67
C1-naphthalene	c1-na	3.86	142.2	609	47	2,108	115
C2-naphthalene	c2-na	3.91	156.2	1,418	142	2,204	208
C3-naphthalene	c3-na	4.90	170.3	1,395	171	1,172	184
Acenaphthylene	acy	4.08	152.2	9.3	1.1	11	1.3
Acenaphthene	ace	4.08	154.2	4.9	1.5	18	6.3
Fluorene	fl	4.22	166.2	42	4.9	265	34
Anthracene	an	4.53	178.2	ND	—	1.5	0.5
Phenanthrene	ph	4.53	178.2	79	9.2	238	14
C1-phenanthrene	c1-ph	5.15	192.2	241	18	386	32
C2-phenanthrene	c2-ph	5.51	206	320	17	333	31
Dibenzothiophene	dbt	4.38	184	342	14	51	8.5
C1-dibenzothiophene	c1-dbt	4.86	198	827	63	111	15
C2-dibenzothiophene	c2-dbt	5.50	212	1,330	57	106	12
Fluoranthene	fluo	5.24	202.3	ND	—	10	1.9
Pyrene	py	5.07	202.3	4.9	1	20	3.5
Chrysene	ch	5.77	228.3	6.9	1.3	26	3.9
C1-chrysene	c1-ch	6.42	242.2	19	3.3	42	6.8
C2-chrysene	c2-ch	6.88	256.3	25	4.2	37	7.7
Benzo[*a*]anthracene	baa	5.91	228.2	ND	—	11	1.7
Benzo[*b*]fluoranthene	bbf	5.80	252.3	1.7	0.4	4.2	0.9
Benzo[*k*]fluoranthene	bkf	6.00	252.3	ND	—	ND	—
Benzo[*b+k*]fluoranthene	bbkf	—	—	ND	—	5.1	0.6
Benzo[*a*]pyrene	bap	6.04	252.3	ND	—	1.3	0.4
Indeno[*1,2,3,cd*]pyrene	ip	7.00	276.3	ND	—	ND	—
Benzo[*g,h,i*]perylene	bghip	6.50	276.3	ND	—	1	0
Dibenzo[*a,h*]anthracene	daha	6.75	278.3	ND	—	ND	—

METHODS

Organisms

Juvenile turbots (*Scophthalmus maximus* L.) were purchased at a fish farm (Tinfos Aqua as, Farsund, Norway). After shipping, they were stored in tanks with running sand-filtered seawater at a temperature of 19 ± 1°C. During the quarantine period, the individuals were fed the dried food pellets used at the farming site.

The blue mussels (*Mytilus edulis*) were collected along the shore at a non-contaminated site close to the mouth of the fjord north of Stavanger, Norway. After their sampling, they were placed in a large tank with running sand-filtered seawater with no further temperature regulation. During the quarantine period, no food was provided to the animals. Prior to the exposure experiments, the shell of each individual was brushed to remove sessile animals growing on it.

Experimental arrangement

Exposure system. Fish and mussels were exposed in two separate experiments carried out in 1997. Each bioassay was performed with the CFS, a system designed for testing chronic toxicity of complex mixtures of substances with poor water solubility [32]. This system comprises continuous-flow exposure (chronic) of aquatic organisms to natural contaminant levels in closed chambers and in a light- and temperature-controlled room. Each chamber had a volume of 18 L. They contained a central cylinder where a Teflon®-coated main propeller rotated at a speed of approximately 250 rpm for circulation of the water in the chamber and maintenance of the droplets in suspension. The lid of the chamber was conical to reduce coating. Another smaller Teflon propeller, fixed close to the top of the lid, rotated with the main propeller and enhanced water circulation at the inner surface of the lid. In addition, magnetic bars (aquarium principle) were also moved by hand to allow frequent removal of oil coating the lid.

The test solution was supplied through the bottom of the chamber and the outlet was at the top. A flow rate of approximately 250 ml/min was maintained in the chambers. Oil was kept in a dark, sealed storage bottle and pumped directly into the stream of seawater before dispersion and distribution to the chambers. No carrier solvent was added.

Droplets formation was achieved by mechanically driven energy using alternatively a mixing valve (RF-Rogaland Research, Stavanger, Norway) or a Dispax rotator (model DPL3-5E, IKA-Maschinen bau, Staufen, Germany) at 10,000 rpm. These devices were placed at a short distance from the injection point of the oil to minimize coating and optimize the formation of droplets (see [32] for more details of the CFS). The mixture of oil droplets and seawater (= dispersion) was then supplied to the chambers. The average droplet size was 10 μm in diameter, as monitored by frequent Coulter Counter© multisizer measurements (Beckman-Coulter, Fullerton, CA, USA).

Exposure conditions. In the two bioassays, a photoperiod of 12 h light and 12 h dark was used. In the experiment with turbot, seawater temperature was maintained at 20 ± 1°C, whereas in the mussel experiment, fjord temperature (7 ± 1°C) was used with no regulation. Two types of oil were tested, a blended Arabian light topped at 150°C (BAL150) and a North Sea crude oil. The PAH composition of these two oils is described in Table 1.

Adult blue mussels were exposed to a dispersion of BAL150 at a nominal concentration of 1 mg/L. Three exposure

chambers were used for the bioconcentration studies and each of them contained approximately 100 mussels. Uptake clearance kinetics and bioconcentrations were followed during an exposure period of 8 d. In the turbot experiment, three exposure chambers were also used and each of them contained 14 fish with an average weight of 12.5 g. The fish were exposed to dispersed North Sea crude oil at a nominal concentration of 0.5 mg/L. The individuals were exposed for a period of 21 d.

Elimination kinetics were followed after transfer of the remaining organisms to PAH-free seawater. At the end of the exposure period, each set of animals was temporarily moved (some minutes) into a bucket filled with clean seawater. The exposure chambers were then washed with hot water. After cleaning, the animals were immediately replaced in their respective chambers and kept in PAH-free running seawater for measurement of elimination rate constants during periods of 9 d for fish and 10 d for mussels.

Sampling and sample preparation

Seawater. Silicon tubing was connected to the output of the exposure chambers. During a sampling event, a volume of 2 L was collected in prewashed and heated (500°C) amber Duran (Mainz, Germany) glass bottles filled with 16 ml of a 10% hydrochloric acid solution (pH $<$ 2). The seawater sample preparation was then made according to the method described in Baussant et al. [21].

Organisms. Before sampling of organisms, the volume of water in the exposure chamber was reduced by siphoning, the lid was then opened, and the individuals were caught with a net (fish) or by means of tongs (mussels).

One fish was taken from each exposure chamber (n = 3) during each sampling event. The fish was first transferred to a glass containing clean seawater and, after about half a minute, it was placed in another glass with clean seawater. The skin was wiped with paper tissue thereafter. This helped to remove extra mucus secretion where oil particles could be loosely attached. Weight and length were then measured. The whole fish was then cut into small pieces with stainless steel scissors that were rinsed successively in one bath of cyclohexane and two to three baths of distilled water after each sample. Following homogenization, the tissues were transferred into a glass vial sealed with a Teflon lid and stored at −80°C until analysis.

Four mussels were opened per sample. The residual seawater trapped in each shell was removed and the pooled soft tissue was then transferred into a glass beaker. Homogenization was performed without any solvent addition by an Ultra-Turax® (IKA-Maschinen bau, Staufen, Germany). The rod and blades were rinsed successively with cyclohexane and distilled water between samples. The homogenate was transferred to vials sealed with a Teflon lid and stored at −80°C until analysis.

The biological samples were thereafter prepared according to the method described in Baussant et al. [21].

Chemical analyses of the samples

Polycyclic aromatic hydrocarbons. Bioconcentration was studied for all PAH species based on the U.S. Environmental Protection Agency-recommended list ranging from the two aromatic (naphthalene) to the hexa aromatic (benzo[*ghi*]perylene). However, in this presentation, we will limit our description to the two-, three-, and four-ring PAHs (Table 1). The methylated forms of naphthalene (c1–c3), phenanthrene (c1, c2), and chrysene (c1, c2) were also quantified. Parental and methylated forms (c1, c2) of dibenzothiophene were likewise analyzed in the tissue homogenates. The PAH analyses of all samples were performed by a gas chromatograph (GC; HP5890, Hewlett Packard, Avondale, PA, USA) connected to a mass spectrometer (MS; Finnigan SSQ7000, Bremen, Germany) set 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 and 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. The calculation method and quality assurance are described in Baussant et al. [21].

All solvents and chemicals used for extraction procedures were of SupraSolv quality (Merck, Darmstadt, Germany). Distilled water was deionized (18.2 MΩ) prior to use. The glassware was scrupulously cleaned. First, a detergent powder in a Miele laboratory dishwasher with hot water (90°C) was used. Tap water and distilled water were used for rinsing. Turbo Vap glass equipment (Zymark, Hopkinton, MA, USA) used for sample extraction was heated at 400°C for 3 h to eliminate any trace of contaminants after the washing. Other glassware equipment was heated in a furnace at 500°C for 3 h with slow temperature increase and decrease. Samples were protected from light during extraction and analysis.

Lipids. The final level of contaminant was expressed either as amount of PAHs to the body wet weight or lipid weight. A liquid/liquid extraction procedure based on the method established by Folch et al. [33] and on the recommendations of Christie [34] was used. The lipid extraction was performed with a mixture of chloroform and methanol in the ratio 2:1. The total lipid extraction was carried out with approximately 1.5 g of homogenized tissue for turbot and was an average 3% of the body wet weight. For mussels, 2.5 g of tissue was analyzed and the total lipid content was approximately 2% of the body wet weight.

Data analysis

Fish experiment. Despite the use of a continuous-flow system with closed exposure chambers, the concentrations of the PAHs in the seawater decreased with time. The bioconcentration data were fit by nonlinear regression to a model that includes a term for decline in the water concentration. This model can be seen as simulating an in situ dilution process with water concentration gradually decreasing. The rationale for using this approach can be found, e.g., in the papers of Landrum [14] and Widianarko and van Straalen [35]. The model is described by

$$\frac{dC_f}{dt} = k_1 C_w^0 e^{-\lambda t} - k_2 C_f$$

which, after integration, gives

$$C_f = \frac{k_1 C_w^0 (e^{-\lambda t} - e^{-k_2 t})}{(k_2 - \lambda)} \quad (1)$$

where C_f = the concentration of the compound in the fish (μg/kg wet wt or mg/kg lipid wt)
C_w^0 = concentration of compound in seawater at the start of the exposure (μg/L)
k_1 = uptake clearance rate constant of compound from seawater (L seawater/kg organism/d)

k_2 = elimination rate constant (d^{-1})
λ = rate constant for the decline of compound in the seawater (d^{-1})
t = time (d)

The term $e^{-\lambda t}$ describes the rate of compound nonavailable to the fish because of the (assumed exponential) decline in the seawater. Uncontaminated food was provided to the fish during the experiment and, consequently, the uptake of compound through food consumption was likely to be negligible because excess pellets were removed by siphoning shortly after the feeding event. Elimination rate constants, k_2, used in Equation 1 were estimated by the slope of the linear regression line for plots of ln C_f versus time fit to a first-order decay as

$$\ln C_f = \ln C_f^0 - k_2 t$$

where C_f^0 is the concentration of the compound in the fish at the beginning of the elimination period.

Mussel experiment. Because the data suggested that mussels were still in a linear phase of uptake after the end of exposure (see [21]) and that elimination rates were low, a simple first-order linear model was used to fit the data and estimate the initial uptake clearance rate, k_1, in mussels as

$$C_m = k_1 C_w t \quad (2)$$

where C_m = concentration of the compound in mussels (μg/kg wet wt or mg/kg lipid wt).

Only the first data points (day 0.33–day 2; $n = 4$) were used in the calculations of k_1 because mussels started to spawn after that period. Also, elimination rate constants could not be calculated accurately because of fluctuations in the data during the first days of the elimination phase. They will not be described in this article.

Analyses of bile metabolites in fish

In parallel with the parent PAH analysis by GC/MS on whole fish, the presence of PAH metabolites in the bile of fish was investigated during each sampling event by fixed wavelength fluorescence (FF) performed on a Perkin Elmer LS 50 B luminescence spectrofluorometer (Norwalk, CT, USA) [36]. The excitation/emission wavelength pair 290/335 nm was used as representative of metabolites of two- to three-ring PAHs. Ethanol (48%) was used to dilute bile samples (1:1,600) prior to fluorescence measurements.

Statistics

Nonlinear regressions of bioconcentration data were modeled using JMP software (*Windows*, Ver 3.1, SAS Institute, Cary, NC, USA). Elimination rate constants were calculated with a macro program using Excel (*Windows*, Ver 7.0, Microsoft, Redmond, WA, USA). The slope of the regression line was considered significant at $p < 0.05$.

RESULTS

Uptake and bioconcentration in fish

Figure 1 illustrates the bioconcentration kinetics of three PAHs representative of two-, three-, and four-ring compounds. The two- and three-ring PAHs presented a similar temporal pattern as exemplified by na and ph (see Table 1 for PAH codes). Typically, a rapid uptake was observed shortly after the start of the exposure. A maximum level in fish was reached after approximately 3 d, then a decrease in PAH concentrations occurred. The kinetics of ch, a four-ring PAH, was slightly different, although more variability was measured in the fish

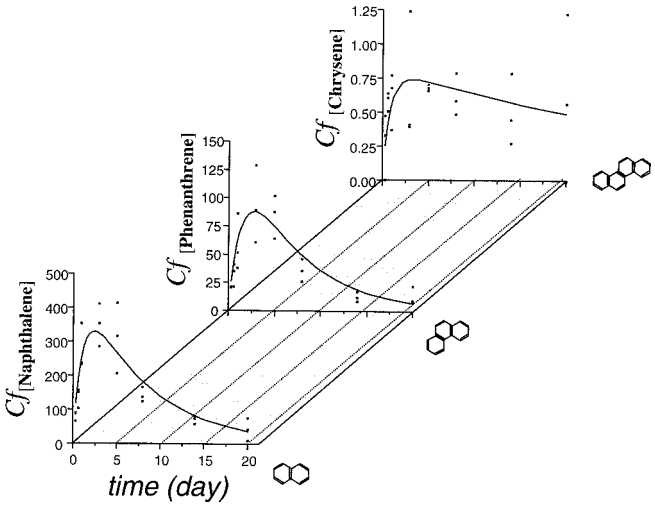

Fig. 1. Kinetics of naphthalene, phenanthrene, and chrysene in fish during the exposure period. Data points are displayed with small x symbols. The curve corresponds to the nonlinear fit of the data using the model expressed in Equation 1. Units are μg/kg wet weight. Note different scales for polycyclic aromatic hydrocarbon (PAH) concentrations. C_f = concentration of hydrocarbon in the fish tissue.

for this compound. The decline over time seemed to be relatively less pronounced than for na and ph.

The model fit rather well to the description of all compound kinetics, and the assumption of first-order decay for exposure concentration with time seemed to be adequate. The parameters for these uptake kinetics are summarized in Table 2. The estimated coefficient λ varied from 0.024 to 0.285/d. The lowest values were obtained for the largest molecules, i.e., fluo and ch, suggesting relatively less decline in water phase. Consequently, a simplification of model 1 might have been relevant for the four-ring PAHs. However, the standard deviation of λ was also relatively high, indicating fluctuations in the data for these compounds and that more replicates would have been an improvement.

Larger uptake clearance rate constants were estimated for the small, relatively more water-soluble molecules, i.e., alkylated na (c1-na and c2-na), acy, and parent dbt. c1-dbt and c2-dbt were decreasing very early after the start of exposure. Hence, it was not possible to estimate the k_1s with this sampling strategy for the methylated forms of dbt. In contrast with the naphthalenes, k_1 constants for the phenanthrenes decreased with increased methylation. The uptake rate for c2-ph was low and represented only 1% of that of c2-na. The uptake rate for ch was 5% of that of c2-na. Alkylated forms of ch were not detected during the experiment. The py was detected in the fish, but values were too variable to allow reliable k_1 estimation.

The relationship between uptake clearance rate constants based on lipid weight, k_{L1}, and the molecular lipophilicity expressed by log K_{ow} is illustrated in Figure 2. The plot showed that the uptake rate constant for fish increased slightly with increasing log K_{ow} up to ≈4 and then declined with larger log K_{ow}. This curvilinear shape of the data was well represented by a polynomial expression ($n = 10$; $r^2 = 0.846$). Hence, only the uptake rate constants for the low-molecular weight PAH compounds seemed to fit the partitioning theory in which the bioconcentration factor of a substance is directly related to the partition coefficient, K_{ow}.

Table 2. Uptake (k_1) and elimination (k_2) rate constants and λ parameters for the kinetics of the polycyclic aromatic hydrocarbons in fish as estimated from model 1;[a] bioconcentration factor (BCF) estimated from k_1/k_2 (ND = not detected; NS = regression line not significant; S = regression line significant)

			Wet weight data (μg/kg)							Lipid weight data (mg/kg)						
Compound	Code	log K_{ow}	k_1	λ	k_2	n	r^2	$p <$ 0.05	BCF	k_{L1} ($\times 10^4$)	λ	k_2	n	r^2	$p <$ 0.05	BCF
Naphthalene	na	3.34	396 ± 32	0.131 ± 0.021	0.94 ± 0.19	8	0.80	S	421	1.10 ± 0.06	0.124 ± 0.013	0.90 ± 0.13	8	0.89	S	12,248
C1-naphthalene	c1-na	3.86	975 ± 82	0.098 ± 0.018	0.91 ± 0.17	8	0.83	S	1,071	2.73 ± 0.17	0.089 ± 0.012	0.88 ± 0.12	8	0.90	S	31,120
C2-naphthalene	c2-na	3.91	1,232 ± 109	0.083 ± 0.017	0.87 ± 0.18	8	0.79	S	1,416	3.45 ± 0.24	0.075 ± 0.012	0.83 ± 0.14	8	0.85	S	41,660
C3-naphthalene	c3-na	4.90	606 ± 56	0.085 ± 0.018	0.77 ± 0.19	8	0.73	S	787	1.69 ± 0.14	0.077 ± 0.014	0.73 ± 0.16	8	0.78	S	23,264
Acenaphthylene	acy	4.08	1,055 ± 147	0.060 ± 0.023	—	—	—	—		—	—	—	—	—	—	
Acenaphthene	ace	4.08	525 ± 49	0.107 ± 0.019	—	—	—	—		—	—	—	—	—	—	
Fluorene	fl	4.22	885 ± 87	0.119 ± 0.022	0.61 ± 0.20	8	0.60	S	1,451	2.44 ± 0.19	0.111 ± 0.017	0.57 ± 0.16	8	0.68	S	42,942
Anthracene	an	4.53	758 ± 96	0.193 ± 0.040	—	—	—	—		—	—	—	—	—	—	
Phenanthrene	ph	4.53	646 ± 65	0.159 ± 0.03	0.69 ± 0.15	8	0.79	S	936	1.78 ± 0.14	0.152 ± 0.022	0.65 ± 0.11	8	0.84	S	27,388
C1-phenanthrene	c1-ph	5.15	144 ± 17	0.145 ± 0.033	0.74 ± 0.14	7	0.85	S	195	0.38 ± 0.04	0.136 ± 0.027	0.68 ± 0.10	7	0.89	S	5,701
C2-phenanthrene	c2-ph	5.51	15 ± 2	0.108 ± 0.025	0.69 ± 0.21	6	0.73	S	22	0.04 ± 0.005	0.087 ± 0.021	0.65 ± 0.18	6	0.76	S	631
Dibenzothiophene	dbt	4.38	912 ± 100	0.285 ± 0.05	0.61 ± 0.21	7	0.63	S	1,495	2.47 ± 0.23	0.29 ± 0.041	0.55 ± 0.16	7	0.70	S	44,971
C1-dibenzothiophene	c1-dbt	4.86	—	—	—	—	—	—		—	—	—	—	—	—	
C2-dibenzothiophene	c2-dbt	5.50	—	—	—	—	—	—		—	—	—	—	—	—	
Fluoranthene	fluo	5.24	210 ± 62	0.063 ± 0.026	—	—	—			0.56 ± 0.16	0.057 ± 0.024	—	—	—		
Pyrene	py	5.07	—	—	—	—	—	—		—	—	—	—	—	—	
Chrysene	ch	5.77	64 ± 9	0.024 ± 0.017	1.18 ± 0.46	4	0.77	NS	54	0.12 ± 0.02	0.035 ± 0.019	0.66 ± 0.41	4	0.56	NS	1,865
C1-chrysene	c1-ch	6.42	ND	—	ND	—	—	—		ND	—	ND	—	—	—	
C2-chrysene	c2-ch	6.88	ND	—	ND	—	—	—		ND	—	ND	—	—	—	

[a] Model: $C_f = k_1 C_w^0 e^{-\lambda t} - e^{-k_2 t})/(k_2 - \lambda)$.

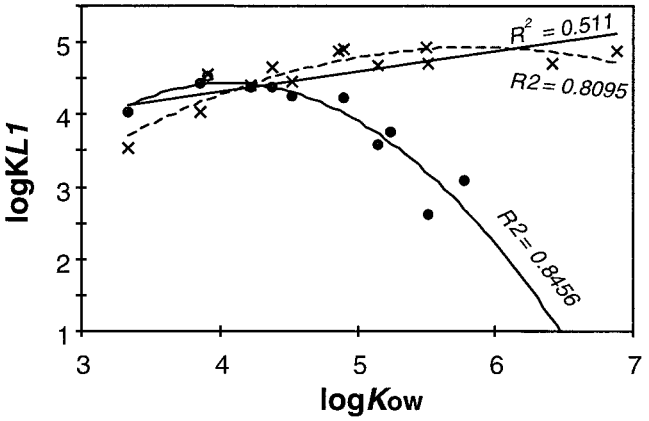

Fig. 2. Plot of the log of uptake clearance rate constants, log k_{L1}, versus the log of the hydrophobicity expressed by the octanol/water partition coefficient, log K_{ow}, in fish (● = polynomial fitting) and mussels (× = linear ——— and polynomial – – – fittings).

Uptake in mussels

Uptake clearance rate constants of PAHs in mussels were fitted to the first-order linear model expressed by Equation 2. The kinetic values are reported in Table 3. All compounds were found to fit significantly well to this model except for ace and parental ch, for which more variability was observed during the initial phase of uptake. The uptake rates of the larger molecules considered in this article, the four-ring PAHs, were much higher than in the fish. Also, there were increasing uptake clearance rate constants with increasing number of substitutions for all compounds, and the largest k_1 values were calculated for c3-na, c2-dbt, and c2-ch. The relationship of k_{L1} with log K_{ow} is well represented by a linear model, as depicted in Figure 2. Uptake rate constants were significantly increasing with hydrophobicity of the PAHs ($r^2 = 0.511$; $p < 0.05$). Thus, for mussels, the partitioning theory was verified and uptake constants increased with increasing lipophilicity of the PAHs. Since k_{L1} for molecules with log K_{ow} above five tended to reach a maximum level, the application of nonlinear fit curves (polynomial expression) was, however, also good ($r_2 = 0.809$).

Elimination rates in fish

The initial elimination rate constants in fish appeared to follow first-order kinetics (Table 2). To express these kinetics, we have computed the time to decrease by 95% (T_{95} in days) of the initial concentration measured at the start of the elimination period by

$$T_{95} = 3.0/k_2$$

where k_2 is the elimination rate constant measured from the fish data during the elimination phase.

These values were compared with a predicted T_{95pre} based on a predicted k_{2pre} estimated following Spacie and Hamelink [9] as

$$\log k_{2pre} = 1.47 - 0.414 \log K_{ow}$$

There was no large difference between the elimination rates of the various compounds in the fish. The T_{95} for all compounds ranged from 3 to 5 d, demonstrating the ability for the fish to eliminate quite rapidly the PAH compounds upon transfer to clean seawater. Good agreement was found between the calculated and the predicted T_{95} for compounds with log K_{ow} below four, but the fit to the model was poorer for compounds with log K_{ow} above four (Fig. 3). Consequently, there was no correlation between the elimination rates and the octanol-water partition coefficient for the larger compounds with log $K_{ow} > 4$.

Relationships of estimated BCF with log K_{ow} *in fish*

The estimated bioconcentration factors were calculated from the ratio of k_1/k_2 by using the values calculated for these rate constants shown in Table 2. The various BCFs were plotted against log K_{ow} and were compared with the BCF provided by

Table 3. Uptake rate constants for the various polycyclic aromatic hydrocarbons in mussels as estimated from model 2 (NS = regression line not significant; S = regression line significant)[a]

			Wet weight data (μg/kg)				Lipid weight data (mg/kg)			
Compound	Code	log K_{ow}	k_1	n	r^2	$p <$ 0.05	k_{L1} (× 10^4)	n	r^2	$p < 0.05$
Naphthalene	na	3.34	61 ± 9	8	0.89	S	0.34 ± 0.05	8	0.89	S
C1-naphthalene	c1-na	3.86	201 ± 28	8	0.89	S	1.11 ± 0.17	8	0.87	S
C2-naphthalene	c2-na	3.91	652 ± 79	8	0.92	S	3.60 ± 0.47	8	0.91	S
C3-naphthalene	c3-na	4.90	1,439 ± 187	8	0.91	S	7.97 ± 1.07	8	0.90	S
Acenaphthylene	acy	4.08	887 ± 91	8	0.94	S	4.92 ± 0.56	8	0.93	S
Acenaphthene	ace	4.08	210 ± 286	4	0.21	NS	1.18 ± 1.68	4	0.20	NS
Fluorene	fl	4.22	468 ± 58	8	0.91	S	2.57 ± 0.38	8	0.88	S
Anthracene	an	4.53	—	—	—	—	—	—	—	—
Phenanthrene	ph	4.53	529 ± 124	8	0.75	S	2.92 ± 0.68	8	0.75	S
C1-phenanthrene	c1-ph	5.15	879 ± 217	8	0.73	S	4.91 ± 1.06	8	0.78	S
C2-phenanthrene	c2-ph	5.51	906 ± 267	8	0.66	S	5.04 ± 1.35	8	0.70	S
Dibenzothiophene	dbt	4.38	797 ± 115	8	0.89	S	4.39 ± 0.69	8	0.87	S
C1-dibenzothiophene	c1-dbt	4.86	1,348 ± 199	8	0.88	S	7.43 ± 1.17	8	0.87	S
C2-dibenzothiophene	c2-dbt	5.50	1,551 ± 252	8	0.86	S	8.58 ± 1.46	8	0.85	S
Fluoranthene	fluo	5.24	—	8	—	—	—	8	—	—
Pyrene	py	5.07	—	8	—	—	0.20 ± 5.8	8	0.00	NS
Chrysene	ch	5.77	556 ± 403	8	0.24	NS	3.02 ± 2.24	8	0.23	NS
C1-chrysene	c1-ch	6.42	955 ± 281	8	0.66	S	5.17 ± 1.67	8	0.61	S
C2-chrysene	c2-ch	6.88	1,371 ± 401	6	0.75	S	7.73 ± 2.72	6	0.67	S

[a] Model: $C_m = k_1 C_w t$.

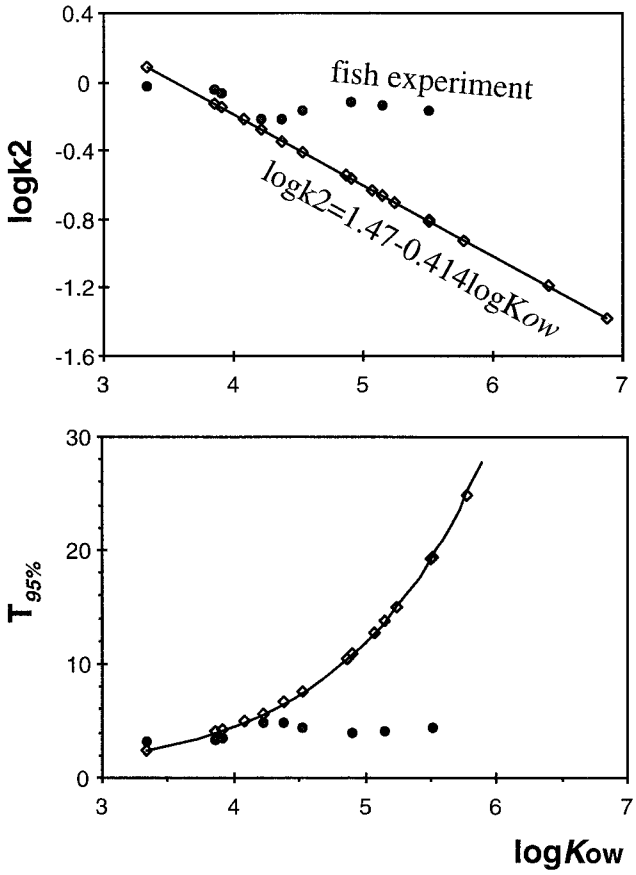

Fig. 3. (**Top**) Plot of the log of the elimination rate constants versus the log of the octanol-water partition coefficient. (**Bottom**) Plot of T_{95} versus log K_{ow}. ♦ = prediction from log k_2 = 1.47 − 0.41 log K_{ow} [9]; ● = estimated values from our data.

the model of Veith et al. [37]. In this model, BCF is predicted to be a linear function of K_{ow} as

$$\log \mathrm{BCF}_{pre} = 0.85 \log K_{ow} - 0.70$$

Since the elimination rate constants in fish were in a narrow range of values for the different PAHs, the BCFs reflect the pattern described for uptake rate constants plotted against log K_{ow}. Figure 4 illustrates this relationship. An increase of BCF was observed with increasing log K_{ow} up to ≈4 (i.e., mainly

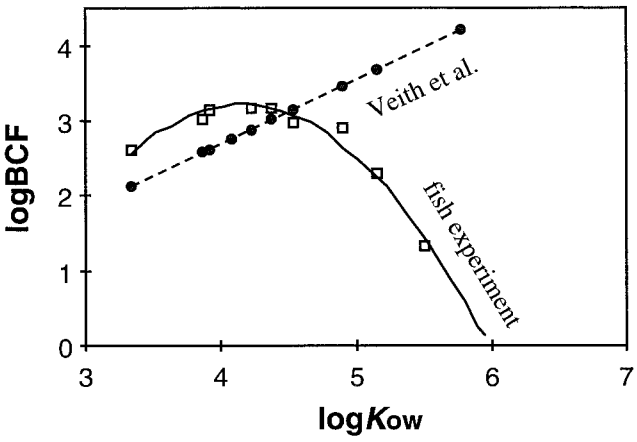

Fig. 4. Relationship between log BCF and log K_{ow}. ● = prediction from the equation of Veith et al. [37]; □ = estimated BCF from the ratio of k_1/k_2. Chrysene was excluded from the plot (k_2 not significant).

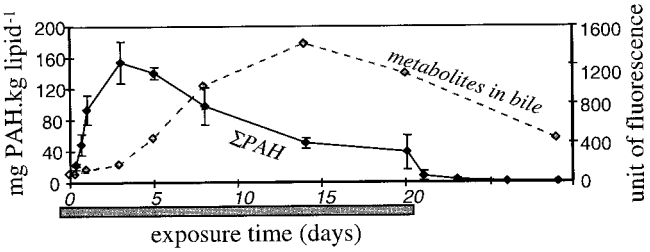

Fig. 5. Plot of the sum of parent and alkylated PAH body burdens measured in whole fish (ΣPAH) by gas chromatography/mass spectrometry analysis and corresponding fluorescence of metabolites in the bile of fish over time.

naphthalenes), but there was a decline of BCF for PAHs with larger log K_{ow}. Consequently, the log BCF–log K_{ow} relationships were expressed by a polynomial equation rather than by a linear expression, and BCF prediction based on hydrophobicity seemed to apply well only in a limited range of log K_{ow} covering the most water-soluble substances.

Evidence of biotransformation in bile of fish by FF

The FF values are expressed as arbitrary fluorescence units. The FF showed a time-displaced peak that related to the pattern of ΣPAHs (parent and alkylated PAHs) over time (Fig. 5). A gradual increase of PAH metabolites was observed after a few days of exposure when ΣPAH started to decrease. A maximum in FF measurements was recorded at day 14, approximately 10 d after the maximum concentration of ΣPAH in the fish.

DISCUSSION

Uptake clearance rate constants for the various PAH compounds followed different patterns in mussels and fish compared with the expected patterns based on the partition coefficients. Based on the log K_{ow}, uptake rates of the larger PAHs should bioconcentrate to a larger degree. Uptake rates in fish were highest for C2-naphthalenes, while a considerably lower uptake was estimated for the C3 form of naphthalene, the alkylated forms of phenanthrene, and larger, four-ring PAH molecules. The mussels, to the contrary, appeared to accumulate compounds proportionally to their log K_{ow}s, and higher rates for the four-ring PAHs were hence calculated.

Temperature was different in the two experiments since fish were maintained in warmer seawater than the mussels. This parameter influences uptake because oxygen consumption increases with temperature up to the tolerance limits. However, the effect of temperature is also species dependent and the two types of organisms were placed in seawater at optimum temperature for their physiological requirements.

A factor that could be argued in the results we described is that the ratios between the various PAHs were different in the two assays because two different type of oils were used. Yet kinetics are assumed to be independent of the concentrations of PAHs in the oil mixture. Although testing single [^{14}C] compounds, Spacie et al. [13] have shown that varying exposure concentrations of anthracene in water had no significant effect on the initial uptake rate by bluegill sunfish. In our experiments, the mussels were exposed to dispersed topped crude oil (BAL150) where naphthalenes and dibenzothiophenes were the major components (52% and 37%, respectively). Dispersed North Sea crude oil was used in the experiment with fish. Eighty percent of the components were represented by naphthalenes but dibenzothiophenes represented

only 3%. From Tables 2 and 3, the ratios of the uptake constants for those PAHs with log $K_{ow} < 4$ (naphthalene, c1, and c2 alkylated forms), which are more likely passively eliminated, can be determined as 396:975:1,232 for fish and 61:201:652 for mussels. These ratios were relatively comparable to the ratios found in the two different oils for these molecules (Table 1), respectively, 1,169:2,108:2,204 (fish—North Sea oil) and 148:609:1,418 (mussels—BAL150). Log K_{ow} was a good descriptor of the bioconcentration kinetic in both organisms for these relatively water-soluble PAHs. However, there was a large discrepancy for PAH molecules with log $K_{ow} > 4$ in fish. Skadsheim et al. [25] reported that, as body size and organism structural complexity increased from unicellular algae via crustaceans to fish, where all were subjected to the same exposure, there was a concurrent change in the PAH profile in the body toward an increasing dominance of smaller molecular PAHs in the larger organisms.

The fish body burden pattern showed that PAHs with log K_{ow} above ≈4 exhibited lower uptake rates than predicted by the partitioning theory. This reduction is species dependent, since the mussels displayed a pattern more in compliance with the partitioning theory. The observed differences in kinetic rates and bioconcentration between mussels and fish may be explained by the bioavailability of PAHs from oil droplets for the two species. In addition, higher PAH metabolism efficiency and a possible lower uptake of hydrocarbons from oil droplets in the gut of fish may have contributed to the pattern.

The binding of organic, nonpolar substances like PAHs on suspended or dissolved particles and on sediments has been shown to modify drastically their bioavailability to organisms [14,18,38,39]. This property appears to be essentially determined by the lipo- or petrophilicity of the compounds. McCarthy and Jimenez [18] showed that the rate coefficients for uptake of PAHs (benzo[*a*]pyrene [B*a*P] in that study) bound to dissolved humic material were only 0 to 10% of the uptake of dissolved PAHs (naphthalene) in bluegill sunfish. The same observation was made by Spacie et al. [13], who demonstrated that dissolved humic material did not affect anthracene uptake but significantly reduced the B*a*P uptake rate in the same fish. Because of their higher lipophilicity (high log K_{ow}), the larger molecular substances have a tendency to bind more to organic particles and organic dissolved matter. A similar line of arguments may describe the binding and hence reduced bioavailability of the larger PAHs contained in dispersed oil droplets in our CFS experiments, when keeping in mind that lipophilicity and petrophilicity are much the same.

Another consideration is the gut retention time. A possibly contributing factor is that vacuole formation around particulate matter may favor the retention of large PAH in mussels. Like most bivalves, mussels are filter-feeding organisms that can trap particles in the gills from the passing water. Particles as small as 1 or 2 μm can be stopped and directed into the gut, where they can be ingested into vacuoles in the epithelium of the hepatopancreas. Oil particles may also be retained and included in intracellular vacuoles for a varying period of time, eventually being assimilated to the tissue lipids [23,40]. Thus, a significant amount of the larger PAHs bound to oil particles may have been retained and accumulated in intracellular vacuoles or in the tissue lipids during the exposure period. Axelman et al. [41] found that blue mussels deployed at a smelter site contaminated by discharges of PAHs bioconcentrated dominantly molecules bound to particles or colloids over those present in the dissolved phase. The chemical determination of the dissolved phase was not directly performed in this study, is quite complex, and has not been resolved to date. Baussant et al. [21] showed that semipermeable membrane devices deployed simultaneously with blue mussels in the same dispersed oil had none of the largest PAHs present in the exposure water. Moreover, past exposure shows the semipermeable membrane devices release more rapidly naphthalene and the c1 and c2 alkylated forms of naphthalenes than do blue mussels while c3-naphthalenes were apparently sequestrated longer in the devices, as in the mussels. If the dissolved fractions of PAHs would have been determined, BCFs estimates from uptake and elimination rates would have probably been underestimated relative to the experimentally determined BCFs.

The observation that the uptake clearance rate constants were flattening for log K_{ow} above five may be due to the nonachievement of steady-state concentrations within the relatively short exposure period [23], and it is also possible that some metabolic induction was initiated [26].

In fish, the accumulation of nonpolar organic compounds that have a log K_{ow} in the range of 2 to 6.5 is essentially determined by exchange across the gills while ingestion of substances through dietary uptake is negligible because of natural low feeding rates, poor absorption efficiency, and rapid elimination rates [42,43]. The apparent proportionally larger accumulation of naphthalenes in turbot compared with the mussels may reflect three processes, i.e., a larger bioavailability of two-ring PAHs, like naphthalenes, across the gills because of their relatively higher water solubility and their fast partitioning into the water from oil particles; a reduced efficiency of absorbing oil particles containing larger PAHs through the digestive epithelium of the gut; and a rapid and efficient biotransformation of the largest PAHs in the tissues (i.e., liver) of the fish. Evidence of this greater efficiency to degrade the larger, more hydrophobic PAHs has been demonstrated in several studies on fish [13,28,29]. The kinetic patterns in our experiment revealed a rapid decrease in PAH concentrations in whole fish tissue within the first 3 d. The same observations were reported by Djomo et al. [44] in the zebrafish exposed to PAH initially sorbed sediments. They also attributed this decrease to, notably, the induction of the enzymatic systems and consequent biotransformation. In this article, we have reported evidence of biotransformation of parental PAHs into PAH-metabolites measured by fluorescence spectrometry in the bile of the turbot. The same biochemical response was measured in an experiment with cod exposed to dispersed crude oil in the CFS [45]. Consequently, in addition to a reduced bioavailability of the larger PAHs bound to oil particles (because of their slow dissolution rate), efficient enzymatic degradation seem to explain the uptake rate patterns and the bioconcentrations of the larger PAHs from dispersed oil in fish, conversely to mussels.

CONCLUSION

The present experimental exposures in a continuous-flow system and the results that were obtained are in line with other studies on PAH exposure to bivalves and fish [38,39,46]. The apparent bioconcentration factors may be lower than expected based on the simple partitioning model due to both a reduced bioavailability and an extensive enzymatic degradation by organisms. To our knowledge, however, this study is one of the first to highlight the importance of these parameters during chronic exposure of marine organisms to dispersed crude oils. These considerations may have important implications for the

management and risk assessment of produced water discharges from offshore production fields by industries. The bioaccumulation potential and risk assessment of substances discharged at sea have frequently been linearly related to the lipo- or petrophilicity of the substance as expressed by K_{ow}. Our data seem to indicate that this relationship may only be valid within a limited range of low log K_{ow} values corresponding to the relatively more water-soluble fraction of hydrocarbons in oil. For example, for larger and less water-soluble PAHs (log $K_{ow} > 4$), the BCFs predicted from the partitioning model are considerably greater than calculated values based on the ratio k_1/k_2 obtained from our experimental data with fish. More insight into the dissolution rates of PAHs from dispersed oil droplets is required in order to more accurately predict bioaccumulation of this complex type of exposure to marine biota. The low bioconcentration factors estimated from k_1/k_2 in fish are in disagreement with what was expected from the partition coefficients of the larger PAHs. Yet the potential environmental impact recognized for these larger PAHs should not be disregarded since the toxic effects of parent and alkylated PAHs are activated after biotransformation by oxidative enzymatic processes. Even if the BCFs of PAHs with log $K_{ow} > 4$ is low, as observed in these experiments with fish, it is important not to neglect these compounds in environmental risk assessment because they can be metabolized apparently quickly to substances with mutagenic and carcinogenic potential for the exposed organisms. Consequently, future risk assessment models should perhaps consider the fate and effects of the PAH metabolites to marine biota in which metabolization is an important excretion pathway.

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