Identification of Ah Receptor Agonists in Sediment of Meiliang Bay, Taihu Lake, China

MIN QIAO, YINGYING CHEN,
QINGHUA ZHANG, SHENGBIAO HUANG,
MEI MA, CHUNXIA WANG, AND
ZIJIAN WANG*

Research Center for Eco-Environmental Sciences, Chinese
Academy of Sciences, P.O. Box 2871 Beijing 100085, China

Deterioration of ecosystem and water quality in Taihu Lake, the third largest freshwater lake in China, is of great concern in China. However, most evaluations on water and sediment were carried out by chemical analysis, which could hardly gain information on the risk stressors affecting the system. Sediment samples from Meiliang Bay, Taihu Lake were assayed for AhR-mediated EROD induction using a rat hepatoma cell line (H4IIE). The known AhR agonists including PCDD/Fs, PCBs, and PAHs were analyzed by instruments. The cause–effect relationship between the observed EROD activity and chemical concentrations of AhR agonists and their dose–effect relationship were examined. Our results showed that sediment extracts could induce significant AhR effects, and the chemical-derived TCDD equivalents (TEQ_{cal}) were significantly correlated to bioassay-derived TCDD equivalents (TEQ_{bio}) ($R = 0.85$, $p < 0.01$). Among different contributors, PAHs could account for 70–93% and PCDD/Fs could contribute less than 30% of TEQ_{cal} . PCBs exhibited almost no contribution. Therefore, integration of EROD bioassay and chemical analysis provided an effective way for identifying the potential risk stressors. Under this circumstance, a quantitative dose–effect relationship between TEQ_{PAH} and EROD activity could be established, suggesting that the observed AhR effect was mostly derived from PAHs. The implication is that the EROD assay and the identified risk stressors (PAHs) may be used in lieu of detailed environmental analysis to follow the progression of a remediation strategy.

Introduction

Hydrophobic organic compounds with high toxicity and great persistence are widespread in the environment. They are deposited in the sediments by becoming adsorbed on dust particles and various surfaces (1). They can be accumulated and biomagnified in the food chain (2) because of their hydrophobic property. It has been reported that certain planar halogenated and nonhalogenated hydrocarbons, such as some polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), as well as polycyclic aromatic hydrocarbons (PAHs) with chemical structures similar to dioxin can induce the CYP1A1 (3). Since these different compounds appear to act in a similar manner by binding to the hydrocarbon receptor (AhR) transcription factor thereby resulting in a similar type of effects, toxic equivalency factors (TEF) have

been used to compare their relative potency (4). Thus, the total toxic potency of an environmental sample with mixed contaminants of similar mode of action could be expressed in a single TCDD equivalents (TEQ) dose or TEQ_{cal} . The TEQ_{cal} is considered to be an important indicator in ecological risk assessment.

Under the field conditions, chemical analyses of target compounds provide little information on the potential adverse biological effects of complex mixtures. In the past decades, *in vitro* and *in vivo* bioassays based on ethoxyresorufin-*O*-deethylase (EROD) induction were developed. Numerous studies have illustrated the utility of AhR-based cell, such as H4IIE rat hepatoma cell (5), fish liver cell line (6), and others (7), to evaluate the relative ability of a compound to induce EROD activity. This enzymatic assay is a sensitive biomarker to characterize the existence of toxic organic pollutants in a complex environmental media that can induce CYP1A enzyme system. A combination of bioassays and chemical analysis was used to identify the stressors present in sediment samples collected in the field (8). However, the explanation of the results was mainly based on qualitative description and statistical regression analysis of the variables.

Nowadays, dioxin and dioxin-like pollution are of great concern in China. Jin et al. (9) first estimated that the total emission of PCDD/F ranged from 7144 to 13 575 g I-TEQ/yr on the basis of the emission factor approach. On a national basis, PCB levels in all environmental media were relatively low, with little evidence of major contamination in China's main regions and rivers. Some areas, mainly the industrial pollution sites or PCB equipment storage locations, were seriously polluted with the highest PCB residue level of 150 000 ng/g (10). The contamination levels of PAHs seemed to be related to the size and to the industrial activities. Most data were acquired in recent years from south China, and the sediment samples from Macao Harbor had the highest concentrations of 12 741 ng/g (11). However, most of the research is mainly based on the chemical analysis and the relative importance of different contaminants in China environment is unknown yet.

Taihu Lake is the third largest freshwater lake in China and is a typical shallow lake with an average water depth of about 2 m. It is not only a large fishery base and a famous scenery spot, but it is also the potable water source of many cities and towns in eastern China including Shanghai, Suzhou, and Wuxi (12). The Taihu Lake Region (TLR) with high rice production in China provides more than 14% of the gross domestic production (GDP). Meiliang Bay in the northern part of Taihu Lake supplies one-third of the drinking water for Wuxi City, an industrial city located about 2 km northeast of the lake (13). Taihu Lake accepts industrial and agricultural pollutants and sewage mainly from small towns on the west side of the lake. There are several scenic spots and sanitariums around Meiliang Bay, and it also receives much wastewater from Wuxi City. Water quality of Taihu Lake is deteriorating rapidly and poses a threat to water supply and recreation. Therefore, Taihu Lake is listed as one of the key environmental rehabilitation projects of the nation's Green Engineering Plan for the years from the 1990s to the 2010s. In recent years, an ecological restoration project was implemented in Meiliang Bay to ensure a safe drinking water supply.

So far, most of the studies about the lake were focused on the water eutrophication (14, 15), and chemical monitoring was mainly based on the instrument analysis. Genotoxicity in Taihu Lake was reported, but the cause–effect relationship was unclear (12, 13). In addition, most of the contamination

* Corresponding author phone: +86-10-62849140; fax: +86-10-62923543; e-mail: wangzj@cees.ac.cn.

FIGURE 1. Map of study area and sampling sites.

data were obtained from surface water samples in the lake. Because of the hydrophobic nature of most of the toxic compounds, it can be suspected that the sediment of the lake is even more contaminated than the water. This is particularly important because the lake is very shallow and particle resuspension under windy condition is very common (16). Therefore, the hydrophobic chemicals in sediment can affect benthic community and release into water easily.

The objective of this study is to assess AhR-mediated potency in sediment samples and to identify the potential risk stressor based on a combination of EROD assay and chemical analysis. Then, the EROD bioassay and the identified risk stressors (PAHs) may be used in lieu of detailed environmental analysis to follow the progression of a remediation strategy.

Materials and Methods

Sampling and Extraction. Surface sediments were sampled by a core sampler from eight sites of Meiliang Bay, Taihu Lake in July, 2003 with the aid of a global positioning system (GPS; Figure 1). The samples were scooped into aluminum jars that have been prewashed with dichloromethane (DCM) and were kept at -20°C until further analyses.

The freeze-dried and sieved ($425\text{-}\mu\text{m}$ sieve) sediment samples were extracted with 200 mL DCM/acetone (1:1, v/v) in a Soxhlet apparatus. Activated copper was added for desulfurization. The extracts were preconcentrated to about 2 mL on a rotary evaporator (Büchi R-200, Switzerland) and were solvent-exchanged to hexane. The concentrated hexane extracts were evaporated to near dryness under a gentle stream of nitrogen gas, were redissolved in 0.5 mL dimethyl sulfoxide (DMSO), and were stored at -20°C before EROD bioassay.

EROD Bioassay. H4IIE rat hepatoma cells were cultured in Dulbecco's modified eagle medium DMEM (GIBCO Germany) supplemented with 10% (v/v) fetal bovine serum, 100 units/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. The cells were incubated at 80% humidity and 5% CO_2 at 37°C . The routine maintenance of cells was followed according to Donato's method (17, 18). Briefly, stock cultures of H4IIE cells were trypsinized, counted, and diluted into growth medium to achieve the desired number of cells ($1.5 \times 10^4/\text{mL}$). Cells were seeded into 96-well culture plates at 100 μL per well, and the plates were stored at 37°C under 5% CO_2 atmosphere in an incubator (Sanyo, Japan). After 24 h when a monolayer was formed consisting of 70–80% cell cover, the culture medium was removed and was replaced by a culture medium containing TCDD standard solutions or the test solutions. The standard solutions (0.01, 0.03, 0.05, 0.11, 0.22, 0.44, 0.88, 7, 28 ng/mL DMSO, $n = 9$) were prepared by serial dilution of TCDD stock solution that could yield a full dose–response curve of EROD induction. They were included on each plate as positive controls and for calibration purposes. Six concentration levels of test solutions were prepared by 2-fold dilution of each sediment raw extract. In each well,

the final concentration of DMSO was about 0.5%. A 0.5% DMSO solution was also used as solvent control. Each test solution was assayed in triplicate, and three solvent controls and three blanks were assayed simultaneously on each 96-well plate. The edges of the 96-well culture plates were wrapped with Parafilm and were incubated for 72 h at 37°C .

After incubation, EROD enzyme activity was analyzed following the methods of Donato et al. (17) and Wang et al. (18). Briefly, the culture medium was removed and was replaced with 100 μL fresh medium containing 10 μM 7-ethoxyresorufin (Sigma, E3763, United States) as substrate and 10 μM dicumarol (Sigma, M1390, United States). Cells were incubated for another 60 min at 37°C in an atmosphere of 95% air and 5% CO_2 . The reaction was terminated by the addition of 130 μL of methanol. The cell medium was carefully mixed in the titer plate shaker and a 100- μL aliquot from each well was transferred to a new 96-well plate for fluorescence measurement using a microplate reader (TECAN-Genios, Austria). The excitation and emission wavelengths were 535 nm and 590 nm, respectively. Protein concentrations were determined as described by Bradford (19). Resorufin standard curve was analyzed at the same time to convert fluorescent units to picomoles of resorufin. EROD enzyme activity was expressed as mean picomoles of resorufin produced per minute per milligram of microsomal protein ($\text{pmol min}^{-1} \text{mg protein}^{-1}$).

Data were analyzed by a sigmoid nonlinear curve-fitting module. The function is a four-parameter logistic equation (20, 21):

$$Y = \frac{A - D}{1 + (X/C)^B} + D$$

where Y is the EROD response, X is the inducer dose, A is the maximum EROD response, B is the slope coefficient, C is the EC_{50} , and D is the minimum EROD response. EC_{50} is the concentration of TCDD eliciting 50% of the maximal inducible EROD activity. The bioassay-derived TCDD equivalents (expressed as TEQ_{bio}) in sediment samples were then obtained by comparing with the dose–response curve of the standard solution on the same plate. If necessary, the extract of sediment sample was diluted to fit the linear part of the TCDD dose–response curve.

Blanks that extracted with no sediment were used to exclude EROD induction during the working procedure because of experimental conditions. The mean solvent control response was subtracted from both sample and standard responses on a plate-by-plate basis. The detection limit for the H4IIE assay was 18.5 fg TCDD/well, which corresponds to an activity equal to 3 times the standard deviation of the mean solvent control response.

Chemical Analysis. For PAHs analysis, Soxhlet extracts were subjected to cleanup on a silica gel/aluminum oxide column. PAHs fraction was obtained by elution with 70 mL of a mixture of hexane:DCM (7:3, v/v). The eluates were evaporated and were dissolved in hexane for GC-MS analysis. (22). For PCBs and PCDD/Fs analysis, the sample was cleaned up by multilayered silica gel, acid alumina, and florisil chromatographic columns and was analyzed by HRGC/HRMS on an Agilent 6890 gas chromatography coupled with an Autospec Ultima mass spectrometer (Waters Micromass, Manchester, United Kingdom) (23). All data were subjected to strict quality control procedures to meet EPA requirements for field research. Phenanthrene- d_{10} and ^{13}C -labeled surrogate standards were spiked to determine recoveries. Blanks were analyzed to check for interference or contamination arising from solvents or glassware during the working procedure. Detailed instrumental conditions and method evaluation are described elsewhere (22, 23).

TABLE 1. Concentrations^a and the Calculated TEQs for PAHs, PCDD/Fs, and PCBs in Sediments from Meiliang Bay, Taihu Lake^b

chemical	TEF/REP ^c	concentrations at sampling sites							
		1	2	3	4	5	6	7	8
B[a]A	0.0000014	140.8	221.5	303.5	288.8	137.6	296.0	227.4	294.7
Chry	0.0000016	93.6	140.5	201.0	193.0	113.9	195.0	152.2	186.9
B[b]F	0.000004	284.2	440.5	630.7	612.3	316.5	612.1	501.9	565.6
B[k]F	0.00011	84.0	137.1	195.0	184.7	96.0	175.7	150.7	178.2
B[a]P	0.0000013	196.4	310.2	476.1	382.1	134.7	422.6	332.8	448.8
I[cd]P	0.000013	271.9	429.6	615.4	575.5	275.3	588.6	509.7	527.8
D[ah]A	0.000004	6.5	68.7	109.1	91.1	44.3	91.5	76.8	98.0
TEQ _{PAH}		14.5	23.6	33.8	31.8	16.1	31.1	26.5	30.4
2378-TCDF	0.1	2.1	1.3	2.5	0.8	2.4	2.6	2.2	2.4
12378-PeCDF	0.05	1.2	1	1.6	1.2	1.9	1.8	1.4	0.9
23478-PeCDF	0.5	1.6	0.4	2	1.5	0.4	0.4	0.5	0.7
123478-HxCDF	0.1	5	0.8	1.2	2.5	3.1	1.8	1.6	0.8
123678-HxCDF	0.1	2.6	1	1.7	1.4	2.3	1.7	1.1	0.7
234678-HxCDF	0.1	0.9	0.6	1.3	1.5	1.6	1.9	1.2	0.5
123789-HxCDF	0.1	0.5	0.7	0.4	0.3	0.4	0.2	0.2	0.3
1234678-HpCDF	0.01	7.3	4.7	6.6	7.6	6.6	7.4	5.7	6.8
1234789-HpCDF	0.01	0.8	0.2	0.5	0.5	1.6	2.9	0.5	0.2
OCDF	0.0001	10.1	6.4	11	10.9	6.3	8.6	7.6	10.6
2378-TCDD	1	1	0.5	0.4	0.7	0.8	0.3	0.2	0.3
12378-PeCDD	1	2	0.8	1	0.9	0.3	0.2	0.4	0.6
123478-HxCDD	0.1	3	1.5	0.8	0.9	0.7	0.8	0.6	0.3
123678-HxCDD	0.1	1.3	1.4	1.4	0.7	0.9	0.9	0.7	0.3
123789-HxCDD	0.1	4	1.5	1.5	0.7	2.1	2.5	0.8	0.7
1234678-HpCDD	0.01	9.2	6.6	12.1	8.2	25.3	8.4	8.5	9.2
OCDD	0.0001	79.6	77.2	111.1	87.6	203.3	86.5	94.9	94.1
TEQ _{PCDD/F}		6.0	2.6	3.8	3.5	3.1	2.2	1.9	2.1
PCB-77	0.0001	25.3	48.6	35.8	31.1	25.0	27.5	21.1	24.3
PCB-81	0.0001	1.6	3.3	0.6	1.4	1.2	0.7	1.0	0.8
PCB-105	0.0001	28.0	57.9	38.8	35.1	27.3	31.8	24.5	29.3
PCB-114	0.0005	4.3	7.7	6.0	5.2	3.1	1.1	2.7	1.1
PCB-118	0.0001	68.4	143.9	93.3	86.2	65.3	68.8	59.4	67.1
PCB-123	0.0001	14.3	23.0	20.0	16.2	11.6	13.5	9.4	12.1
PCB-126	0.1	2.4	3.8	3.2	2.6	2.8	2.5	1.9	2.7
PCB-156	0.0005	7.9	17.7	10.9	10.3	7.6	8.4	6.8	7.3
PCB-157	0.0005	3.1	4.3	4.3	3.1	2.7	3.0	2.3	2.4
PCB-167	0.00001	3.5	8.1	4.9	4.7	3.0	4.1	3.0	3.4
PCB-169	0.01	0.5	0.6	0.9	0.4	0.5	0.4	0.5	0.4
PCB-189	0.0001	0.9	1.6	1.4	1.2	1.1	1.1	1.0	1.0
TEQ _{PCB}		0.3	0.4	0.4	0.3	0.3	0.3	0.2	0.3
TEQ _{cal} ^d		20.8	26.6	37.9	35.6	19.5	33.6	28.6	32.8
TEQ _{bio}		23.2	32.0	35.8	35.1	17.8	27.4	26.5	28.9

^a Concentration is expressed as ng/g dw for PAH; pg/g dw for PCDD/Fs, PCBs, and TEQs. ^b Detected concentrations are presented in bold type. Values in plain type mean not detected and are presented by limit of determination (LOD). WHO-TEQs were calculated by assuming that all values of the different congeners less than the LOD were equal to the LOD. ^c TEF/REP values were obtained from references 24, 25, 27. ^d TEQ_{cal} is the sum of the TEQ_{PCB}, TEQ_{PCDD/F}, and TEQ_{PAH}.

The TEQ_{cal} was obtained as the sum of TEQ for an individual compound by assuming additive responses to chemicals in the mixture. The World Health Organization (WHO) has provided TEFs for individual chemicals (24) that include 7 PCDDs, 10 PCDFs, as well as 12 PCBs. The TEQs for PCDDs, PCDFs, and PCBs were calculated by multiplying the measured concentration by the corresponding TEF. PAHs are commonly metabolized through cytochrome P4501A1 activity, and prolonged exposure may yield typical dioxin-like effects. Several PAHs, including benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), benzo[b]fluoranthene (B[b]F), chrysene (Chry), benz[a]anthracene (B[a]A), indeno[1,2,3-cd]pyrene (I[cd]P), and dibenz[a,h]anthracene (D[ah]A), have been shown to elicit dioxin-like responses or to induce cytochrome P4501A1 activity in vitro (25, 26). Several researchers have reported that the TEF/relative potencies (REPs) to TCDD for selected PAHs derived from various cell lines and the reported TEF/REP values for PAHs vary significantly in the published literature. Therefore, in the present study, REPs related to TCDD and obtained from bioassay using in vitro recombinant form of H4IIE cells (25, 27) have been used to estimate TEQs contributed by PAHs (Table 1).

Results and Discussion

TEQ_{bio} from EROD Assay. All the sediment extracts induced a significant response in the H4IIE cells (Table 1). The TCDD equivalents ranged from 17.8 to 35.8 pg TEQ_{bio}/g-dw. The EROD-inducing potency was higher for sampling sites close to the nearby waterworks and was lower for sampling sites in the middle of the bay.

Concentration Levels and TEQ_{cal} from Chemical Analysis. The chemical data were shown in Table 1. The levels of seven dioxin-like PAHs were high when compared to those obtained from some other rivers in China, such as Shiziyang, Xijiang River, and Lingding Bay (28). Nevertheless, these were lower than those found in contaminated sediments from Germany (29) and in Boston Harbor sediments from the United States (30). In comparison, the levels of total PCDD/Fs were much lower than those in sediments from Ya-Er Lake (China) (31) and those in Passaic River sediment (United States) (32). In the case of PCBs, the concentrations were 2 or 3 orders of magnitude lower than those from the sediment of the river Spittelwasser in the industrial region of Bitterfeld (Germany) (33) and those in the Ariake Sea (Japan) (27). From chemical data, the TEQ_{cal} ranged between 19.5 and 37.9 pg

TABLE 2. Comparison of PAH Diagnostic Ratios of Meiliang Bay, Taihu Lake with Those in Published Literatures

PAH source	B[b]F/B[k]F	I[cd]P/BghiP	B[a]A/Chry	Phe/An	Flu/Pyr	Flu/(Flu+Pyr)	LMW/HMW
pyrolytic ^a origin	NA ^c	NA	>1	<10	>1	>0.5	low
petrogenic ^a origin	NA	NA	<1	>15	<1	<0.5	high
automobiles ^b	1.26 ± 0.19	0.33 ± 0.06	0.53 ± 0.06	NA	NA	NA	NA
coal/coke ^b	3.70 ± 0.17	1.09 ± 0.03	1.11 ± 0.06	NA	NA	NA	NA
wood ^b	0.92 ± 0.16	0.28 ± 0.05	0.79 ± 0.13	NA	NA	NA	NA
smelters ^b	2.69 ± 0.20	1.03 ± 0.15	0.60 ± 0.06	NA	NA	NA	NA
this study	3.17–3.48	1.20–1.41	1.21–1.58	2.97–5.36	1.03–1.29	0.51–0.56	0.05–0.08

^{a,b} From references 38–40. ^c NA, not available.

FIGURE 2. Response of in vitro EROD assay with H4IIE rat hepatoma cells to 2,3,7,8-TCDD standards and sediment extracts. Doses expressed as ng TEQ/mL media present in the test solution for TCDD standards or for dioxin-like PAHs. Responses expressed as EROD activity.

TEQ/g-dw. Therefore, the sediment samples could be judged as moderately to heavily polluted by dioxin-like chemicals by comparing with the safe sediment value of 20 pg TEQ/g-dw. (34). By chemical analysis, it was observed that the TEQ contributed by PCDD/F congeners ranged from 1.9 to 6.0 pg TEQ/g-dw, that by PCBs ranged from 0.2 to 0.4 pg TEQ/g-dw, and that by PAHs ranged from 14.5 to 33.8 pg TEQ/g-dw. Therefore, it appears that the TEQ_{cal} was mainly contributed by dioxin-like PAHs in the sediment. The TEQ_{cal} was about the same order of magnitude as TEQ_{bio} (Table 1).

The contribution of halogenated aromatic hydrocarbons (HAHs) to the TEQ_{cal} was less than 20% and that of PAHs was more than 80% except for site 1. Among the different types of dioxins, PCDDs accounted for 45.5–71.0% TEQ_{PCDD/F} throughout the samples, where congener 1,2,3,7,8-PeCDD and 2,3,7,8-TCDD accounted for 22.5–50.9% TEQ_{PCDD/F}. Contribution of PCB congeners was almost negligible. Nevertheless, PCB126 contributed more than 88% of TEQs from all PCB congeners throughout the sediment samples. PAHs contributed the greatest proportion to the TEQ_{cal} because of their relatively high levels in the sediment samples. Among different PAHs, B[k]F accounted for approximately 62.2–65.5%, followed by I[cd]P and B[b]F for 22.2–25.0% and 7.4–7.9%, respectively. There was a significant dose-dependent relationship between TEQ_{cal} and EROD activity of sediment raw extracts ($R = 0.85$, $P < 0.01$, $n = 8$). A nearly linear dose–effect relationship existed between TEQs for dioxin-like PAHs and EROD activity ($R = 0.80$, $P < 0.05$, $n = 8$). Furthermore, the TEQ_{PAH} value calculated from the REPs and concentrations was in the linear part of the calibration curve for standard solutions (Figure 2) which confirmed that PAHs could be the stressors for the observed potency.

Comparison between TEQ_{cal} and TEQ_{bio} provided a means to evaluate the importance of the known target contaminants

in the complex mixture. TEQ_{bio} was significantly correlated with TEQ_{cal} and TEQ_{PAH}, although instrumental values for most of the sites were higher. There were no significant correlations between TEQs contributed by PCDD/Fs or by PCBs and the TEQ_{bio}. Many investigators have found that the polyaromatic fractions of organic extracts from aquatic sediment had high potency in inducing EROD activity (35–37). However, the attempts to attribute the potency to identified priority PAHs had often failed (35–37). The sediment from a large commercial harbor of Onsan Bay, Korea, induced significant AhR-mediated gene expression on the basis of the H4IIE-luc in vitro cell bioassay (8), which could not be explained by the 16 priority PAHs. Similarly, in the work carried out on Lake Shihwa, Korea (35), and on creek Spittelwasser in Saxony-Anhalt (Germany) (33), strongly induced AhR-mediated gene expression in H4IIE-luc cell and in hepatic cell of rainbow trout appeared to have been caused by undetectable compounds. In the present work, the dioxin-like PAHs could account for most of the CYP1A induction potency. In view of the potential uncertainty in the TEQ estimates, our results suggested that in certain circumstances the known AhR agonists could account for the potency observed in bioassay.

Since PAHs were the greatest contributors to dioxin-like activity in sediments, we further evaluated the composition and sources of PAHs. Most components of 16PAHs were present in all sediment samples. The predominance of four- and five-ring aromatic hydrocarbons in sediment samples was clearly observed. Numerous studies have reported that isomer ratios (38–40) are useful indicators of PAH sources. The results of this study suggested that the predominantly pyrolytic origin for PAHs in the surface sediments and for the PAH profile corresponds most closely with that associated with coal combustion (Table 2).

Acknowledgments

We gratefully acknowledge the support of National Basic Research Program of China (2003CB415005) and National Natural Science Foundation of China (20337020, 40471129) for conducting this research. Thanks are also due to Dr. Shahamat U. Khan in George Mason University, United States, for carefully revising the manuscript.

Literature Cited

- (1) Wang, L. C.; Lee, W. J.; Lee, W. S.; Ping, G.; Chien, C.; Tsai, P. J. Characterizing the emissions of polychlorinated dibenzo-*p*-dioxins and dibenzofurans from crematories and their impacts to the surrounding environment. *Environ. Sci. Technol.* **2003**, *37*, 62–67.
- (2) Wu, W. Z.; Schramm, K. W.; Kettrup, A. Bioaccumulation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in the foodweb of Ya-Er Lake, area, China. *Water Res.* **2001**, *35*, 1141–1148.
- (3) Fent, K. Fish cell lines as versatile tools in ecotoxicology: assessment of cytotoxicity, cytochrome P4501A induction potential and estrogenic activity of chemicals and environmental samples. *Toxicol. in Vitro* **2001**, *15*, 477–488.
- (4) Toyoshima, H.; Walker, N. J.; Bailer, A. J.; Portier, C. J. Evaluation of toxic equivalency factors for induction of cytochromes P450

- CYP1A1 and CYP1A2 enzyme activity by dioxin-like compounds. *Toxicol. Appl. Pharmacol.* **2004**, *194*, 156–168.
- (5) Tillitt, D. E.; Giesy, J. P. Characterization of the H4IIE rat hepatoma cell bioassay as a tool for assessing toxic potency of planar halogenated hydrocarbons in environmental samples. *Environ. Sci. Technol.* **1991**, *25*, 87–92.
 - (6) Miller, K. A.; Addison, R. F.; Bandiera, S. M. Hepatic CYP1A levels and EROD activity in English sole: biomonitoring of marine contaminants in Vancouver Harbour. *Mar. Environ. Res.* **2003**, *57*, 37–54.
 - (7) Kennedy, S. W.; Lorenzen, A.; Norstrom, R. J. Chicken embryo hepatocyte bioassay for measuring cytochrome P4501A-based 2,3,7,8-tetrachlorodibenzop-dioxin equivalent concentrations in environmental samples. *Environ. Sci. Technol.* **1996**, *30*, 706–715.
 - (8) Koh, C. H.; Khim, J. S.; Villeneuve, D. L.; Kannan, K.; Giesy, J. P. Analysis of trace organic contaminants in sediment, pore water, and water samples from onsan bay, korea: instrumental analysis and in vitro gene expression assay. *Environ. Toxicol. Chem.* **2002**, *21*, 1796–1803.
 - (9) Jun, J.; Hao, P.; Tang, X. Y. An inventory of potential PCDD and PCDF emission sources in the mainland of China. *Organohalogen Compd.* **2004**, *66*, 852–858.
 - (10) Xing, Y.; Lu, Y. L.; Dawson, R. W.; Shi, Y. J.; Zhang, H.; Wang, T. Y.; Liu, W. B.; Ren, H. C. A spatial temporal assessment of pollution from PCBs in China. *Chemosphere* **2005**, *60*, 731–739.
 - (11) Mai, B. X.; Qi, S. H.; Zeng, E. Y.; Yang, Q. S.; Zhang, G.; Fu, J. M.; Sheng, G. Y.; Peng, P. A.; Wang, Z. S. Distribution of polycyclic aromatic hydrocarbons in the coastal region off Macao, China: assessment of input sources and transport pathways using compositional analysis. *Environ. Sci. Technol.* **2003**, *37*, 4855–4863.
 - (12) Kong, Z. M.; Zang, Y.; Wu, Q. L. Monitoring the genotoxicity of Lake Taihu, using two kinds of micronucleus tests. *Environ. Pollut.* **1998**, *99*, 279–283.
 - (13) Shen, L.; Lin, G. F.; Tan, J. W.; Shen, J. H. Genotoxicity of surface water samples from Meiliang Bay, Taihu Lake, Eastern China. *Chemosphere* **2000**, *41*, 129–132.
 - (14) Hu, W. P.; Salomonsen, J.; Xu, F. L.; Pu, P. M. A model for the effects of water hyacinths on water quality in an experiment of physico-biological engineering in Lake Taihu, China. *Ecol. Modell.* **1998**, *107*, 171–188.
 - (15) Zuo, Q.; Lu, C. A.; Zhang, W. L. Preliminary study of phosphorus runoff and drainage from a paddy field in the Taihu Basin. *Chemosphere* **2003**, *50*, 689–694.
 - (16) Shen, J. H.; Gutendorf, B.; Vahl, H. H.; Shen, L.; Westendorf, J. Toxicological profile of pollutants in surface water from an area in Taihu Lake, Yangtze Delta. *Toxicology* **2001**, *166*, 71–78.
 - (17) Donato, M. T.; Gómez-Lechón, M. J.; Castell, J. V. A microassay for measuring cytochrome P4501A1 and P450IIB1 activities in intact human and rat hepatocytes cultured on 96-well plates. *Anal. Biochem.* **1993**, *213*, 29–33.
 - (18) Wang, C. X.; Wang, Y.; Kiefer, F.; Yediler, A.; Wang, Z. J.; Kettrup, A. Ecotoxicological and chemical characterization of selected treatment process effluents of municipal sewage treatment plant. *Ecotoxicol. Environ. Saf.* **2003**, *56*, 211–217.
 - (19) Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
 - (20) DeLean, A.; Munson, P. J.; Guardabasso, V.; Rodbard, D. *A User's Guide to ALLFIT, Simultaneous fitting of families of sigmoidal dose response curves using the four-parameter logistic equation*; Laboratory of theoretical and physical biology national institute of child health and human development, National Institutes of Health: Bethesda, MD, 1988.
 - (21) Wan, X. Q.; Ma, T. W.; Wu, W. Z.; Wang, Z. J. EROD activities in a primary cell culture of grass carp (*Ctenopharyngodon idellus*) hepatocytes exposed to polychlorinated aromatic hydrocarbonas. *Ecotoxicol. Environ. Saf.* **2004**, *58*, 84–89.
 - (22) Qiao, M.; Wang, C. X.; Huang, S. B.; Wang, D. H.; Wang, Z. J. Composition, sources and potential toxicological significance of PAHs in the surface sediments of the Meiliang Bay, Taihu Lake, China. *Environ. Int.* **2006**, *32*, 28–33.
 - (23) Zhang, Q. H.; Jiang, G. B. Polychlorinated dibenzo-p-dioxins/furans and polychlorinated biphenyls in sediments and aquatic organisms from the Taihu Lake, China. *Chemosphere* **2005**, *61*, 314–322.
 - (24) Van den Berg, M.; Birnbaum, L.; Bosveld, A. T. C.; Brunstrom, B.; Cook, P.; Feeley, M.; Giesy, J. P.; Hanberg, A.; Hasegawa, R.; Kennedy, S. W.; Kubiak, T.; Larsen, J. C.; van Leeuwen, F. X. R.; Liem, A. K. D.; Nolt, C.; Peterson, R. E.; Poellinger, L.; Safe, S.; Schrenk, D.; Tillitt, D.; Tysklind, M.; Younes, M.; Wærn, F.; Zacharewski, T. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* **1998**, *106*, 775–792.
 - (25) Villeneuve, D. L.; Khim, J. S.; Kanna, K.; Giesy, J. P. Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. *Environ. Toxicol.* **2002**, *17*, 128–137.
 - (26) Bols, N. C.; Schirmer, K.; Joyce, E. M.; Dixon, D. G.; Greenberg, B. M.; Whyte, J. J. Ability of polycyclic aromatic hydrocarbons to induce 7-ethoxyresorufin- o-deethylase activity in a trout liver cell line. *Ecotoxicol. Environ. Saf.* **1999**, *44*, 118–128.
 - (27) Nakata, H.; Sakai, Y.; Miyawaki, T.; Takemura, A. Bioaccumulation and toxic potencies of polychlorinated biphenyls and polycyclic aromatic hydrocarbons in tidal flat and coastal ecosystems of the Ariake Sea, Japan. *Environ. Sci. Technol.* **2003**, *37*, 3513–3521.
 - (28) Mai, B. X.; Fu, J. M.; Sheng, G. Y.; Kang, Y. H.; Lin, Z.; Zhang, G.; Min, Y. S.; Zeng, E. Y. Chlorinated and polycyclic aromatic hydrocarbons in riverine and estuarine sediments from Pearl River Delta, China. *Environ. Pollut.* **2002**, *117*, 457–474.
 - (29) Brack, W.; Schirmer, K. Effect-directed identification of oxygen and sulfur heterocycles as major polycyclic aromatic cytochrome P4501A-inducers in a contaminated sediment. *Environ. Sci. Technol.* **2003**, *37*, 3062–3070.
 - (30) Wang, X. C.; Zhang, Y. X.; Chen, R. F. Distribution and partitioning of polycyclic aromatic hydrocarbons (PAHs) in different size fractions in sediments from Boston Harbor, United States. *Mar. Pollut. Bull.* **2001**, *42*, 1139–1149.
 - (31) Wu, W. Z.; Xu, Y.; Schramm, K. W.; Kettrup, A. Persistence of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) in Ya-Er Lake area, China. *Environ. Int.* **2001**, *26*, 323–326.
 - (32) Pruell, R. J.; Taplin, B. K.; McGovern, D. G.; McKinney, R.; Norton, S. B. Organic contaminant distributions in sediments, polychaetes (*Nereis virens*) and American lobster (*Homarus americanus*) from a laboratory food chain experiment. *Mar. Environ. Res.* **2000**, *49*, 19–36.
 - (33) Brack, W.; Segner, H.; Möder, M.; Schüürmann, G. Fixed-effect-level toxicity equivalents—a suitable parameter for assessing ethoxyresorufin-o-deethylase induction potency in complex environmental samples. *Environ. Toxicol. Chem.* **2000**, *19*, 2493–2501.
 - (34) Evers, E. H. G.; Laane, R. W. P. M.; Groeneveld, G. J. J.; Olie, K. Levels, temporal trends and risk of dioxins and related compounds in the Dutch aquatic environment. *Organohalogen Compd.* **1996**, *28*, 117–122.
 - (35) Khim, J. S.; Villeneuve, D. L.; Kannan, K.; Koh, C. H.; Giesy, J. P. Alkylphenols, polycyclic aromatic hydrocarbons, and organochlorines in sediment from Lake Shihwa, Korea: instrumental and bioanalytical characterization. *Environ. Toxicol. Chem.* **1999**, *18*, 2424–2432.
 - (36) Khim, J. S.; Kannan, K.; Villeneuve, D. L.; Koh, C. H.; Giesy, J. P. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 1. instrumental analysis. *Environ. Sci. Technol.* **1999**, *33*, 4199–4205.
 - (37) Khim, J. S.; Kannan, K.; Villeneuve, D. L.; Koh, C. H.; Giesy, J. P. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 2. in vitro gene expression assays. *Environ. Sci. Technol.* **1999**, *33*, 4206–4211.
 - (38) Baumard, P.; Budzinski, H.; Garrigues, P.; Dizer, H.; Hansen, P. D. Polycyclic aromatic hydrocarbons in recent sediment and mussels (*Mytilus edulis*) from the Western Baltic Sea: occurrence, bioavailability and seasonal variations. *Mar. Environ. Res.* **1999**, *47*, 17–47.
 - (39) Dickhut, R. M.; Canuel, E. A.; Gustafson, K. E.; Liu, K.; Arzayus, K. M.; Walker, S. E.; Edgecombe, G.; Gaylor, M. O.; Macdonald, E. H. Automotive sources of carcinogenic polycyclic aromatic hydrocarbons associated with particulate matter in the Chesapeake Bay region. *Environ. Sci. Technol.* **2000**, *3*, 4635–4640.
 - (40) Soclo, H. H.; Garrigues, P. H.; Ewald, M. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas. *Mar. Pollut. Bull.* **1999**, *40*, 387–396.

Received for review July 26, 2005. Revised manuscript received October 25, 2005. Accepted October 28, 2005.

ES051456P