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Comparison of PAH and Nonylphenol Uptake by Carp (*Cyprinus carpio*) and Semipermeable Membrane Devices (SPMDs) from Water

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PAHs and nonylphenols (NPs) are common contaminants in aquatic environments. PAHs are a class of persistent organic pollutants that are ubiquitous in the environment. PAHs in aquatic environments come from a variety of sources, such as wastewater, industrial and domestic discharges, and atmospheric precipitation (Zheng et al., 2004). NPs are the degradation metabolites of a class of widely used surfactant, alkylphenol polyethoxylates. Both classes of contaminants have rather serious adverse effects on human health with the former often a carcinogen and the latter an endocrine disrupter through mimicking natural hormones in the interaction with estrogen receptors (Ying et al., 2002). Therefore, bioavailability of these contaminants is a critical issue in the study of their environmental behaviors in water.

Methods for determining bioavailable amounts of aquatic contaminants can include: the measurement of concentration changes in aqueous phase; the measurement of accumulative amounts in tissues of sentinel organisms such as mussels and oysters in the context of routine monitoring; and that of absorbed quantities by semipermeable membrane devices (SPMDs) as passive abiotic samplers (Boehm et al., 2005). Due to low concentrations of PAHs and NPs in aquatic environments, the difficulties in detection of their concentration change are often encountered with the first method. For the second method, selection of organisms of uniform physiological characteristics determines the quality of data obtained but can not be easily fulfilled although the organisms such as shellfish and carps have commonly been used (Richardson et al., 2001; Hahn 2002; Verweij et al., 2004). Furthermore, the utility of organisms is limited at highly polluted sites.

SPMDs overcome some of these problems. They passively simulate the process of bioconcentration of hydrophobic organics across biological membranes such as fish gills. Now, SPMDs are employed worldwide to monitor organic contaminants in a variety of media such as marine water, freshwater, air, and sediment (Petty et al., 2000b). Lots of works have been carried out on the PAHs uptake by SPMDs, but few on NPs, although NPs are rather persistent in the aquatic environments and have been found to be concentrated in organisms. In this study, several PAHs and NPs were selected as target chemicals to investigate their uptake kinetics by SPMDs and by carps (*Cyprinus carpio*), so that we can assess the utility of SPMDs to substitute organisms for pollutants monitoring and risk assessment in water.

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MATERIALS AND METHODS

The low-density polyethylene tubings were purchased from the Environmental Sampling Technologies (EST), St. Joseph, MO, USA. The tubings were 2.5 cm wide, 9.14 cm long, with wall thickness ranging from 70~95μm, containing no plasticizer or other additives. The tubings were dipped in hexane for 72 hours to get rid of impurity, and then filled with 0.1mL triolein (purity≥95%, China Medicine Group). Each end was heat sealed, and tubings were stored at -20℃ until use.

Carps (*Cyprinus carpio*) were purchased from Tianjin Aquafarm. Healthy and uniformly carps (about 10 cm long, 100 g each) were selected and domesticated for one week in the lab. No food was added 3 days before the experiment.

The uptake kinetics experiment was carried out in an aquarium, as shown in Figure 1 Mixed contaminants were taken to the uptake tank by a peristaltic pump at 1.0 mL/min from reagent bottle, combined with pure water from the water tank by another peristaltic pump. The two influents met and flowed into the uptake tank at 20 mL/min. Tubings and carps were put into the uptake tank, and the experiment began. Tubings and carps were sampled at regular intervals for pollutants analysis. Initial concentrations of naphthalene (NAP), 1-chloro-naphthalene (1-Cl-NAP), 1-methyl-naphthalene (1-Me-NAP), phenanthrene (PHE), nonylphenol (NP), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO) were 20μg/L in the reagent bottle. The experiment lasted 17 days, and water samples from uptake tank were taken on day 1, 9, and 17 for pollutants analysis to determine the variation of pollutants concentrations in the experiment duration.

Tubings are dipped into 200mL of hexane for 20-30 seconds, and the surfaces are washed by distilled water. Then they are dipped in 1mol/L HCl for about 30 seconds, taken out, rinsed by distilled water, acetone and isopropanol orderly, and air-dried. According to the proportion of 1mL triolein: 180mL hexane, tubings were dialysed for 24 hours in the dark at 18℃. The dialytic solution was rotatory evaporated in water bath at 60℃ to nearly 1 mL. The extract was concentrated to dryness under a gentle N_2 stream, and redissolved with 1 mL of hexane for instrumental analysis.

From each fish about 10 g of fish muscle tissue was dissected, homogenized and dried with pre-extracted sodium sulphate. The samples were extracted with cyclohexane/ acetone/petroleum ether (1:1:1) in a Soxhlet apparatus for 24h. The extracts were rotatory evaporated to nearly 1 mL, concentrated to dryness under a gentle N_2 stream, and redissolved with 1 mL of hexane for instrumental analysis. In order to determine the lipid weight of the samples, 10 g of fish (as control) was homogenized and dried with sodium sulphate. The mixture was Soxhlet extracted as described above, and the extracts were transferred to an accurately pre-weighed flask. The solvent in the flask was air-dried naturally, and the flask was weighed again. The difference of the flask weight was the lipid weight of the fish.

PAHs were analyzed on a HPLC (Waters Model 1525, USA) using a reversed-phase column (Waters, 5C18-MS-II, 4.6×250mm) equipped with a Waters 2475 fluorescence detector. Isocratic elution was taken with acetonitrile/water (80/20, v/v)

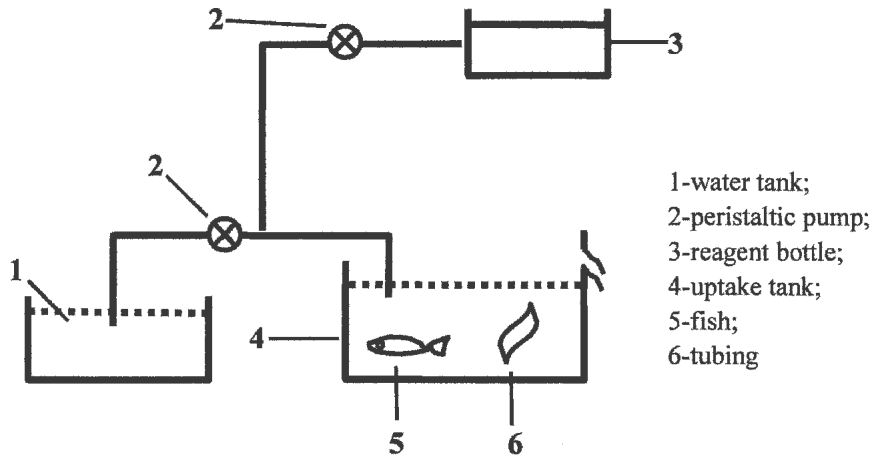

Figure 1. Apparatus for uptake kinetic experiment.

at 1.0 mL/min, and excitation and emission wavelengths were 280 and 355 nm, respectively. NPs were measured with HPLC using a Waters W2108N007 NH_2 column (μBondapak 3.9mm i.d. ×300mm×5μm) equipped with Waters 2475 fluorescence detector. Mixtures of n-hexane/isopropanol (98/2, v/v) and isopropanol/ water (98/2, v/v), named solvents A and B, respectively, were used as mobile phase for HPLC analysis. Gradient elution was carried out with a linear program from 95% A and 5% B to 80% A and 20% B in 10 min with a flow rate of 1.0 ml/min. Excitation and emission wavelengths of the fluorescence detector were 233 and 302 nm, respectively.

RESULTS AND DISCUSSION

In the experiment duration, the average concentrations of each contaminant were shown in Table 1. Averaged water temperature was 18℃, and averaged pH was 6.5.

Table 1. Concentrations of pollutants in the system.

Contaminant	NAP	1-Cl-NAP	1-Me-NAP	PHE	NP	NP1EO	NP2EO
μg/L	0.36	0.21	0.25	0.10	0.23	0.26	0.45
logKow	3.37	3.90	3.86	4.46	4.48	4.17	4.21

NAP: naphthalene, 1-Cl-NAP: 1-chloro-naphthalene, 1-Me-NAP: 1-methyl-naphthalene, PHE: phenanthrene, NP: nonylphenol, NP1EO: nonylphenol monoethoxylate, NP2EO: nonylphenol diethoxylate

Uptake kinetics of PAHs and nonylphenols by carps and SPMDs were shown in Figure 2. It can be concluded that, for all chemicals their concentrations in fish increased rapidly first, then declined. While in SPMDs, chemicals concentrations increased first, and with time went on a steady stage was observed.

Uptake of PAHs by SPMDs was clearly linear during the exposure time (with r^2 values from 0.82 to 0.94 for the four PAHs). After 24h, concentration factors (CFs,

ratios C_L/C_W) for NAP, 1-Cl-NAP, 1-Me-NAP and PHE were 575, 2252, 1976 and 2420, respectively. On day 17, CFs for the above chemicals were 1086, 5952, 4040, and 5740, respectively. The enrichment of PAHs by SPMDs was correlated to the chemical's hydrophobicity (expressed as logKow in Table 1). The rate of equilibration of triolein phase of SPMDs with dissolved PAH in water decreases with increasing PAH molecular weight and octanol/water partition coefficient (Boehm et al., 2005). In Figure 2 it showed that the steady stage of PAH uptake for NAP, 1-Cl-NAP and 1-Me-NAP was obtained on day 10, while for PHE, it was on day 14.

In contrast, uptake of PAHs by carps was something different with SPMDs. On day 1 (24h), bioconcentration factors (BCFs) for NAP, 1-Cl-NAP, 1-Me-NAP and PHE were1233, 2538, 2400, and 2520, respectively, while on day 17, BCFs were 1142, 3762, 2700, and 5080 for the above four PAHs. Unlike CFs in SPMDs, BCFs on day 17 were not the highest values during the exposure time. In the 17-d experiment, the highest BCFs values for NAP, 1-Cl-NAP, 1-Me-NAP and PHE were 2078, 4874, 3744 and 8080, which appeared on day 4, 3, 3, and 5, respectively. This was due to the way of chemicals uptake by fish and the biodegradation of chemicals in fish body. SPMDs passively simulate the bioconcentration of dissolved bioavailable compounds across biological membranes. This process is mainly governed by a simple partitioning of the compound between SPMD and water. After being enriched in the triolein phase of SPMDs, chemicals are not ready to degrade (Petty et al., 2000a). Whilst in organisms like fish and shellfish, the bioaccumulation of compounds is a result of the different uptake and elimination processes of the compounds combined with metabolic clearance (Verweij et al., 2004). This is why the highest CFs values for PAHs in SPMDs appeared on day 17, while the highest BCFs values of fish appeared on day 4, 3, 3, and 5, respectively for NAP, 1-Cl-NAP, 1-Me-NAP and PHE.

The same trends of NPs uptake by SPMDs and carps were observed in the experiment. Although NPs are a kind of polar organic compounds comparing with PAHs, they can be effectively enriched by SPMD. On day 1 (24h), CFs for NP, NP1EO, NP2EO were 765, 435, 364, and BCFs were 5087, 538, and 251, respectively. On day 17, CFs were 1478, 904 and 533, while BCFs were 1913, 1000 and 467, respectively. The highest CFs appeared on day 17, and the highest BCFs values appeared on day 1, 2, 2 for NP, NP1EO, NP2EO, respectively. Stuer-Lauridsen and Kjølholt (2000) used SPMDs to monitor NPs in two sections of the sewage system in the municipality of Roskilde, Denmark, and found that concentration factors of NPs in SPMDs were 57 and 316 after 24h and 6 days deployment, which was lower than the results in this laboratory study.

The enrichment of lipophilic chemicals in SPMDs and organisms is related to chemicals' octanol/water partitioning coefficients. Figure 3 and Figure 4 showed the relationships between PAHs and NPs concentration factors and chemicals' logKow values. The average lipid weight content of fish used in this study was 2.68%. It indicated that concentration factors increased with increasing chemicals' hydrophobicity. Linear regression showed that r^2 values were more than 0.60.

The concentrations of contaminants present in the SPMD extracts can be used to

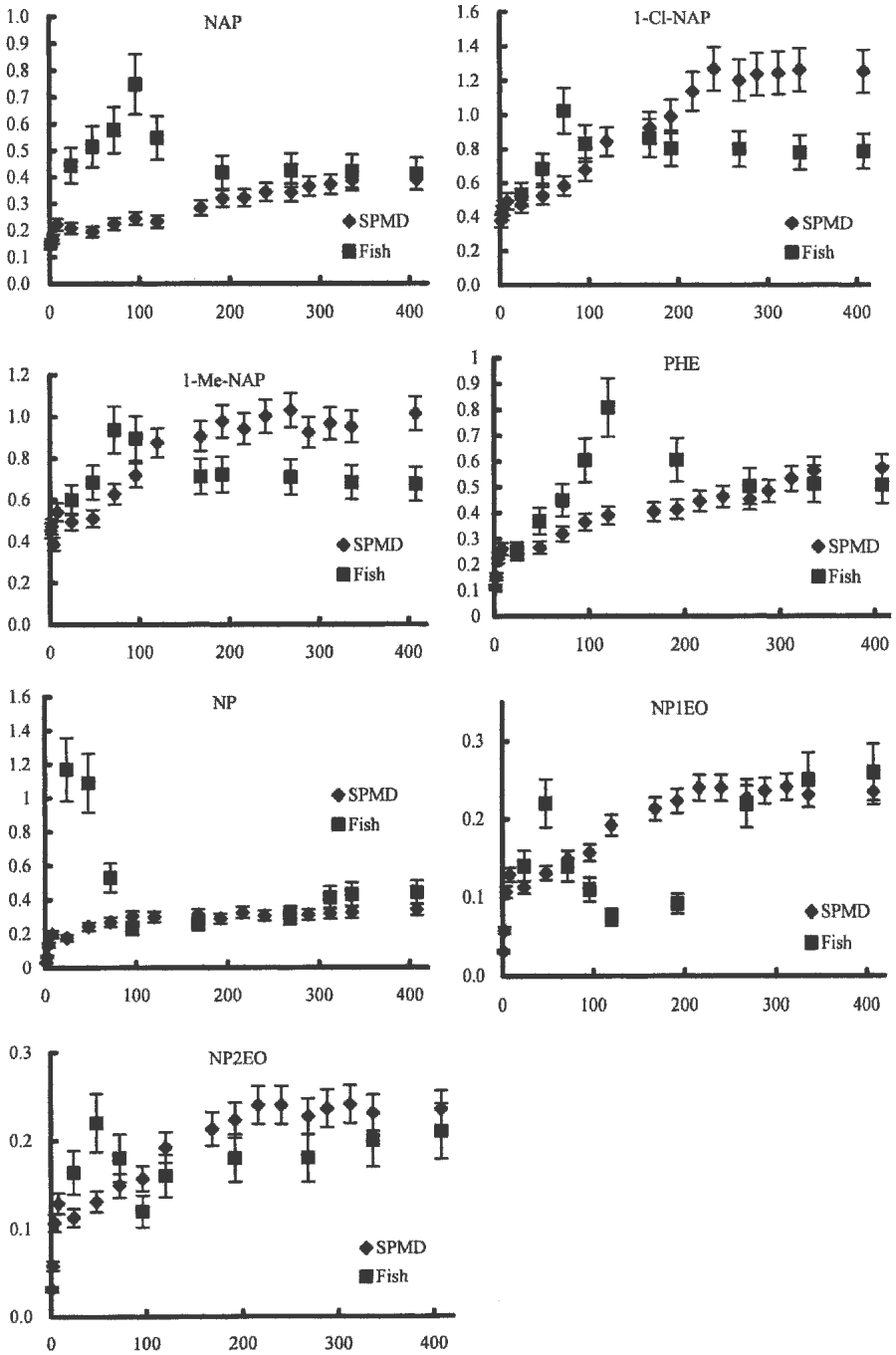

Figure 2. Uptake kinetics of PAHs and nonylphenols by carp and SPMDs. The axis X represents the time, hr. The axis Y represents the chemical's concentration, mg/L.

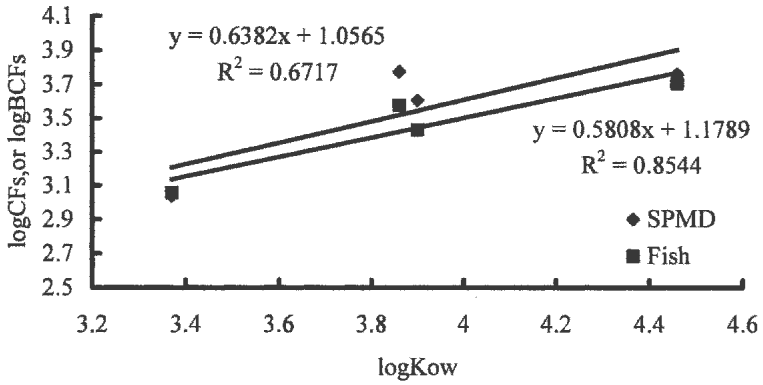

Figure 3. Relationship between logKow and logCFs or logBCFs for PAH uptake.

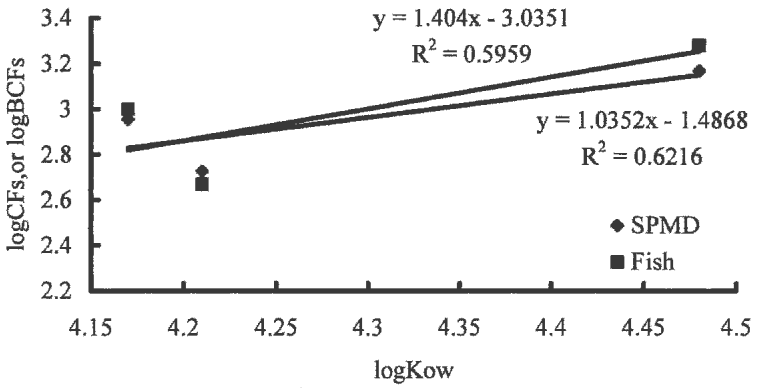

Figure 4. Relationship between logKow and logCFs or logBCFs for NPs uptake.

Table 2. Exchange rate coefficients (k_e) and water sampling rates (Rs) of SPMDs exposed for 17 days at 18℃.

Chemicals	Rs (L/d)	k_e (d^{-1})
NAP	0.5	0.056
1-Cl-NAP	5.04	0.21
1-Me-NAP	6.83	0.26
PHE	5.0	0.058
NP	6.50	0.073
NP1EO	5.39	0.11
NP2EO	5.57	0.10

estimate the ambient concentrations of these pollutants. Huckins et al. (1993) developed a model to calculate ambient contaminant concentrations, as follows:

$$C_W = C_{SPMD} V_L / R_S t$$

where C_{SPMD} is the concentration of the individual analyte in the SPMD and V_L is the volume of triolein in the SPMD (mL), R_S is the SPMD analyte sampling rate (L/d) and t is deployment time in days.

Exchange rate coefficients (k_e) and water sampling rates (Rs) for PAHs and NPs are summarized in Table 2. Of these values, Rs values for NAP and PHE can be compared with that reported by Huckins et al. (1999). Because the SPMD sampling rates can change due to changes in temperature and flow velocity of the water, they reported Rs values of 15 priority pollutant PAHs at 10℃, 18℃, and 26℃, respectively. Compared with NAP and PHE Rs values of 0.9 and 3.6 L/d at 18℃ in their results, in this study Rs values of 0.5 and 5.0 L/d for NAP and PHE were obtained. The difference may be due to the open coexisting system of SPMDs and carps, which leads to some volatilization loss of NAP from the system and the competition adsorption of pollutants between SPMDs and fish. Rs values for NPs could not be compared due to the lack of such information in literatures so far.

Many researchers have investigated organic pollutants from water by SPMDs and by organisms. For example, Wang et al (1998) investigated the uptake of moderately hydrophobic chlorophenols from water by SPMDs and by goldfish. Richardson and his group (2001) used mussels (*Perna viridis*) and SPMDs to monitor chlorinated trace organic contaminants in Hong Kong coastal waters in 2001, and PAHs and petroleum hydrocarbons at the same site in 2003 (Richardson et al., 2003). Verweij et al (2004) assessed bioavailable water concentrations of PAHs, PCBs and OCPs (organochlorine pesticides) at several freshwater sites in and around the city of Amsterdam, using SPMDs and caged carps. Boehm et al (2005) compared the mussels and SPMDs for monitoring PAHs at oil spill sites. Of these studies, some differencecs of pollutants profiles in SPMDs and organisms were observed. The differences indicate that organisms such as mussels and carps better reflect the total exposure (i.e., varying concentrations of dissolved, colloidal, and particulate chemical in water) than do SPMDs. Unlike mussels, SPMDs are designed to sample only dissolved chemicals by a thermodynamically driven partitioning process between the aqueous and triolein lipid phase.

In this study, we conclude that SPMD may be used to sequester trace PAHs and nonylphenols from water effectively. A comparison between chemicals concentrated in SPMDs and in carps demonstrates that SPMD has the similar uptake potential as fish has, and may be used to sample these chemicals from water less costly.

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