PII: S0045-6535(98)00380-4

UPTAKE OF WEAKLY HYDROPHOBIC NITROAROMATICS FROM WATER BY SEMIPERMEABLE MEMBRANE DEVICES (SPMDs) AND BY GOLDFISH (*Carassius auratus*)

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(Received in Germany 4 April 1998; accepted 29 June 1998)

ABSTRACT

Semipermeable membrane devices (SPMDs) filled with triolein, and goldfish (*Carassius auratus*) were simultaneously exposed to nine weakly hydrophobic nitroaromatics in a laboratory continuous-flow experiment. The results showed that the device concentrated the compounds in most cases to a larger extent than fish and that the overall uptake rate constants (K_{μ}) and average pollutant sampling rates (R_S) were similar. We conclude that the SPMDs may be used to determine the levels of weakly hydrophobic nitroaromatics in water. It is obvious, however, that more researches at laboratory in order to establish kinetic database for a wide spectrum of compounds varying in lipophilicity and other properties are needed to elucidate various theoretical and practical aspects of the use of membranes in the environmental research and for related practical applications.

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Key Words : SPMDs, Fish, Nitroaromatics, Bioconcentration

INTRODUCTION

Semipermeable membrane device (SPMD) is a new tool for passive, in situ monitoring concentrations of organics in the aquatic environment (1, 2, 3). In SPMDs, the mechanism behind the uptake of chemicals is based on the process of passive partitioning of a compound between water and a thin film of neutral lipid, enclosed in semipermeable polymeric membrane. Therefore SPMDs can

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be used to mimic the processes by which aquatic organisms concentrate hydrophobic organic contaminants from water (2, 4, 5). The partition coefficients of the contaminants between SPMDs and water may be comparable to or even higher than their octanol/water partition coefficients ($P_{O/W}$) or the bioconcentration factors (BCF) in aquatic organisms (6, 7). In SPMDs, triolein, which constitutes a significant fraction of the lipid pool of most aquatic organisms (8), is generally used as the lipid phase. Polyethylene is generally used as the semipermeable membrane (Triolein-SPMD).

Since the beginning of the 1990s, SPMDs have drawn more and more attention in the monitoring of hydrophobic pollutants in the water and air (6, 9, 10, 11, 12). It has been used to study a variety of organic compounds, mostly the highly hydrophobic contaminants such as PAHs, PCBs, organochlorine insecticides, and pyrethroid insecticides. These compounds have all been shown to be concentrated in SPMDs (4, 5, 13-18) in a manner similar to aquatic organisms. However, less work has been done for weakly and moderately hydrophobic compounds.

In this study, nine nitroaromatics of weakly hydrophobic contaminants and of great importance to environment were selected as model compounds for a laboratory continuous-flow experiment where the uptake of the compounds by goldfish (*Carassius auratus*) and SPMDs was simultaneously followed.

MATERIALS AND METHODS

Test chemicals, SPMDs and Fishes

Nitrobenzene, 1,2-dinitrobenzene, 1,3-dinitrobenzene, 1,4-dinitrobenzene, 2-nitrotoluene, 3-nitrotoluene and 4-nitrotoluene were purchased from Beijing Chemical Company. 2,4-dinitrotoluene, and 2,6-dinitrotoluene (content = 95-99%) were purchased from Sigma Chemical Company (St. Louis, USA). The nine nitroaromatics are important industrial chemicals and intermediate products, their big output and widespread application have resulted in severe pollution in many rivers around the world.

Semipermeable membrane device was prepared in a similar way as described by Huckins *et al.*(1). Layflat polyethylene tubing (width 3.0cm, membrane thickness 40-45 μ m, from Beijing Plastics Company) was cut into pieces of 15cm in length, soaked in cyclohexane for 24h to remove trace contaminants, and dried with ultra pure nitrogen. Then 20 μ l (18.2mg) of high purity triolein

(99%), purchased from Sigma Chemical Company, was coated onto the inner part of the polyethylene tubing (14cm) before the ends were sealed with a plastic sealer (Model SF-300, Lianyungang Microwave Electric Appliance Factory, China). The tube was formed into twisted loops and glass sticks were used as anchors to keep the device in a vertical position when suspended in the test solution.

Goldfish (*Carassius auratus*, 6 months) were obtained from Guanyuan Aquatic Institute in Beijing. The average lengths and weights of the fish were 9.5 (± 2.3)cm and 7.9 (± 2.7)g, respectively. The average lipid content calculated on fresh weight was 2.37 (± 0.72)%. The fish were sterilized in 5% (w/v) salt water and reared under the laboratory condition for two weeks before experiment. The death rate during the domestication was lower than 2%.

Flow-through experiment

40 SPMDs and 40 goldfish were placed in an exposure chamber (volume 70 liters). A concentrated mixture of the nine 9 nitroaromatics was added at a constant rate ($1\text{ml}\cdot\text{min}^{-1}$) into the main water flow to the chamber by a peristaltic pump. The overall flow rate through the exposure chamber was maintained at $20\text{ml}\cdot\text{min}^{-1}$ and the rate of the peristaltic pump was adjusted to obtain relatively stable concentrations of the different chemicals in the exposure chamber (Table 1). The pH and temperature in exposure chambers were 7.5 and 15°C , respectively. In a control chamber with the same volume as the exposure chamber but without addition of nitroaromatics and maintained under the same conditions as described above, 15 goldfish and 15 triolein-SPMDs were placed. The experiment was conducted for 20 days. During the experiment, three SPMDs and three goldfish from the exposure chamber and one SPMD and one goldfish from the control chamber were sampled for analysis at 12, 24, 36, 48, 96, 144, 192, 240, 288, 336, 384, 432, and 480 hours of the exposure, respectively. To monitor the concentrations of the added compounds in the experimental chamber, triplicate samples of the water were taken every two days.

Measurement of nitroaromatics accumulated by SPMDs

After sampling, the SPMDs were rinsed with distilled water, dried with clean paper, transferred

into beakers containing 40 ml of cyclohexane, and dialyzed at 13°C for 24 hours. The SPMDs were removed and the solvent were passed through anhydrous sodium sulfate and evaporated to 2 ml in a rotary evaporator. The nitroaromatic compounds were determined by a Shimadzu model GC-9AG gas chromatograph (GC) with glass column (2.1m × 3.2mm, filled with Chromosorb OV-17). The GC was equipped with an electron capture (^{63}Ni) detector and programmed temperature from 160 to 180°C.

Spiking study was carried out for eight replicates at concentrations similar to those expected in the SPMD samples and showed that the recoveries for the nitroaromatics after dialysis ranged from 66% to 73%. The standard deviation did not exceed 11%. The levels in the SPMDs were accordingly corrected for the loss during dialysis. The weight loss of triolein during the dialysis with cyclohexane were less than 1% and negligible polyethylene waxes entered into the solvent during the dialysis. Therefore, no further clean-up procedure was required before analysis.

Determination of nitroaromatics accumulated by the goldfish

The fish was sacrificed and homogenized in a mortar with three times by weight of anhydrous sodium sulfate. 20g of the mixture was placed in a Soxhlet apparatus and the nitroaromatics were extracted with 30ml of acetone/petroleum ether mixture (59 : 41 =v/v) at 60-65°C for 2 hours. The extract was passed through a column of anhydrous sodium sulfate, then rotary evaporated to approximately 1ml and transferred into polyethylene tubings. The sealed plastic tubings were dialyzed in cyclohexane at 13°C for 24 hours. After removing the tubings, the solvent was passed through anhydrous sodium sulfate, rotary evaporated to 2 ml and analyzed by gas chromatograph as described earlier. The dialysis recovery for 9 nitroaromatics from goldfishes in recovery studies were from 57% to 71% and the standard deviation of the averaged recovery did not exceed 16%. To estimated concentrations of chemicals in the lipid of goldfishes, the measured values were corrected for loss during the dialysis by the correction coefficients derived from the recovery studies.

Measurement of nitroaromatics in water

The studied compounds were extracted with 2 ml of petroleum ether from the water. The extract

was dried with anhydrous sodium sulfate and analyzed with GC/ECD as described earlier. Spiking study was carried out for eight replicates at concentrations similar to those expected in the exposure chamber and the results showed that recoveries of the chemicals were in the range of 91-102%. Therefore, no correction for losses was necessary for this analytical step.

RESULTS AND DISCUSSIONS

Uptakes of the nitroaromatics by triolein-SPMD and fishes in flow-through experiment

The weakly hydrophobic nitroaromatics were taken up very fast by the SPMDs (Fig. 1). After 24 hours, a steady state was achieved with device concentration factors (DCFs, ratio of concentration of compound in triolein of SPMD to concentration in water) ranging from 80 to 400, except for NB, the DCF was less than 10. It was normal that values of DCF and BCF fluctuated to some extent after the uptake reached the steady state. Unfortunately, for 1,3-DNB, some SPMD samples between day 2 and 14 were lost. Bioconcentration factors (BCFs, ratio of concentration of compound in lipid of fish to concentration in water) were similar to or even higher than DCFs for 2,4-DNT, 1,3-DNB and 1,4-DNB, while they were lower than DCFs for NB, 2,6-DNT, 2-NT, 3-NT, 4-NT and 1,2-DNB.

Uptake kinetics

An kinetic equation proposed by Huckins et al.(2) based on two-compartments bioconcentration model was used to describe the overall uptake curves of the chemicals accumulated in SPMDs (equation 1). The same equation was used also to describe accumulation of those chemicals in goldfish.

$$C_L = C_W K_{L/W} (1 - e^{-K_d t}) \quad (1)$$

where C_L is the concentration of a chemical in the SPMD; C_W is the concentration in water; $K_{L/W}$ is the lipid/water partition coefficient; and K_d is the elimination rate constant. $K_{L/W}$ and K_d were estimated through fitting the experimental data using the SPSS 6.0 software for Windows and its Levenberg-Marquardt least-squares algorithm, with t and C_L being independent and dependent variable respectively, and C_W being constant.

A good fit of the experimental data to the kinetic equation (1) was obtained for all the chemicals,

Fig. 1 Uptake of nitroaromatics by SPMD and fish in flow-through experiment.

Fig. 1 (continued) Uptake of nitroaromatics by SPMD and fish in flow-through experiment.

**Table 1. Properties of the Studied Nitroaromatics and Their Concentrations
in the Exposure Chamber.**

Chemicals	Log $P_{o/w}$ ^a	Log BCF ^b (<i>Carassius auratus</i>)	MW	Concentration in the exposure chamber (mg.l ⁻¹ , Mean±SD), n=11
NB	1.89	1.47	123	6.02±0.76
2-NT	2.30	2.28	137	1.63±0.21
3-NT	2.45	2.31	137	1.03±0.15
4-NT	2.34	2.37	137	1.18±0.20
1,2-DNB	1.55	1.02	168	0.29±0.06
1,3-DNB	1.52	1.87	168	0.09±0.01
1,4-DNB	1.46	< 0.70	168	0.15±0.02
2,4-DNT	2.04	2.3	182	0.39±0.04
2,6-DNT	2.02	2.44	182	0.38±0.03

^a Data cited from literature (20).

^b Data cited from literature (21).

Table 2. Estimated Uptake Kinetic Parameters for Nitroaromatics in SPMDs.

Chemicals	$K_{L/W}$		K_u (hrs ⁻¹)		$t_{90\%}$ (days) ¹
	Estimate	CV ² , %	Estimate	CV ² , %	
NB	4.52	6.3	0.38	29.2	6.11
2-NT	312.6	5.4	0.57	26.8	4.08
3-NT	167.4	7.3	1.06	30.3	2.18
4-NT	264.3	8.6	1.10	30.2	2.09
1,2-DNB	104.2	3.1	48.8	- ^f	0.05
1,3-DNB	180.0	7.0	0.83	24.3	2.77
1,4-DNB	106.2	6.1	1.15	25.8	2.00
2,4-DNT	275.1	3.4	4.09	30.7	0.56
2,6-DNT	301.9	3.2	2.79	26.1	0.83

¹ Time required to reach 90% equilibrium concentration in SPMDs

² CV, variation coefficient (%).

^f No accurate values could be obtained.

**Table 3. Estimated Uptake Kinetic Parameters of Nitroaromatics
in goldfish (*Crassius auratus*).**

Chemicals	$K_{L/W}$		K_u		$t_{90\%}$ (days)
	Estimate	CV, %	Estimate	CV, %	
NB	0.4	8.68	0.76	27.0	3.01
2-NT	4.4	19.7	0.35	50.1	6.64
3-NT	2.6	22.2	0.25	44.8	9.10
4-NT	0.5	30.4	0.55	46.4	4.22
1,2-DNB	3.1	15.2	0.10	31.6	23.50
1,3-DNB	356.5	4.2	0.51	23.3	4.52
1,4-DNB	522.9	7.1	0.47	27.8	4.93
2,4-DNT	191.3	5.8	0.47	23.0	4.86
2,6-DNT	71.8	9.5	0.60	30.3	3.81

Annotation is the same as in Table 2.

Table 4. Concentrations of Nitroaromatics In Water As Estimated with SPMDs .

Chemicals	Measured Conc. in Water mg.l ⁻¹	Estimated Conc. in Water *		R _s	
		mg.l ⁻¹	CV, %	liter.hr ⁻¹	CV, %
NB	6.022	6.021	4.6	0.02	10.0
2-NT	0.320	0.319	4.7	2.48	21.7
3-NT	1.031	1.030	5.9	2.03	15.7
4-NT	0.442	0.443	6.8	3.22	16.9
1,2-DNB	0.294	0.295	2.7	4.77	-f
1,3-DNB	0.090	0.091	5.6	0.92	30.9
1,4-DNB	0.150	0.151	4.7	1.72	-f
2,4-DNT	0.391	0.390	1.7	6.02	31.1
2,6-DNT	0.376	0.377	2.9	2.81	36.8

* Estimation was made by equation 1 and the obtained uptake kinetic parameters.

Fig. 2 Comparison of the partition coefficients (Log K_{L/w}) for SPMD/water, for fish/water(Log BCF), and partition coefficients (Log K_{v/w}) for octanol/water.

either concentrated by SPMD or by goldfish. The squared r values for the regressions ranged from 0.80 to 0.96. Variations for coefficients K_L/W and K_u for SPMD were less than 10% and 30%, respectively. Variations for coefficients K_L/W and K_u for goldfish were larger but less than 30% and 50%, respectively (Table 2 and 3).

Except for NB, the partition coefficients of SPMD/water were similar to the $P_{O/W}$ of the tested compounds (Fig. 2). However, the BCF reflected a biological degradation related to the characteristics of the compounds where the number and location of the substitutions in the molecule seem to be of significance. Assuming that the uptake pattern by the fish is similar to that of SPMDs, then the biological degradation of the nitroaromatics seems to proceed along the order nitrobenzene > methyl mononitrobenzene > methyl dinitrobenzene. This order is obviously related to the complexity of the compound and their substitution characteristics. By this comparison, it may be inferred that goldfish obviously cannot be used as an indicator organism in monitoring the occurrence of NB, 2-NT, 3-NT, 4-NT and 1,2-DNB in the field due to its biological degradation. Also, since the fish degrade the compounds, their metabolic systems are operating (or damaged), utilizing energy that otherwise might be used for reproduction etc.

Based on the K_L/W and K_u values, we have calculated the time required to reach 90% steady-state concentration in SPMD and in goldfish, respectively (Table 2, 3). The uptake of the tested weakly hydrophobic compounds reached this level in SPMDs within 6 days, in fish within 9 days (with 1,2-DNB as an exception).

Prediction of chemical concentrations in water

The K_L/W and K_u were used in equation (1) to estimate the concentrations of nitroaromatics in SPMDs and in goldfish based on their concentration in the water or vice versa (Fig. 1 and Table 4). The estimated concentration in the water were close to the measured average water concentrations with variation coefficients not exceeding 7% at steady-state (Table 4). Therefore we conclude that SPMD may be used to predict concentration of weakly hydrophobic nitroaromatics in water.

Another parameter used to predict chemical concentration in water by SPMDs is the sampling rate R_s , which could be used in the linear uptake kinetics region. To obtain the R_s , a mathematical

model used to fit the experimental data (2) . In the model, the concentration of a chemical in the SPMDs (C_L) should be proportional to the sampling rate (R_s) of SPMD when it is linearly proportional to the concentration of the chemical in water (equation 2).

$$C_L = \frac{C_w R_s t}{V_L} \quad (2)$$

where V_L is the volume of the triolein used in the SPMD; R_s is the sampling rate of the SPMD, expressed as extracted volumes of water per hour and is specific for different chemicals; and t is the sampling time.

Equation (2) was fitted by least squares linear regression to the linear portion of the experimental data for the SPMDs and for goldfish. A good fit was obtained for all chemicals with r squared values from 0.80 to 0.99 in the linear regression. Variation coefficients for R_s were less than 37% (Table 4). By definition, R_s is indicative for the potentiality of a chemical entering the SPMD, which in turn depends on both device parameters and the ability of a chemical to be absorbed by the polyethylene membrane ($R_s = K_{M/W} \cdot k_o \cdot A$, where $K_{M/W}$ is the distribution coefficient of a chemical between membrane and water, k_o is overall membrane mass-transfer coefficient and A is membrane surface area [12].

Since 1990s, SPMDs have been tested for a variety of organic compounds, mostly the highly hydrophobic ones such as PAHs, PCBs, organochlorine insecticides, and pyrethroid insecticides. The devices have been also tested in variety of environmental conditions. For example, after two months of exposure in river water, levels of organochlorine compounds were 1.6 times higher in clams than in SPMDs [7]. After 28 days of exposure of SPMDs adjacent to fish in the lake water, they accumulated a greater number of chemicals and at higher concentrations than the fish [20] . In a similar study, SPMDs accumulated more PAHs (fluoranthene, pyrene and chrysene) than fathead minnows and rusty crayfish in an urban stream [21]. It is undeniable, however, that few laboratory experiments have been carried out where the performance of SPMD were used as indicators of bioconcentration, i.e. by comparing the accumulation of chemicals in the SPMDs with the uptake by test organisms under the same exposure conditions. There are insufficient data allowing to establish a reliable correlation between DCF and BCF for a wide spectrum of compounds varying in lipophilicity. The results presented in this study added some kinetic data to this database, which suggested that SPMDs

may concentrate weakly hydrophobic pollutants such as nitroaromatics to the similar extent as that in goldfish. From the results, it could be noticed that the partition coefficients of SPMD/water were similar to the $P_{O/W}$ of the tested compounds, and the estimated concentrations in the water by using kinetic data of SPMDs were close to the measured water concentrations. It infers that SPMD can be used to predict concentration of weakly hydrophobic nitroaromatics in natural water.

In order to evaluate bioavailability and bioconcentration potential of the pollutants, BCF is usually measured in long-time experiments by exposing test organisms (algae, mussel, fish etc.) to a certain concentration of a chemical and determining its concentration in the organism or in tissues (such as in lipid) after the steady-state is attained. Such tests are complex, time-consuming and therefore costly. In addition, one is often interested in the bioconcentration potential of a chemical under much more diverse conditions, and direct measurements of bioconcentration are needed. SPMDs can be exposed to various conditions and therefore provide a good alternative in the measurements of bioconcentration. Triolein contained in SPMDs proved to be very efficient time-integrating and *in situ* concentrators of nonpolar organics in aquatic environments. Further attempts are required to optimize this methodology and improve quantification to increase the accuracy of kinetic data estimates.

CONCLUSION

We conclude that SPMD may be used to sequester nitroaromatics from water, though these compounds are less lipophobic. A comparison between chemicals concentrated in SPMD and in fish demonstrate that SPMD may be used to ensure the potentiality of the biological degradation for chemicals. It is obvious, however, that more researches at laboratory in order to establish kinetic database for a wide spectrum of compounds varying in lipophilicity and other properties are needed to elucidate various theoretical and practical aspects of the use of membranes in the environmental research and for related practical applications.

ACKNOWLEDGEMENT

This paper was supported by National Nature Science Foundation Project 29637010. Thanks are due to Prof. Anders Sodergren in the Lund University, Sweden for careful revise the manuscript.

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