

# Assessing Accumulation and Biological Effect of Hydrophobic Organic Contaminants in Water Using Caged Japanese Medaka and Deployed Triolein-embedded Cellulose Acetate Membranes

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**Abstract** Applicability of triolein-embedded cellulose acetate membrane (TECAM) to accumulation and potential biological effect assessment for hydrophobic organic contaminants (HOCs) was investigated compared with Japanese medaka. The results of field exposure showed that medaka and TECAMs accumulated contaminants in a similar pattern with good correlations between concentrations in medaka and TECAMs based on lipid weight for OCPs ( $r = 0.96$ ,  $p = 0.01$ ,  $n = 9$ ) and PAHs ( $r = 0.73$ ,  $p = 0.01$ ,  $n = 13$ ). Meanwhile, 2,3,7,8-TCDD equivalents (TEQs) of TECAM extracts detected by *in vitro* H4IIE cell bioassay corresponded well to hepatic EROD activities of exposed fish and TEQs of water samples. We concluded that TECAM could be utilized as a surrogate for biomonitoring organisms to assess the bioaccumulation of HOCs and potential biological effect.

**Keywords** TECAMs · Japanese medaka · Bioaccumulation · EROD

Biomonitoring is a very pervasive and directive approach that can combine chemical analysis and bioassays to not only monitor pollutants but also assess pollutants' bioaccumulation and toxicities (Van der Oost et al. 2003). In addition, biomonitoring addresses many of the limitations from time-point sampling methods. However, using of

biomonitoring organisms often involves certain problems, such as geographic distribution, and individual variability by sex, age and physiological state. Moreover, biomonitoring can be time-consuming and expensive, and may not be applied in the harsh environment. All of these make it difficult to compare and interpret results from different locations (U.S. EPA 2000).

Hence, it is highly desirable to develop simplified and suitable sampling tools that can remedy the limitations of biomonitoring organisms. Significant attention has been placed on non-living passive samplers. Many kinds of passive samplers have been developed and applied in recent years (Namiesnik et al. 2005), such as triolein-containing semipermeable membrane device (SPMD) (Huckins et al. 1990). The SPMDs have been widely used for monitoring a variety of hydrophobic organic contaminants (HOCs) in aquatic ecosystem, e.g., polycyclic aromatic hydrocarbons (PAHs), organochlorinated pollutants (OCPs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs) (Huckins et al. 2006). Meanwhile, chemicals sequestered by SPMD are amenable to examination by a variety of bioassays, which enhances an investigator's ability to screen for potential toxicities of HOCs in the environment (Petty et al. 2000a; Ke et al. 2007a). Nonetheless, SPMD often suffers from time-consuming dialysis and complex clean procedures (Petty et al. 2000b).

Triolein embedded cellulose acetate membrane (TECAM) based on the SPMD concept, was developed by Xu et al. (2005). 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) (Xu et al. 2005). TECAM has presented to be a very promising integrative sampler in monitoring HOCs and assessing their bioavailability in aquatic

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ecosystem (Ke et al. 2006, 2007b). However, there have been no studies published until now to test whether the contaminants sequestered by TECAMs are compatible with bioassays to provide information concerning the relative toxicological significance. The objective of this study is to assess the applicability of the biomimetic sampler TECAM to accumulation and potential biological effect assessment of HOCs for environmental monitoring compared to Japanese medaka in a constructed wetland, Guanting Reservoir (40°25' N, 115°47' E) in Beijing, China.

## Materials and Methods

The mixture standard solutions of OCPs ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ -HCH, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT and 16 priority PAHs (naphthalene [Nap], acenaphthylene [Acy], acenaphthene [Ace], fluorene [Flu], phenanthrene [Phe], anthracene [Ant], fluoranthene [Fla], pyrene [Pyr], benzo(*a*)anthracene [BaA], chrysene [Chr], benzo(*b*)fluoranthene [BbF], benzo(*k*)fluoranthene [BkF], benzo(*a*)pyrene [BaP], dibenz(*a,h*)anthracene [DahA], indeno(1,2,3-*cd*)pyrene [IcdP], benzo(*g,h,i*)perylene [BghiP]) were purchased from Supelco (Bellefonte, PA). Standards of phenanthrene-*d*<sub>10</sub>, 2,4,5,6-tetrachloro-*m*-xylene and PCB209 were used as surrogates, and standards of hexamethylbenzene and pentachloronitrobenzene as internal standards for PAHs and OCPs, respectively. Triolein (1,2,3-tri[(*cis*)-9-octadecenoyl]glycerol) of 95% purity was obtained from Sigma-Aldrich (St. Louis, MO). The organic solvents of pesticide grade were obtained from Fisher Scientific (Fair Lawn, NJ).

TECAMs were purchased from Beijing Huao Zhiheng Technology Co., Ltd. The properties had been described previously by Xu et al. (2005). All TECAMs were constructed in the same configuration: 40–50- $\mu$ m-thick, 12 cm wide and 18 cm long, embedded with 7.5% (weight) of triolein. The Japanese medaka (*Oryzias latipes*) had been cultured and maintained for more than 10 generations in our laboratory. Medaka were fully mature (body weight  $0.41 \pm 0.04$  g; body length  $36 \pm 2$  mm). Field exposure of medaka and TECAMs were continued for 30 days from mid-July to mid-August 2006 in stainless cages at six sites in the wetland. All the fish were visually examined before exposure and not fed during the exposure. The water temperature varied from 20 to 25°C, pH from 7.2 to 8.9, and dissolved oxygen (DO) from 5.6 to 7.7 mg/L except site 1 (the inlet of the wetland) where the DO might be only 2.0 to 3.0 mg/L incidentally during experiment time. After deployment, livers of three male fish as a sample in triplicate were kept in liquid nitrogen for 7-ethoxyresorufin-*O*-deethylase (EROD) activity

analysis. No obvious biofouling was found on TECAMs except for several pale yellow colorations. Field blank TECAMs and fish underwent the same processes except exposure in wetland water. Miyun reservoir that is one of drinking water sources for Beijing without being polluted by industrial and agricultural activities was selected as a control site.

Water samples (4L) were collected from each site at day 1, 15 and 30 of the exposure. These samples were immediately filtered through 0.45- $\mu$ m glass fiber prefilters (Millipore) prebaked at 450°C for 4 h. About 2 L of water was spiked with surrogate standards for chemical analysis, the other 2L was unspiked for in vitro bioassay, and was enriched by solid-phase extraction (SPE) with C<sub>18</sub> cartridges (Spe-ed<sup>TM</sup>, 500 mg/6 mL). Whole fish except intestines and gills were spiked with the surrogate standards and ground with enough pre-extracted sodium sulfate, and extracted with 200 mL acetone/hexane (1:1v/v) in a Soxhlet apparatus for 72 h. Lipid content was measured gravimetrically after evaporation of solvent from extracts to constant weight. The extracts for chemical analysis were preconcentrated to 2 mL in a rotary evaporator. Each TECAM sample was dialyzed by 40 mL of hexane for 24 h. The TECAMs were stable in hexane with negligible weight loss during dialysis. No additional cleanup of the samples was required for later analysis (Xu et al. 2005). The extracts of TECAMs were preconcentrated to 2 mL in a rotary evaporator, and split into two portions: one portion was evaporated to dryness with a gentle stream of pure N<sub>2</sub> and redissolved in dimethyl sulfoxide (DMSO, 99.5%, Sigma) for the H4IIE cell bioassay; the other portion was subjected to chemical analysis. Every C<sub>18</sub> cartridge was eluted with 10 mL dichloromethane and 5 mL hexane. The spiked extracts were mixed, concentrated and subjected to a further cleanup procedure for chemical analysis, and the unspiked extracts without cleanup were exchanged to DMSO for bioassay.

Preconcentrated extracts of water samples and fish samples for chemical analysis were subjected to a glass column (10 mm i.d.) containing 18 g of 1:2 alumina/silica gel for cleanup. The column was eluted with 15 mL of hexane, and the elute was discarded. The column then was eluted with 70 mL of dichloromethane/hexane (3:7, v:v), and this fraction contained PAHs and OCPs. Internal standards were added to the final extracts which were concentrated to 0.2 mL prior to instrumental analysis. Analysis of OCPs was carried out with an Agilent 6890 series gas chromatograph equipped with a <sup>63</sup>Ni electron-capture detector. Details of the OCPs analysis have been described elsewhere (Xu et al. 2005). The PAHs were analyzed using an Agilent 6890 series gas chromatograph

equipped with an Agilent 5973 Network mass-selective detector and a 60 m DB-5 MS silica fused capillary column (60 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m). Detection was performed using selected-ion mode, and quantification was done according to internal standard calibration. The recoveries of surrogate standards fell within a fairly narrow range (75%–86%) for OCPs and PAHs. The average recoveries of PAHs and OCPs in matrix spikes were from 56.8%  $\pm$  6.4% to 90.1%  $\pm$  6.5% and from 65.4%  $\pm$  1.5% to 112.1%  $\pm$  3.7%, respectively. Only very small amounts of a few low-molecular-weight PAHs with  $\log K_{ow} < 4.5$  were detected in fish and TECAM field blanks. No OCPs were detected in these blanks.

Liver samples of male medaka were homogenized in ice-cold phosphate buffer solution (PBS,  $H = 7.4$ ) with 10% (v/v) phenylmethylsulfonylfluoride (PMSF) at a ratio of 1:10 (liver tissue/buffer solution). Homogenates were then centrifuged at 12000  $\times g$  for 10 min at 4°C to obtain supernatants. EROD activity was analyzed as described by Ma et al. (2005) and expressed as pmol/min/mg. After 30 days exposure, no significant differences of EROD activities of fish livers were found between control, field blanks and laboratory blanks. The H4IIE cell bioassay was done on H4IIE rat hepatoma cells which were cultured in Dulbecco's modified eagle medium (DMEM, GIBCO, Germany). Details of the bioassay have been described elsewhere (Qiao et al. 2006). The bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) of samples were obtained by comparing with the dose-response curve of the standard 2,3,7,8-TCDD. If necessary, the sample extracts were diluted to fit the linear part of the standard TCDD dose-response curve. TEQs of procedural blanks for water samples (ultrapure water) had no differences compared to negative blanks (DMSO), and TEQs detected in field TECAM blanks were very low.

## Results and Discussions

No fish could survive at site 1 maybe due to incidental low DO concentration of 2–3 mg/L. It was reasonable to assume that majority of the target contaminants in both TECAMs and medaka approached steady-state after 30 days exposure according to previous study (Ke et al. 2007b). Lipid-normalized OCPs' and PAHs' concentrations of fish and TECAMs are presented in Tables 1 and 2.

Logarithm values of bioaccumulation factors of medaka (*BAF*, the ratio of target contaminant lipid-normalized concentration in fish to the concentration in water) for OCPs and PAHs were within the range of 3.3 ( $\beta$ -HCH)  $\sim$  5.1 (*p,p'*-DDE) and 3.3 (Nap)  $\sim$  4.1 (BaP), respectively.  $\log BAF$ s were 0.5–1.1 logarithm units lower than

logarithm values of TECAM concentration factors (*TCF*, the ratio of target contaminant lipid-normalized concentration in TECAM to the concentration in water) for OCPs. Meanwhile,  $\log BAF$ s were about 0.3–1.2 logarithm units lower than  $\log TCF$ s for PAHs except *DahA*, *IcdP* and *BghiP* which were under method detection limits in fish samples. Nevertheless, there existed correlations between  $\log BAF$ s of medaka and  $\log K_{ow}$ s for target contaminants ( $r = 0.75$ ,  $p = 0.01$ ,  $n = 22$ ), and between  $\log TCF$ s of TECAM and  $\log K_{ow}$ s ( $r = 0.77$ ,  $p = 0.01$ ,  $n = 24$ ). These correlations indicate that accumulation of individual PAHs and OCPs by medaka and TECAMs can be partly predicted by their  $\log K_{ow}$ s, and suggest that similar processes may be involved in accumulation of TECAMs and medaka for PAHs and OCPs. Furthermore, good match was observed between  $\log BAF$ s and  $\log TCF$ s for OCPs ( $r = 0.96$ ,  $p = 0.01$ ,  $n = 9$ ) and limited match for PAHs ( $r = 0.73$ ,  $p = 0.01$ ,  $n = 13$ ), which implies that TECAMs may have some fundamental similarities to medaka in controlling accumulation of hydrophobic contaminants. For example, the triolein used in TECAMs is a major neutral lipid in fishes, which is the largest storage site of persistent HOCs in many aquatic organisms (Huckins et al. 2006). Meanwhile, the accumulation of HOCs by TECAM is solely mediated by passive diffusional and partitioning processes (Ke et al. 2007b), which are the basis of equilibrium partitioning (EP) theory. Several studies (Utvik and Johnsen 1999; Ke et al. 2007b) have shown that there were good correlations between residue concentrations of side-by-side exposed biomonitoring organisms and SPMDs (or TECAMs), suggesting that passive partitioning and diffusional processes may also be an important step in HOCs' accumulation in biomonitoring organisms.

It was found that TECAMs accumulated higher levels of OCPs and PAHs than medaka (Tables 1 and 2). Other studies (Gale et al. 1997; Ke et al. 2007b) have found the same phenomenon. Complex active biological processes of test organisms are considered to be responsible for some causes of this phenomenon (Huckins et al. 2006). Moreover, a few of high-ring PAHs were not detected in our fish samples at certain sites but were all detected in TECAMs (Table 1). This observation was consistent with this phenomenon, and showed that the contaminants which the fish were exposed to could not be detected by fish tissue but could by TECAM.

Because TECAMs uptake HOCs in similar patterns with biomonitoring organisms, and only sample bioavailable, dissolved phase of the contaminants in water (Ke et al. 2007b), the marriage of TECAMs and the H4IIE cell bioassay could simulate the processes of first accumulation of contaminants and later induction of AhR-mediated effect. Water samples from the wetland were found to have much higher levels of TEQs with the range of 9.4–23.7 pg/L than

**Table 1** Concentrations of OCPs and PAHs in fish (ng/g lipid,  $n = 3$ ) and their logBAFs

Compounds	Site 2 <sup>a</sup>	Site 3	Site 4	Site 5	Site 6	log $K_{ow}$ <sup>b</sup>	logBAF <sup>c</sup>
Lipid content	12.9%	13.8%	12.6%	12.5%	14.1%		
$\alpha$ -HCH	4	2	3	2	3	3.8	$3.3 \pm 0.2$
$\beta$ -HCH	22	5	13	7	8	3.8	$3.3 \pm 0.2$
$\gamma$ -HCH	8	9	7	3	7	3.7	$3.6 \pm 0.2$
$\delta$ -HCH	10	5	5	4	5	4.1	$3.3 \pm 0.1$
Heptachlor	29	9	15	9	11	5.3	$4.5 \pm 0.2$
Aldrin	17	16	11	7	8	5.3	$4.4 \pm 0.2$
$p,p'$ -DDE	44	31	25	23	11	6.1	$5.1 \pm 0.2$
$p,p'$ -DDD	35	39	31	25	18	6.0	$4.8 \pm 0.1$
$p,p'$ -DDT	10	8	7	7	5	6.4	$4.5 \pm 0.1$
$\sum$ OCPs	179	124	117	88	77		
Nap	423	514	463	493	360	3.5	$3.3 \pm 0.1$
Acy	36	30	41	50	37	4.1	$3.3 \pm 0.1$
Ace	90	103	103	120	95	4.2	$3.6 \pm 0.1$
Flu	39	35	29	27	33	4.4	$3.4 \pm 0.1$
Phe	1095	1242	1089	1253	962	4.5	$3.6 \pm 0.1$
Ant	143	107	146	181	123	4.5	$3.7 \pm 0.1$
Fla	702	543	581	549	572	5.2	$3.8 \pm 0.1$
Pyr	239	174	185	173	194	5.3	$4.0 \pm 0.2$
BaA	145	91	98	72	83	5.9	$3.9 \pm 0.2$
Chr	96	142	126	164	104	5.6	$3.9 \pm 0.2$
BbF	59	132	60	43	41	5.8	$3.7 \pm 0.1$
BkF	ND <sup>d</sup>	58	25	56	ND	6.2	$3.7 \pm 0.1$
BaP	54	59	ND	ND	ND	6.4	$4.1 \pm 0.1$
$\sum$ PAHs	3120	3232	2946	3180	2604		

<sup>a</sup> No fish survived at site 1<sup>b</sup>  $K_{ow}$  = octanol/water partition coefficient. Selected values are from Mackay et al. (1992)<sup>c</sup> Mean values of five sites where fish survived<sup>d</sup> ND = below the method detection limit (MDL)

those from control site with TEQ of 4.2 pg/L (Table 3). Meanwhile, obviously high levels of liver EROD activities were induced in fish after exposure at five sites where fish survived compared to controls (Table 3). TEQs of TECAM extracts from the wetland were also higher than those from the control, and corresponded well to both induced EROD activities of fish livers and TEQs of water samples (Table 3 and Fig. 1). This could provide information concerning early-warning sign for potentially harmful biological effects and toxicological significance of the exposed aquatic organisms. Significantly induced EROD activities also indicated of activation of metabolic processes, which is regarded as one of determinative biological processes responsible for the divergence of accumulation between fish and TECAMs.

Furthermore, we used relative potency factors related to 2,3,7,8-TCDD obtained from in vitro recombinant form

of H4IIE cell bioassay (Villeneuve et al. 2002) to estimate TEQ contribution ratio of PAHs as described by Qiao et al. (2006). It was found that chemically calculated TEQs (expressed as TEQ<sub>PAHs</sub>) of AhR-active PAHs could only account for 6%–15% and 17%–47% of total bioassay-derived TEQs for water samples and TECAM samples in the wetland, respectively (Table 3). It appeared likely that in addition to PAHs the fish and TECAMs had been exposed to a mixture of other organic contaminants capable of inducing EROD activity. Further investigations were needed to evaluate the cause and effect.

In conclusion, the great similarity between fish and TECAMs for accumulating HOCs, and the compatibility with the H4IIE cell bioassay together confirm the applicability of TECAM as a good tool for environmental biomonitoring and assessment.

**Table 2** Concentrations of OCPs and PAHs in TECAMs (ng/g lipid, n = 3) and their logTCFs

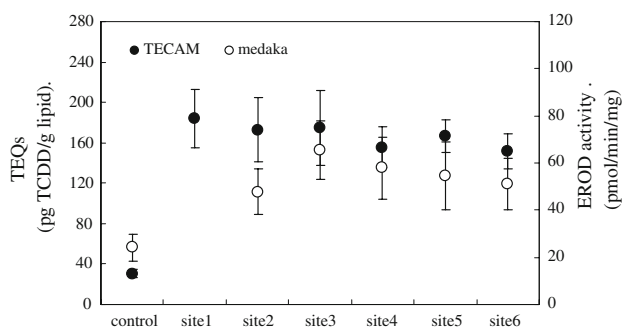
Compounds	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	logTCF
$\alpha$ -HCH	17	13	15	12	10	10	4.0 $\pm$ 0.1
$\beta$ -HCH	43	117	46	58	12	34	4.0 $\pm$ 0.3
$\gamma$ -HCH	19	12	25	15	23	17	4.0 $\pm$ 0.1
$\delta$ -HCH	57	45	51	31	28	41	4.2 $\pm$ 0.1
Heptachlor	81	124	69	111	70	72	5.2 $\pm$ 0.1
Aldrin	26	41	60	41	13	22	4.9 $\pm$ 0.2
<i>p,p'</i> -DDE	148	131	139	94	112	105	5.8 $\pm$ 0.1
<i>p,p'</i> -DDD	219	153	185	229	131	157	5.6 $\pm$ 0.1
<i>p,p'</i> -DDT	124	64	104	36	56	85	5.6 $\pm$ 0.2
$\Sigma$ OCPs	735	700	695	628	457	543	
Nap	1713	1825	1380	1713	1141	1647	3.8 $\pm$ 0.1
Acy	242	103	119	156	139	178	3.9 $\pm$ 0.2
Ace	390	227	188	180	164	193	3.9 $\pm$ 0.1
Flu	341	287	315	293	232	327	4.3 $\pm$ 0.1
Phe	3293	2996	3887	3407	2732	2365	4.1 $\pm$ 0.1
Ant	451	310	792	453	279	281	4.2 $\pm$ 0.2
Fla	2967	1619	2709	2437	2353	2739	4.4 $\pm$ 0.1
Pyr	1980	1297	2032	1804	1160	1585	4.9 $\pm$ 0.1
BaA	1567	1269	1365	1684	1164	1148	5.0 $\pm$ 0.1
Chr	1159	984	1024	955	867	721	4.8 $\pm$ 0.1
BbF	1228	752	1002	866	404	504	4.7 $\pm$ 0.1
BkF	695	465	184	472	458	328	4.6 $\pm$ 0.1
BaP	131	117	103	194	112	84	4.4 $\pm$ 0.1
IcdP	171	87	85	107	59	109	4.8 $\pm$ 0.2
DahA	119	88	76	78	79	84	4.8 $\pm$ 0.1
BghiP	54	49	45	52	46	57	NA <sup>b</sup>
$\Sigma$ PAHs	16501	12475	15306	14851	11388	12351	

<sup>a</sup> Lipid content of TECAM was 7.5%<sup>b</sup> Mean values of all six sites<sup>c</sup> Not available because BghiP's concentrations in water samples were under MDL**Table 3** EROD activities of fish livers and TEQs of water and TECAM samples in the wetland (n = 3)

	TEQs				EROD activities of fish liver (pmol/min/mg)
	TEQs of water (pg TCDD/L)	TEQ <sub>PAHs</sub> of water (pg TCDD/L)	TEQs of TECAMs (pg TCDD/g lipid)	TEQ <sub>PAHs</sub> of TECAMs (pg TCDD/g lipid)	
Procedural blank (or field blank)	0 <sup>a</sup>	0 <sup>a</sup>	3 $\pm$ 0.5	0 <sup>a</sup>	19.7 $\pm$ 5.5
Control	4.2 $\pm$ 0.7	0.7 (17%) <sup>b</sup>	30.5 $\pm$ 4.2	19.4 (64%)	24.1 $\pm$ 5.8
Site 1	17.5 $\pm$ 3.7	1.9 (11%)	184.3 $\pm$ 28.7	87.3 (47%)	NA <sup>c</sup>
Site 2	14.1 $\pm$ 5.9	1.3 (9%)	172.8 $\pm$ 31.8	59.0 (34%)	47.7 $\pm$ 9.7
Site 3	9.4 $\pm$ 4.0	1.4 (15%)	174.4 $\pm$ 37.2	29.1 (17%)	65.3 $\pm$ 12.4
Site 4	23.7 $\pm$ 6.1	1.4 (6%)	155.4 $\pm$ 20.4	60.7 (39%)	57.8 $\pm$ 13.1
Site 5	15.3 $\pm$ 1.8	1.3 (8%)	166.6 $\pm$ 15.8	56.4 (34%)	54.6 $\pm$ 14.2
Site 6	8.6 $\pm$ 1.2	1.0 (12%)	151.8 $\pm$ 17.4	42.3 (28%)	51.1 $\pm$ 10.8

<sup>a</sup> No significantly increased compared to solvent blank (DMSO) for TEQ of water and no PAHs that can induce EROD activity detected for TEQ<sub>PAHs</sub><sup>b</sup> Chemically calculated TEQs from PAHs and PAHs' contribution ratios of total TEQs in brackets<sup>c</sup> Not available because of no fish





**Fig. 1** EROD activities of fish livers and in vitro H4IIE bioassay-derived 2,3,7,8-TCDD equivalents (TEQs) of TECAM extracts in the wetland and control site

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