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Predicting Bioavailability and Accumulation of Organochlorine Pesticides by Japanese Medaka in the Presence of Humic Acid and Natural Organic Matter Using Passive Sampling Membranes

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Adsorption to dissolved organic matter (DOM) may significantly decrease the freely dissolved concentration of many hydrophobic organic compounds and, hence, result in reduced bioavailability to aquatic organisms. Here, the suitability of using triolein-embedded cellulose acetate membrane (TECAM) as a biomimetic surrogate to assess the bioavailability of organochlorine pesticides (OCPs) in water in the presence of DOM was explored. The accumulation of OCPs was measured in TECAM and pelagic Japanese medaka (*Oryzias latipes*) in the laboratory after 12 h exposure to water containing different levels of Aldrich humic acid. Further, OCP uptake by TECAM and medaka in real aqueous environments was evaluated after 30 d exposures in two sites. Laboratory results showed that OCP uptake by medaka consistently decreased with increasing levels of humic acid in the range of 0−15 mg C/L in sample solutions. This tendency was closely mimicked by OCP accumulation in TECAM under the same conditions. Field results showed that TECAM accumulated similar OCP patterns as medaka (r^2 = 0.92 for site 1 and r^2 =0.94 for site 2), although comparison of the in-field eight OCP concentrations in TECAM to those in medaka yielded approximately a factor of 3 (on a wet weight basis). These results suggest that the TECAM method can be used as a simple and useful tool to predict the bioavailability and bioaccumulation potential of poorly biotransformed organic compounds in pelagic fish in aqueous environment.

Introduction

Hydrophobic organic compounds (HOCs) are the subject of worldwide attention due to their ubiquitous nature. These substances often resist chemical and biological breakdown in the environment because of their chemical structure and, consequently, can accumulate in organisms (e.g., bioaccumulation), biomagnify in food chains, and are potentially toxic to humans and wildlife. It is appropriate, therefore, to estimate their environmentally relevant concentrations and to yield adequate information on the fate and distribution of these chemicals in the environment. HOCs in water may accumulate in aquatic food chains and can reach levels that threaten fish-eating organisms such as humans and predatory birds. Dissolved organic material (DOM), such as humic acid, plays an important role in controlling the bioavailability of HOCs in the aquatic environment (*1*). A decrease in the freely dissolved aqueous concentration of HOCs has been found in the presence of DOM (*2*). Since it is the freely dissolved form of contaminant which is the driving force for the transport, distribution, and bioaccumulation, this decrease is responsible for reduced bioavailability and/or toxicity. Therefore, current HOC contamination assessment methods based on the total chemically extractable concentration are often unreliable and poorly predictive. Although traditional biomonitoring is the most direct approach to test HOC bioaccumulation and toxicity, the measurements are complicated, tedious, very expensive (especially in field conditions), and interpretation of the results is often ambiguous. Hence, it is highly desirable to develop a simplified "biomimetic" sampling method that can provide a good means to better predict when organisms are at risk.

The freely dissolved concentration of a hydrophobic chemical is difficult to measure directly because of the requirement for separation of the bound and free forms. Although a range of methods has been proposed for this purpose (e.g., fluorescence quenching, solubility enhancement, dialysis membranes, headspace equilibration, ultrafiltration), these methods are either labor intensive, time-consuming, or require tight experimental control, and they are unsuitable for field use. Recently, two types of passive sampling devices, solid-phase microextraction (SPME) (*3*) and semipermeable membrane device (SPMD) (*4*), have proven successful in the prediction of the bioavailability of aqueous residues of various HOCs (*5*−*8*).

For practical reasons, the application of passive sampling devices can be divided into equilibrium and nonequilibrium (i.e., integrative) sampling modes. Both sampling approaches have advantages and disadvantages, and the appropriateness of their use depends on the goals of a given study. In aquatic systems where chemical concentrations often vary over time as well as location, integrative samplers are particularly important to obtain the time-weighted average contaminant concentration as a fundamental part of ecological risk assessment. In this context, assessment of the contaminant uptake in fish is important for various reasons. Fish are commonly used as integrative indicators because of their high levels of lipid; they are at the top of the aquatic food web and are often consumed by humans. Despite SPMD is widely used as a "virtual fish" (*8*), it often suffers from time-consuming dialysis and complex cleanup procedures (*9*). Moreover, although many studies of using chemical methods to predict aqueous HOC bioavailability are reported, they are mostly a comparison between SPME and invertebrate organisms and are restricted to laboratory research. Passive samplers were scarcely used to quantify the effect of DOM on the bioavailability of HOCs to fish and validated in the field until recently.

The triolein-embedded cellulose acetate membrane (TECAM), developed by Wang and co-workers (*10*), is a very

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promising passive sampler similar to SPMD (*11*). A TECAM consists of two immiscible phases: an outer hydrophilic cellulose acetate membrane and inner hydrophobic triolein phase. Once the TECAM is submerged in water, chemicals diffuse through the membrane and become trapped in the triolein, which can subsequently be extracted by organic solvents (e.g., *n*-hexane) and then concentrated for analysis. The use of TECAM as a sampling technique appears to be cost-effective, easy to prepare, and involves simple pretreatment procedures (commonly no additional cleanup is needed).

Considering the uptake routes of HOCs differ greatly between benthic and pelagic fish, the primary aim of the present study was to investigate the suitability of TECAM to predict the bioavailability and accumulation of persistent organochlorine pesticides (OCPs) for pelagic Japanese medaka (*Oryzias latipes*) in the presence of organic matter adsorption phases. Comparisons of accumulation were made between TECAMs and medakas in short-term laboratory tests and long-term field tests. On the basis of the data, we discuss the possibilities for application of TECAM for biomimetic sampling. Only one representative class of HOCs, OCPs, was tested in this study because our focus was the method itself and its applicability for environmental studies.

Experimental Section

Materials. The individual standard stock solution of chemicals: dieldrin, endrin, and 4,4′-DDT were purchased from Supelco (Bellefonte, PA). The standard solution containing three OCPs was prepared by diluting the stock solution with methanol to 1 mg/L. Humic acid sodium salt was purchased from Aldrich (Steinheim, Germany). Although commercial humic acids showed some differences from the natural aquatic humic substances, they do possess the similar property of associating with the HOCs (*2*). Thus, Aldrich Humic acid was used as the probe of the naturally aquatic humic substance. A stock solution of humic acid was prepared by dissolving humic acid sodium salt in 0.01 M sodium hydroxide solution. The solution was stirred for about 24 h and centrifuged at 27 100*g* (20 °C) for 1 h to remove most of the particles, and then filtered to 0.45 μm with prerinsed cellulose acetate nitrate membrane filters (Whatman, Clifton, NJ) to remove any remaining particulate material. The organic carbon content of the humic acid stock solution was measured with a total organic carbon analyzer (phoenix-8000, Tekmar-Dohrmann, Mason, OH). Humic acid concentrations are expressed as milligrams of organic carbon per liter of water. The ultrapure water (DOC < 0.1 mg C/L) used in this study was purified by a Milli-Q Gradient system (Millipore, Bedford, MA). "Artificial freshwater" (AFW) used as test water was prepared by dissolving the analytical grade reagents $CaCl_2 \cdot H_2O$ (294 mg), $MgSO_4 \cdot 7H_2O$ (123 mg), $NaHCO_3$ (65 mg), and KCl (5.8 mg) in 1000 mL ultrapure water and continuously aerating before use (*12*).

Development of TECAM Methods. The procedure for preparing TECAM was the same as described previously (*10*). All TECAMs were constructed in the same configuration: 40−50 μm thick, 12 cm wide, and 18 cm long, embedded with 7.5 wt % of triolein. Triolein is a major lipid in fishes and is known for its high hydrophobicity (*13*). In the configuration of TECAM, triolein was closely encapsulated by the outer cellulose acetate membrane polymers (*10*, *14*). Small pieces of TECAM (4 × 6 cm, average wet weight 0.159 g) were used for all laboratory experiments unless otherwise indicated. All TECAMs were kept in ultrapure water before use.

Since our previous study has proved that only the freely dissolved chemical partitions to the TECAM (*14*), TECAM is suitable for measuring free fractions of HOCs. Hence, a negligible depletion extraction method using TECAM (nd-TECAM) was developed to measure the free pesticide concentration in the test samples containing DOM (*14*). Negligible depletion extraction implies that only a very small amount is extracted from a solution, so that the existing equilibrium between the bound and free form of a chemical in the solution is not disturbed (*15*). Briefly, in a 1 L conical flask, a piece of TECAM was immersed in the 1 L of test solution for 1 h sampling. The flask was sealed to prevent losses of analytes due to volatilization. Calibration standards with known pesticide concentrations were prepared with DOC-free AFW and analyzed under the same conditions as described above. The calibration factors (i.e., slope of the regression line) were determined to translate chemical concentration in TECAM to aqueous concentration in exposure solution. All the experiments were performed on a constant temperature-reciprocating shaker with controlled conditions (90 rpm, 20 °C).

Laboratory Bioaccumulation Experiments. The Japanese medaka were cultured and maintained for more than five generations in our laboratory before fish were used for experiments. The medakas were kept in dechlorinated tap water (using active carbon column) at a constant temperature (25 ± 2 °C), with a constant photoperiod of 16:8 h (light: dark). Female medaka selected for the bioconcentration experiments were approximately 5 months post hatch, fully mature (mean body weight, 500 ± 100 mg; mean body length, 24 ± 3 mm), and were outside of spawning period. They were not fed for 24 h prior to the experiment.

A short-term laboratory bioaccumulation experiment with medaka was carried out to see whether the freely dissolved fraction was of importance for the bioavailability of OCPs in fish, and, further, to investigate if TECAM can mimic the bioconcentration pattern of OCPs in biota with and without DOM. A relatively short exposure time was used to minimize the consumption of humic acid as well as OCP standards and because steady-state conditions were not necessary to compare the effect of humic acid treatments on the uptake of chemicals. The sample solutions were produced through diluting the humic acid stock solution with AFW to reach organic carbon content of 1, 2, 5, 10, and 15 mg C/L, which is consistent with commonly occurring environmental ranges (*16*). AFW was also used as a control medium. Before use, the test solutions were adjusted to a pH of 7.5 ± 0.2 with 0.1 M HCl and 0.1 M NaOH. One liter of each solution was transferred to each 1 L conical flask and spiked with three test OCPs to give a nominal concentration of 50 ng/L for each analyte. The flasks were kept sealed and shaken gently for 4 d in the dark before medakas were added. Several studies have indicated that short contact time (<24 h) is enough to establish an interaction between DOM and HOC that reduces the bioavailability of the HOC in laboratory test system (*2*, *17*, *18*). The bioavailability here refers to the readily available fraction of OCP, which is in equilibrium with the water surrounding the DOM (*19*). Following equilibration, one medaka was introduced into each flask, and the test flasks were kept at room temperature. Three replicate flasks were made for every treatment. Exposures continued for 12 h to approach a sufficient accumulation of test chemicals in medaka. To accurately assess HOC uptake, it was necessary to maintain a constant aqueous concentration of free analyte. Accordingly, the test solution in the exposure flasks was renewed every hour to compensate for the decrease of analyte due to physical adsorption to surfaces and absorption by medaka. The free concentrations (C_f) of test OCPs in the exposure water were determined by nd-TECAM extraction method (sampling for 1 h on the shaker with 90 rpm), and total concentrations (C_t) were determined by exhaustive liquid−liquid extraction (LLE) with dichloromethane. Non-steady-state bioconcentration factors (BCF) after 12 h of exposure are calculated as the ratio of the concentration of

contaminant in the medakas (ng/g wet weight) and in the exposure water (ng/mL, C_t measured with LLE and C_f using TECAM).

Uptake Experiments with TECAM. The TECAM accumulation study was performed under identical conditions as the medaka uptake experiment but in a separate exposure system. The exposure also lasted for 12 h to coincide with the bioaccumulation experiment. The maximum loss measured for each OCP was below 10% within the 1 h time frame of test solution renewal in the experiment and thus met the requirement of negligible depletion (*14*).

Field Accumulation Study. Field exposure of TECAMs and medakas was conducted at two contaminated sites in Guanting Reservoir in the Northwest of Beijing, China. This field site was selected because relatively constant concentrations of OCPs have been detected in the water and sediment (*20*, *21*). Three TECAMs and 25 medakas were deployed (0.5 m below water surface) for a period of 30 days in two separate stainless steel cages (approximately 0.3 × 0.3 × 0.3 m), which were fixed on a stake at each of the two sites. All the medakas were visually examined before initiation of the exposure and found to be in good condition. The water temperature at the sites varied from, approximately, 23 to 25 °C during the exposure. After deployment, TECAMs and medakas were retrieved and stored in the laboratory at −20 °C. Five medakas were pooled as a sample. Natural surface water was collected from the two sites and filtered through 0.45 μm membrane filters, and the concentration of total dissolved organic carbon (DOC) was approximately 8 mg C/L.

Sample Processing. Prior to the extraction, the gut and gill of all medakas were removed, and then the fish were weighed, ground with 5 g of precombusted sodium sulfate each, and dried. The resulting powder was ultrasonically extracted with 10 mL of *n*-hexane/dichloromethane (1:1) for 10 min. The solvent was replaced by a new portion of 10 mL *n*-hexane/dichloromethane (1:1), and the extraction was repeated two times. The three fractions were combined and reduced by rotary evaporation to approximately 2 mL. The extracts of fish samples was subjected to a glass column (10 mm i.d.) containing 8 g of 1:2 alumina/silica gel for cleanup and fractionation (*11*). The eluates containing studied OCPs were concentrated to 0.2 mL under a gentle stream of N_2.

TECAMs were rinsed with distilled water and wiped up with clean paper tissue. Each TECAM was ultrasonic extracted three times with 10 mL (lab samples) or 20 mL (field samples) of hexane as solvent for 10 min cycles. After extraction, solutions were combined and concentrated to 0.2 mL under a stream of N_2. Preliminary experiments showed that the recovery of this extraction procedure was >88% for the three OCPs.

The extracts of LLE were preevaporated and solvent-exchanged into hexane, then were blowdown by N_2 to a final volume of 0.2 mL.

Instrumental Analysis. Analysis of OCPs was performed with an Agilent 6890 series GC equipped with a ^{63}Ni electron capture detector (Agilent, Palo Alto, CA). A 30 m × 0.25 mm i.d. × 0.25 μm HP-5 column (J&W, Folsom, CA) was used for pesticide identification and quantification. Details of the OCPs analysis were described elsewhere (*10*).

Data Analysis. According to Suffet et al. (*22*), in water with DOM, the fraction of freely dissolved analyte (*f*) is given by the following equation:

$$f = \frac{1}{1 + K_{doc}C_{doc}} \quad (1)$$

where K_{doc} is the organic carbon based partition coefficient and C_{doc} being the dimensionless mass ratio of two phases between which partitioning is occurring, which can be measured as dissolved organic carbon concentration in solution (kg C/L).

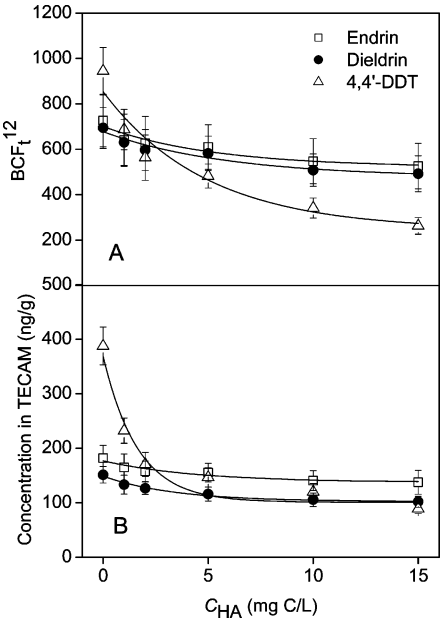

FIGURE 1. Pesticide accumulation in Japanese medaka and in TECAM in water samples containing different levels of Aldrich humic acid. Vertical lines are standard deviations of three replicates. BCF_t^{12} = 12 h bioconcentration factor based on total aqueous concentration.

When TECAMs and medaka are used to accumulate the freely dissolved OCPs, the concentration in the sampling matrix is proportional to the free aqueous concentration. Therefore, we have the equation analogous to eq 1 (*23*):

$$C_{TM} = \frac{C_{TM(0)}}{1 + K_{doc}C_{doc}} \quad (2)$$

with C_{TM} being the OCP concentration in the TECAM and medaka exposed to water with DOM and $C_{TM(0)}$ being the OCP concentration in the matrix exposed to water without DOM. Using eq 2 and the experimentally determined values, calculations of K_{doc} were performed with Origin 7.0 (OriginLab, Northampton, MA) by an iterative procedure using the Marquardt−Levenberg algorithm for least-squares estimation of parameters.

Results and Discussion

Effects of DOM on Bioaccumulation. In the laboratory bioaccumulation experiments, pesticide uptake by medaka consistently decreased with increasing humic acid levels in exposure water for dieldrin, endrin, and 4,4′-DDT. Consequently, the non-steady-state 12 h BCF based on total aqueous concentration (BCF_t^{12}) of all three OCPs were largest in the DOM-free AFW controls and consistently decreased in sample solutions containing various content of humic acid, although the effect was less pronounced for the less hydrophobic dieldrin and endrin (Figure 1A).

In a critical review, Haitzer et al. (*2*) came to the conclusion that the presence of DOM generally causes lowered bioconcentration of HOCs. Although some studies have also reported enhancements in bioconcentration of certain HOCs at low DOM levels (<10 mg C/L) (*2*), Haitzer et al. (*12*) suggested that the enhancements are more likely the result of random, experimental variations. In the present study, we do not observe the temporary increase of bioconcentration in the range of low concentrations of DOM (Figure 1A). This result is in line with an earlier study that found low levels of

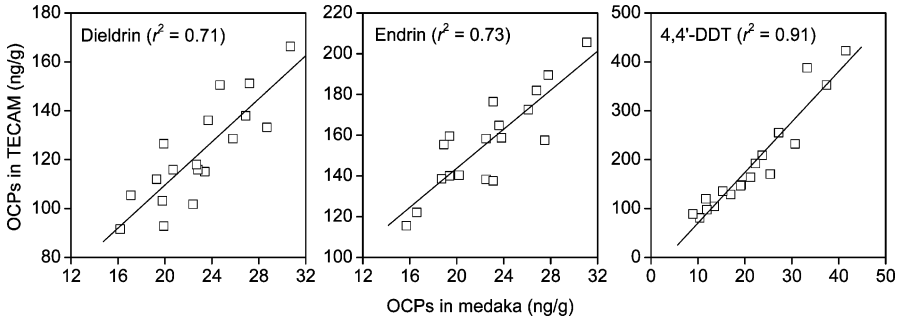

FIGURE 2. Correlation between organochlorine pesticide concentrations in medaka and in TECAM exposed to sample solutions contain different Aldrich humic acid contents (AFW control, 1, 2, 5, 10, and 15 mg C/L, each concentration treatment including three measurements, $n = 18$). Concentrations in medaka are based on whole body wet weight and concentrations in TECAM are based the total weight that includes membrane plus triolein.

TABLE 1. 12 h Bioconcentration Factor of Three Test Organochlorine Pesticides in Medaka Exposed to Sample Solutions with Different Levels of Aldrich Humic Acid and Resulted DOC/Water Partition Coefficients

		bioconcentration factor ($n = 3$)[b]						log K_{doc}[c]	
chemical	log K_{ow}[a]	AFW(control)	1 mg C/L	2 mg C/L	5 mg C/L	10 mg C/L	15 mg C/L	TAM	medaka
Endrin	5.20	726 ± 116(726)	640 ± 115(709)	626 ± 119(740)	610 ± 98(815)	547 ± 99(808)	526 ± 100(802)	4.44	4.49
Dieldrin	5.40	694 ± 90(694)	630 ± 101(714)	597 ± 90(716)	582 ± 76(773)	508 ± 71(743)	492 ± 79(760)	4.64	4.52
4,4'-DDT	6.36	944 ± 104(944)	687 ± 89(986)	563 ± 100(1037)	482 ± 53(1091)	341 ± 44(925)	263 ± 37(1010)	5.64	5.32

[a] Octanol/water partition coefficients obtained from Mackay et al. (*31*). [b] Bioconcentration factors are based on total aqueous concentration (values in brackets are based on freely dissolved concentrations by nd-TECAM method). [c] Log K_{doc} values are calculated from the 12 h laboratory accumulation study according to eq 2.

DOM, either natural or commercial, consistently reduced the uptake of contaminants by aquatic organisms (*12*). The bioconcentration reduction in the presence of DOM is due to the formation of aggregates too large or too polar to cross biological membranes (*22*), since only the freely dissolved chemicals are assumed to be bioconcentrated. However, no clear mechanistic explanations can be given at present for enhancements of bioconcentration at low levels of DOM (*12*).

Bioavailability Prediction by TECAM Method. Accumulation of three model OCPs in TECAM was simultaneously determined in the identical samples used for measuring bioaccumulation. As shown in Figure 1B, the effect of humic acid on the accumulation of OCPs in TECAM (C_{TECAM}) was similar to the effect on BCF_t[12] for the chemicals in medaka. Furthermore, the relationship between the accumulated concentrations in TECAM and DOM concentration was nonlinear for all model compounds, the similar trend was also observed in the bioaccumulation test. Fitting the experimental accumulation data of medaka and TECAM to eq 2 resulted in the log K_{doc} values listed in Table 1. For all model compounds, the K_{doc}s derived from the bioconcentration study were very close to those measured by the TECAM method. All these results indicate that the TECAM method provides an accurate and useful prediction on the decrease in bioavailability of OCPs in medaka in the presence of DOM. To our knowledge, the K_{doc} values for dieldrin and endrin are unavailable in the literature, so we only compared the K_{doc} values of 4,4′-DDT from this experiment with published K_{doc}s using Aldrich humic acid (*24*). Several values are reported for 4,4′-DDT ranging from 5.1 to 5.7, and our values are in excellent agreement with them (Table 1). This result showed that the freely dissolved fraction was of great importance for the bioavailability of HOCs, and their partition coefficients toward DOM could be estimated with the proposed TECAM method. Bioaccumulation of OCPs was closely related to pesticide accumulation on TECAM exposed in the same samples. Linear correlations between body residues in medaka and C_{TECAM} showed close relationships for dieldrin ($r^2 = 0.71$), endrin ($r^2 = 0.73$), and 4,4′-DDT ($r^2 = 0.91$) when data from six humic acid treatments were pooled (including controls) (Figure 2).

The freely dissolved concentrations of dieldrin, endrin, and 4,4′-DDT in the sample solutions of bioaccumulation experiments were determined using the nd-TECAM extraction method and the calibration curve. It is observed that, for all test OCPs, the bioaccumulation in medaka was shown to depend on the freely dissolved fraction of these chemicals in exposure solutions. The average BCF based on the free aqueous concentration (i.e., BCF_f[12]) and that based on the total concentration (BCF_t[12]) were compared, as shown in Table 1 for the test chemicals. By determining the BCFs based on free concentrations, the values remained fairly constant (no significantly differences $p > 0.05$) throughout the DOM concentration series (DOC 0−15 mg C/L). This result is similar to Ramos et al. (*5*), who found that BCF_t of pentachlorobenzene or PCB 77 for *D. magna* decreased in the presence of Aldrich humic acid, but that BCF_f remained relatively independent of humic acid concentrations. All these results show that DOM-absorbed fraction is unavailable for bioaccumulation in medaka and TECAM may be used to predict the actual bioavailability and bioaccumulation potential of OCPs for water samples containing adsorption phases.

Field Verification. The next step was to validate the use of TECAM as a biomimetic tool to predict the effect of natural DOM on the relative long-term accumulation of OCPs in medaka under subchronic field conditions. From an environmental point of view, the validation of TECAM performance in comparison to in vivo bioaccumulation tests under long-term conditions is highly relevant. Since most of medakas appeared to be healthy and no visible adverse effects were observed during the caging period, we assumed that the exposure conditions (e.g., pH, dissolved oxygen, and water temperature, etc.) of caging sites in the present study were suitable for medaka. After the 30 d exposure period, in both TECAM and medaka samples the detected OCP profiles were dominated by eight compounds: α-HCH, β-HCH, γ-HCH, heptachlor, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, and aldrin (Table 2). In bioaccumulation tests with animals, the time required to reach equilibrium depends on the capacity (i.e., volume of the biological compartment available for accumulation) for the contaminants of interest. Thus, smaller organisms will approach steady-state earlier for a given contaminant.

TABLE 2. Concentrations of Organochlorine Pesticides Detected in TECAMs and Medakas. Exposed to the Water of Guanting Reservoir for 30 d[a]

	TECAM (ng/g) ($n = 3$)		medaka (ng/g) ($n = 5$)	
chemical	site 1	site 2	site 1	site 2
α-HCH	0.7	0.9	0.1	0.4
β-HCH	1.1	1.3	0.4	0.6
γ-HCH	1.9	1.1	0.6	0.4
Heptachlor	0.5	0.7	0.3	0.2
4,4′-DDE	0.7	1.1	0.2	0.3
4,4′-DDD	0.6	1.3	0.4	0.4
4,4′-DDT	0.3	0.5	0.1	0.2
Aldrin	4.6	4.3	1.2	1.2
ΣOCPs	10.4	11.2	3.3	3.7

[a] Concentrations of analytes are based on wet weight.

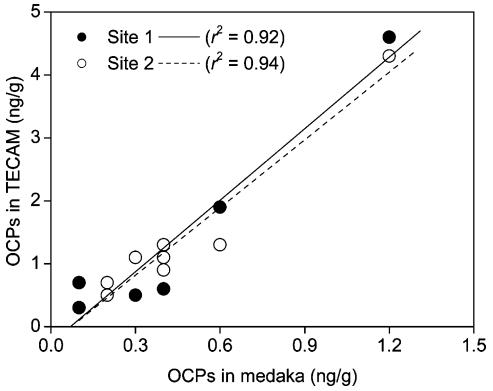

FIGURE 3. Correlation between organochlorine pesticide concentrations in medaka and in TECAM exposed to natural waters containing 8 mg C/L dissolved organic matter in Guanting Reservoir, Beijing. The symbols represent different pesticides and their concentrations in medaka, and in TECAM, are based on wet weight.

Van der Oost (*25*) has observed that a steady-state in bioaccumulation of most OCPs in caged carp was reached after 4 weeks of exposure. The average whole-body wet weight of medakas used in the present study is approximately 0.6 g, which is much less than the whole body weight of carp they used. This implies that the contaminants accumulated in medakas would require shorter time to approach steady-state. Our previous field study has indicated that most PAHs (with similar hydrophobicity to the OCPs studied in the present work) required less than 40 d to achieve steady-state in TECAMs (*11*). Hence, it is reasonable to assume that OCPs accumulation in both TECAMs and medakas approached steady-state conditions after 30 d exposure.

On a wet weight basis, total OCP concentrations in medaka were 3.3 ng/g and 3.7 ng/g, respectively, whereas concentrations in TECAM were 10.4 ng/g and 11.2 ng/g at the end of the exposure from sites 1 and 2, respectively (Table 2). Although comparison of the in-field eight OCP concentrations in TECAM to those in medaka yielded, approximately, a factor of 3, concentrations in the TECAMs exposed to the water of sites 1 and 2 are highly correlated to the concentrations in medakas ($r^2 = 0.92$ for site 1 and $r^2 = 0.94$ for site 2, respectively) (Figure 3). This result suggests that TECAM is a suitable surrogate for bioaccumulation under subchronic environmental conditions. Knowing OCP accumulation in TECAM and concentration of DOC, the decrease in bioavailability of OCPs in medaka caused by the presence of DOM could be predicted according to eq 1 with the help of K_{doc} (which can be derived by nd-TECAM method). Also, it is possible to use TECAM as a fast and inexpensive alternative for estimating internal concentrations of contaminants in aquatic organisms.

Comparison of Accumulation in Medaka with Partitioning to TECAM. Regardless of the marked differences in physiological/physical properties between TECAM and medaka, there are some fundamental similarities in the characteristics and processes affecting the accumulation of HOCs in the two matrices. For example, diffusion of nonpolar compounds through nonporous organic polymers such as the outer membrane of TECAM has been shown to be similar to solute diffusion across biological membranes. Furthermore, the triolein used in TECAMs is a major lipid in fishes (*4*) and is representative of fats or the neutral lipid class (*13*), which is the largest storage site of persistent HOCs in many aquatic organisms. The accumulation of HOCs by TECAM is solely mediated by passive diffusional and partitioning processes, which are the basis of equilibrium partitioning (EP) theory (*26*), whereas accumulation of HOCs by medaka is often via direct uptake from water by gill or skin, and/or by dietary uptake route (i.e., assimilation from contaminated food and organic matter particles). However, Opperhuizen (*27*) found that the feeding rate of fish [0.02 g/(g•d)] compared to the ventilation rate [2000 mL/(g•d)] is very low. Chemical uptake from water phase is the first step and the dominant route for most aquatic organisms even for those consuming heterotrophs and fungi as food (*8*, *28*). Bruggeman et al. (*29*) found that food chain or dietary uptake of chemicals exceed respiratory uptake by fish only when chemicals were quite hydrophobic. Since our DOM consumption experiment indicates that medaka is unable to consume DOM from the water as food source (see the Supporting Information), we conclude that the uptake of the majority of compounds is regulated by simple partitioning from water phase for medakas and the contribution of dietary uptake is very small in this study. Weston and Mayer (*30*) showed that not all ingested bound chemicals but only the fraction that can be solubilized by digestive fluids (i.e., fast-desorbing fraction) are bioavailable, and the slow-desorbing fraction was resistant to gut fluids at relevant time scales (i.e., not bioavailable). Furthermore, contaminants associated with ingested food particles also must cross biological membrane within the gut before accumulation. In the present study, the residues extracted from medakas were wholly bioavailable because the gut and gill of medakas were removed before sample extraction. It is reasonable to further assume that chemical transfer based on EP theory occurs within the solution-phase regardless of the membrane type or location within the organism (e.g., gill, gut, or dermis). Strong support for this hypothesis is found in experiments by You et al. (*7*) which revealed that diffusional-partitioning based methods (i.e., matrix-SPME and Tenax extraction) were good predictors to assess the bioavailability of the fast-desorbing fraction of sediment-associated organic contaminants. In light of the present research and related literature, it is reasonable to conclude that the whole-body accumulation of usual poorly biotransformed nonpolar organic contaminants in medaka follows the EP theory regardless of the uptake route. Therefore, it is not surprising to observe a good correlation between TECAM concentration and medaka concentration for studied OCPs from both laboratory and field data.

In summary, TECAM-based methods, as verified in this study, offer convenient and useful tools to accurately measure freely dissolved compounds. They are time- and cost-effective biomimetic screening tools for estimating bioavailability of bioconcentratable compounds to exposed organisms. Clearly, more research is needed to optimize TECAM in the utility for biomimetic sampling, such as comparison of the accumulation of contaminants with deposit-feeding organisms, because it is unrealistic to expect a single passive sampler to be biomimetic of all biomonitoring organisms and to expect one or two species of biomonitoring organisms mimic bioaccumulation in all organisms of concern (*8*).

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Supporting Information Available

Results of consumption of dissolved organic matter by medaka. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Hassett, J. P. Dissolved natural organic matter as a microreactor. *Science* **2006**, *311*, 1723−1724.
(2) Haitzer, M.; Höss, S.; Traunspurger, W.; Steinberg, C. Effects of dissolved organic matter (DOM) on the bioconcentration of organic chemicals in aquatic organisms. *Chemosphere* **1998**, *37*, 1335−1362.
(3) Authur, C. L.; Pawliszyn, J. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **1990**, *62*, 2145−2148.
(4) Huckins, J. N.; Tubergen, M. W.; Manuweera, G. K. Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavaiiability of lipophilic contaminants and estimating their bioconcentration potential. *Chemosphere* **1990**, *20*, 533−552.
(5) Ramos, E. U.; Meijer, S. N.; Vaes, W. H. J.; Verhaar, H. J. M.; Hermens, J. L. M. Using solid-phase microextraction to determine partition coefficients to humic acids and bioavailable concentrations of hydrophobic chemicals. *Environ. Sci. Technol.* **1998**, *32*, 3430−3435.
(6) Leslie, H. A.; Ter Laak, T. L.; Busser, F. J. M.; Kraak, M. H. S.; Hermens, J. L. M. Bioconcentration of organic chemicals: is a solid-phase microextraction fiber a good surrogate for biota? *Environ. Sci. Technol.* **2002**, *36*, 5399−5404.
(7) You, J.; Landrum, P. F.; Lydy, M. I. Comparison of chemical approaches for assessing bioavailability of sediment-associated contaminants. *Environ. Sci. Technol.* **2006**, *40*, 6348−6353.
(8) Huckins, J. N.; Petty, J. D.; Booij, K. *Monitors of Organic Chemicals in the Environment: Semipermeable Membrane Devices*; Springer Publishers: New York, 2006.
(9) Wezel, K. D.; Vrana, B.; Hubert, A.; Schüürmann, G. Dialysis of persistent organic pollutants and polycyclic aromatic hydrocarbons from semipermeable membrane devices. A procedure using an accelerated solvent extraction device. *Anal. Chem.* **2004**, *76*, 5503−5509.
(10) Xu, Y.; Wang, Z.; Ke, R.; Khan, S. U. Accumulation of organochlorine pesticides from water using triolein embedded cellulose acetate membranes. *Environ. Sci. Technol.* **2005**, *39*, 1152−1157.
(11) Ke, R.; Xu, Y.; Wang, Z.; Khan, S. U. Estimation of the uptake rate constants for polycyclic aromatic hydrocarbons accumulated by semipermeable membrane devices and triolein-embedded cellulose acetate membranes. *Environ. Sci. Technol.* **2006**, *40*, 3906−3911.
(12) Haitzer, M.; Akkanen, J.; Steinberg, C.; Kukkonen, J. V. K. No enhancement in bioconcentration of organic contaminants by low levels of DOM. *Chemosphere* **2001**, *44*, 165−171.
(13) Chou, C. T. Partition coefficients of organic compounds in lipid-water systems and correlations with fish bioconcentration factors. *Environ. Sci. Technol.* **1985**, *19*, 57−62.
(14) Ke, R.; Wang, Z.; Huang, S.; Khan, S. U. Accurate quantification of the freely dissolved organochlorine pesticides in water in the presence of dissolved organic matter using triolein-embedded cellulose acetate membrane. *Anal. Bioanal. Chem.* **2007**, *387*, 2871−2879.
(15) Vaes, W. H. J.; Ramos, E. U.; Verhaar, H. J. M.; Seinen, W.; Hermens, J. L. M. Measurement of the free concentration using solid-phase microextraction: binding to protein. *Anal. Chem.* **1996**, *68*, 4463−4467.
(16) Steinberg, C. E. W.; Paul, A.; Pflugmacher, S.; Meinelt, T.; Klocking, R.; Wiegand, C. Pure humic substances have the potential to act as xenobiotic chemicals−a review. *Fresenius Environ. Bull.* **2003**, *12*, 391−401.
(17) Nikkilä, A.; Kukkonen, J. V. K. Effects of dissolved organic material on binding and toxicokinetics of pyrene in the water flea *Daphnia magna*. *Arch. Environ. Contam. Toxicol.* **2001**, *40*, 333−338.
(18) Akkanen, J.; Kukkonen, J. V. K. Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter. *Environ. Toxicol. Chem.* **2003**, *22*, 518−524.
(19) Semple, K. T.; Morriss, A. W. J.; Paton, G. I. Bioavailability of hydrophobic oragnic contaminants in soils: fundamental concepts and techniques for analysis. *Eur. J. Soil Sci.* **2003**, *54*, 809−818.
(20) Wang, X.; Chu, S.; Xu, X. Organochlorine pesticide residues in water from Guanting Reservoir and Yondding River, China. *Bull. Environ. Contam. Toxicol.* **2003**, *70*, 351−358.
(21) Xue, N.; Zhang, D.; Xu, X. Organochlorinated pesticide multi-residue in surface sediment from Beijing Guanting reservoir. *Water Res.* **2006**, *40*, 183−194.
(22) Suffet, I. H.; Javfert, C. T.; Kukkonen, J. V. K.; Servos, M. R.; Spacie, A.; Williams, L. L.; Noblet, J. A. In *Bioavailability*; Hamelink, J. L., Landrum, P. F., Bergman, H. L., William, H. B., Eds.; CRC: Boca Raton, FL, 1994; pp 93−107.
(23) Miège, C.; Ravelet, C.; Croué, J. P.; Garric, J. Semi-permeable membrane device efficiency for sampling free soluble fraction of polycyclic aromatic hydrocarbons. *Anal. Chim. Acta.* **2005**, *536*, 259−266.
(24) Mott, H. V. Association of hydrophobic organic contaminants with soluble organic matter: evaluation of the database of K_{doc} values. *Adv. Environ. Res.* **2002**, *6*, 577−593.
(25) Van der Oost, R. Fish Biomarkers for inland water pollution, an environmental field survey on the relationships between bioaccumulation and biochemical responses in freshwater fish. Thesis, Vrije Universiteit Amsterdam, 1998.
(26) Di Toro, D. M.; Zarba, C. S.; Hansen, D. J.; Berry, W. J.; Swartz, R. C.; Cowan, C. E.; Pavlou, S. P.; Allen, H. E.; Thomas, N. A.; Paquin, P. R. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ. Toxicol. Chem.* **1991**, *10*, 1541−1583.
(27) Opperhuizen, A. In *Organic Pollutants in the Aquatic Environment*, Proceedings of the 6th European SETAC Symposium, Lisbon, Portugal, May 22−24, 1990; Angeletti, G.; Bjorseth, A., Eds.; 1990
(28) Connell, D. W. *Bioaccumulation of Xenobiotic Compounds*. CRC Press: Boca Raton, FL, 1990.
(29) Bruggeman, W. A.; Martron, L. B. J. M.; Kooiman, D.; Hutizinger, O. Accumulation and elimination kinetics of di-, tri- and tetrachlorobiphenyls in goldfish after dietary and aqueous exposure. *Chemosphere* **1981**, *10*, 811−832.
(30) Weston, D. P.; Mayer, L. M. Comparison of in vitro digestive fluid extraction and traditional in vivo approaches as measures of polycyclic aromatic hydrocarbon bioavailability from sediments. *Environ. Toxicol. Chem.* **1998**, *17*, 830−840.
(31) Mackay, D.; Shiu, W. Y.; Ma, K. C. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Compounds, Volume V*; Lewis Publishers, Chelsea, MI, 1992.

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