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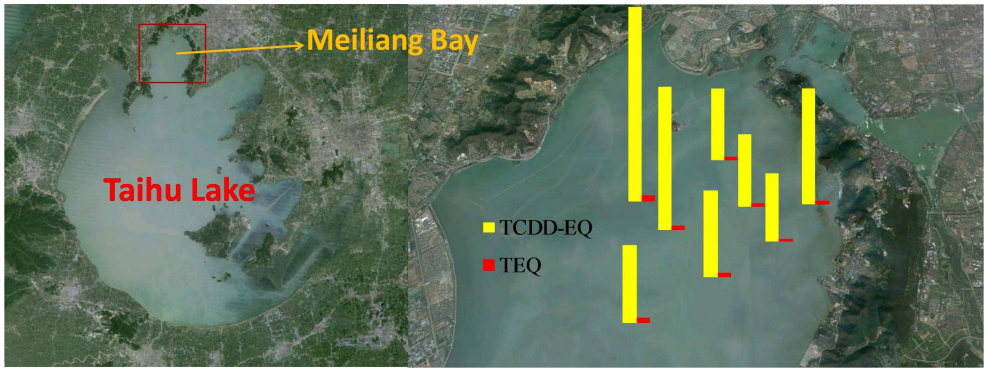
Graphical Abstract



Greater concentrations of TCDD-EQs and lesser concentrations of TEQs were found and a gradual decrease of AhR potency was confirmed.

AhR-mediated activities and compounds in sediments of Meiliang Bay, Taihu Lake, China determined by in Vitro bioassay and instrumental analysis

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Abstract: To better understand the historical deterioration and the recent restoration of the ecosystem in Meiliang Bay, Taihu Lake, the third largest freshwater lake in China, extracts of nine surficial sediments were analyzed for their ability to induce dioxin-like activities *in vitro*. The sediment samples were tested as raw extracts (REs), acid-treated extracts (AEs) and fractionated extracts (FEs), respectively. Based on the initial screening of the REs, all of the sediment samples exhibited significant dioxin-like activity in H4IIE-*luc* bioassay. Calculated from the raw extracts, the sediment contained 359-1018 pg TCDD-EQ (2,3,7,8-tetrachlordibenzo-p-dioxin toxicity equivalents) g^{-1} DW (dry weight) derived from concentration for 20% of maximal effect (EC20). Instrumental analysis of the FE samples revealed that moderately polar (F2) and maximum polarity (F3) fractions were responsible for majority of the significant reporter gene expression in H4IIE-*luc* bioassay. Sediment associated with F2 and F3 samples was estimated to contain 94–260 pg TCDD-EQ g^{-1} DW and 26–106 pg TCDD-EQ g^{-1} DW respectively. Four F1 samples were either cytotoxic or caused morphological changes in H4IIE-*luc* cells. Similar toxicity was also observed in their corresponding REs and AEs, which indicated that the matters causing cytotoxicity were acid stable and cannot be removed by Florisil adsorption process. By use of H4II-*luc* specific relative potencies (RePs), the toxicity equivalents (TEQs) of dioxin-like polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDFs), and polycyclic aromatic hydrocarbons (PAHs) with respect to TCDD were estimated to be 0.7-1.6 pg g^{-1} DW, 2.7-6.9 pg g^{-1} DW and 11.1-22.9 pg g^{-1} DW, respectively. The total instrumental-derived TEQs (12.4-30.9 pg g^{-1} DW) in sediment appeared to account for only a small portion of the dioxin-like responses observed in bioassay. Compared to the results of other studies, dioxin-like activities have decreased significantly in the past decade, which indicates that the large national ecological remediation project carried out in Meiliang Bay, Taihu Lake has been successful.

Keywords: Bioassay, H4IIE-*luc*, Dioxin-like, PCBs, PAHs

Introduction

Persistent organic pollutants (POPs) released from sources can easily pass into sediments, soils, waters and air, as well as a portion entered in biota along food chains. Due to their hydrophobic and persistent natures, they are predominantly sorbed to organic matters in soils and sediment. As a result, the ecological risks posed by persistent organic pollutants are of great concerns[1-4]. Among the POPs, some known persistent halogenated organic contaminants, such as PCBs, PCDDs, PCDFs and certain PAHs having planar conformations similar to 2,3,7,8-TCDD, are able to elicit a wide variety of adverse effects in organisms mediated by an aryl hydrocarbon receptor (AhR)–dependent mechanism[5]. Analytical methods for the characterization of PCBs, PCDD/PCDFs and PAHs in sediments have been well developed[1,4,6-9]. However, instrumental analysis alone provides little information regarding the integrated biological potency. Therefore, bioassay is often employed to supplement the deficiency of chemical analysis in characterizing toxicity of environmental samples. In the past two decades, recombinant rat hepatoma cells (H4IIE-*luc*) with a stable transfected luciferase reporter gene under the control of dioxin-responsive elements (DREs) were utilized to characterize sediment samples for their capacity and potency to induce AhR-mediated (so called dioxin-like) responses *in vitro* [6,7,10-13].

Taihu Lake, the third largest freshwater lake in China, locates among 30º55'–31º33'N and 119º55'–120º36'E with a land area of 2338 km^2 and an average water depth of 1.9 m. Due to decades of intensive industrial development and explosion of city population, Taihu Lake has been seriously polluted by domestic and industrial wastewater effluent and the water quality deteriorated rapidly from 1970 to 2000s[8,14-16]. Meiliang Bay, a crucial sublake located in the northern part of Taihu Lake, supplies one-third of the drinking water of Wuxi City, a famous industrial city with a population over 1 million[8,15]. The sublake suffers from extensive eutrophication and serious blooms of cyanobacteria occurred frequently[16]. Moreover, a variety of AhR-active compounds have been found in Meiliang Bay, including both persistent organic pollutants (PCBs, PCDD/PCDFs) and PAHs[2,8,14-18]. To protect the source of drinking water in the Meiliang Bay, a large national project was carried out from 2003 to 2005 using ecological principles to improve water quality[2,16]. However, the performance of the ecological remediation approach was difficult to evaluate due to the lack of

consistent toxicity assessment approaches, as well as the complex matrix effects attributed to different physic-chemical and biological properties[2,8,13,18-21]. Usually, the bioassay-derived TCDD-EQs were compared with the instrumental-derived TEQs to better understand the contribution of known AhR-mediated compounds[6,12,21-23]. TEQ values were simply calculated by multiplying the measured concentrations of the AhR active compounds and their corresponding toxic equivalency factors (TEFs) and/or RePs. Therefore, the selection of suitable TEF/ReP is crucial for the TEQ calculation. The World Health Organization (WHO) TCDD equivalency factors for PCDD/PCDFs and dioxin-like PCBs are commonly used for estimating of relative potencies in mixtures[24,25]. However, WHO TEFs are limited in comparing the results of H4IIE-*luc* bioassay to the TEQs estimated from the instrumental analyses due to the inconsistence of chemically derived response factors from a number of endpoints for several distinct species and the observed response factors in bioassay[26]. Similarly, assay-specific RePs are necessary for mass balance/potency-balance analyses involving PAHs to achieve greater accuracy[27].

The overarching objectives of the present study were to 1) determine the total dioxin-like activities in the sediment of Meiliang Bay prior to the completion of the national ecological remediation project by use of both instrumental and bioanalytical methods; 2) find out a relative accurate TEQ calculation method to compare the current results with other studies to better understand the detoxicification process taken place in Meiliang Bay, Taihu Lake.

2. Materials and methods

2.1. Chemicals and Reagents

The following standards and reagents were utilized in the present study:16 priority PAH standards (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene (Pyr), chrysene (Chry), benzo[*a*]anthrene (B[*a*]A), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), indeno[1,2,3-*cd*]pyrene (I[*cd*]P), dibenzo[*a,h*]anthracene (D[*ah*]A), and benzo(*g,h,i*)perylene (B[*ghj*]P)) (Sigma, St. Louis, MO, USA), 17 PCDD/PCDFs, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin(2,3,7,8-TCDD), 12 dioxin-like (co-planar) PCB congers (IUPAC Nos.77, 105, 114, 118, 123, 126, 128, 156, 157, 167, 169, 189), anhydrous sodium sulfate(Na_2SO_4), cooper powder and activated florisil,

60-100 mesh (Sigma, St. Louis, MO, USA), high purity n-hexane, dichloromethane (DCM), methanol (Pesticide or HPLC grade, Fisher Scientific, USA)

2.2. Sample Collection

Surficial sediments (0-5 cm) were collected by use of a stainless steel grab sampler from nine locations of Meiliang Bay in August 2004 (Fig.1). Among the sites, Site 1 was close to the source water inlet of Chongshan Drinking Water Plant, Wuxi City. Sediment samples were mixed thoroughly, transferred to hexane-rinsed glass jars and kept on ice during transportation to the laboratory. All sediment samples were frozen at -20 ºC until further analysis.

(Insert Fig.1)

2.3. Sample Extraction and Cleanup

Sediment samples were freeze-dried, and 20 g of each dry sediment sample was then Soxhlet extracted for 18 hr using 400 ml of high purity dichloromethane and hexane (3:1, v/v). Raw extracts (REs) were treated with acid-activated copper powder to remove sulfur and then preconcentrated down to approximately 2.0 ml by rotary evaporation at 39ºC (Buchi, Switzerland)， followed by solvent-exchanging to hexane. The exact volume of 1.0 ml was achieved by evaporating the extracts under a stream of high purity nitrogen (> 99.995%). Two hundred microliter of RE was taken for bioassay and the remaining 0.8 ml of RE were column cleaned up with activated Florisil in accordance with literature method[1]. In brief, the 0.8 ml of each RE sample was fractionated into three fractions through a 10 mm i.d. glass column packed with 10 g of activated Florisil (60-100 mesh; Sigma, St. Louis, Mo, USA). The non-polar fraction (F1), which was eluted with 100 ml of n-hexane contained PCBs and a portion of organochlorine (OC) pesticides PCDD/PCDFs. The mid-polar fraction (F2), which was eluted with 100 ml of n-hexane/dichloromethane (4:1, v/v) contained moderately polar substances including most of the PAHs, a large portion of OC pesticides and the remaining PCDD/PCDFs. Fraction (F3), which was eluted with 100 ml of 50% dichloromethane in high purity methanol contained the most polar substances, including alkylphenols, other polar compounds, such as the degradation products of steroids and some coloring matter. The efficiency of Florisil separation was confirmed by a spike recovery test. Recoveries of the target anayltes ranged from

82% to 105%. Procedural blanks were analyzed every six samples. One hundred microliter of the sediment raw extracts was treated by 98.3% H_2SO_4 to remove PAHs and coloring matter interference for bioassay test.

2.4. Cell culture and bioassay

Detailed procedures for culturing H4IIE-*luc* cells have been described elsewhere[11,12]. Briefly, the H4IIE-*luc* cells were cultured in Dulbecco's Modified Medium (Sigma, D-2902, Sigma, St. Louis, MO, USA) with 10% fetal bovine serum (FBS, Hylone, Logan, UT, USA). Cells were continuously cultured in 100 mm tissue culture plates in an incubator at 37ºC and 5% CO_2 with a humidity of 90%. Fresh culture media were replaced every two to three days. Twenty four hours before dosing, cells were transferred to new media which contained 10% charcoal dextran-treated FBS. Cells were suspended from the tissue culture plate then the 0.25 ml of cells were added to each of the 60 interior wells of a 96-Well ViewPlates™ (Packard Instruments, Meriden, CT, USA) with a concentration of 7.5×10^4 cells ml^{-1}. Prior to dosing, the plates were pre-incubated overnight until full attachment was achieved. 2.5 µl of the appropriate extract and solvent were added to the wells as test and solvent control, respectively. Standard wells received a dilution series of TCDD. Blank wells received no dose. The final concentration of solvent in each well was 1%. Triplicate wells were dosed for each concentration and incubated for 72 hr prior to measuring viability of cells and light produced by expression of the reporter gene luciferase..

Condition of cells was first checked by use of the LIVE/DEAD Viability/Cytotoxicity Kit (Invitrogen, Carlsbad, CA, USA) following the manufacture's procedures. Thereafter, the viability buffer was removed, and cells were rinsed with phosphate buffer solution (PBS) three times prior to adding 75 µl of PBS with Ca^{2+} and Mg^{2+}. Then, 75 µl of reconstituted LucLite substrate was added to each well. The operations were all conducted under subdued light conditions. After incubating for 25 min in dark at room temperature, luciferase activity was measured at 30^{o}C with an automate luminimeter (Dynatech ML 3000 Luminometer; Chantilly, VA, USA). Luciferase activity was interpolated with the TCDD standard curve to determine a percentage of the maximum response observed for TCDD[6]. The bioassay-derived TCDD-EQs were estimated at EC20, EC50 and EC80 to assess whether

assumptions of parallel slopes and equal efficacies had been met[23,28]. To minimize biases caused by not meeting the assumption of parallelism, the EC20 was reported.

2.5. Chemical analysis

Concentrations of PCBs and OC pesticides were determined using a Hewlett-Packard 6890 series II gas chromatograph equipped with sequential dual columns, DB-5 and DB-XLB(60 m length × 0.25 mm i.d. and 0.25 μm film thickness, J&W Scientific Inc., USA) , and with two individual electron capture detector (GC-ECD) operated in splitless mode. The oven temperature program began with an 100ºC hold for 10 min, followed by an increase of 10 ºC min^{-1} to 130 ºC, 1ºC min^{-1} to 255 ºC, 2 ºC min^{-1} to 285 ºC, and a 5-min final hold at 285 ºC. Injector and detector temperatures were set at 250 ºC and 300 ºC, respectively. Helium was used as the carrier gas. This dual column GC-ECD system can enhance the accurate of identification and confirmation of PCBs and OC pesticides. Peaks appeared in both detectors at suitable retention times were selected for quantification. Briefly, concentrations of organochlorines were calculated from the peak area of the sample to a corresponding internal standard adjusted by its response factor. Recoveries of target analytes through this analytical method were 92.2±4.0% for PCBs. Seventeen PCDD/PCDFs and twelve dioxin-like (coplanar) PCBs（Co-PCBs）were further quantified using HRGC/HRMS following the analytical method described elsewhere [8]. Sixteen priority pollutant PAHs, including nine confirmed AhR-mediated ones were measured by a Hewlett Packard 6890 series gas chromatograph coupled with a mass selective detector (GC-MSD). Individual PAHs were identified using their respective standards, and the quantification was based on peak areas relative to the internal standard m-terphenyl and corresponding response factors. Recoveries determined by spiking PAH standards to the sediment samples ranged from 76% to 115%. None of the concentrations were corrected for recoveries. The instrumental-derived TEQs were calculated using TEFs published by WHO in 1998, 2005, and/or bioassay-specific ReP values[25,26].

3. Results and discussion

3.1. H4IIE-*luc* assay

TCDD standard curves were generated using the mean values of luciferase response/TCDD-max versus their corresponding TCDD concentrations, which fitted sigmoidal model[12](Fig.2). Coefficients of variation (CV) for all triplicate standards and testing samples were all less than 15%. Concentrations of TCDD-EQs were estimated by comparing the dose-response curves of the testing samples and the standards[23,29]. Limits of detection (LOD) based on EC20, EC50 or EC80 were 0.16±0.01 pg well^{-1}(4.2±0.9% TCDD-max), 0.58±0.12 pg well^{-1}, 2.58±0.49 pg well^{-1} and 9.47±1.33 pg well^{-1}, respectively.

(Insert Fig.2)

3.2. AhR-mediated activity of raw sediment extracts

Overall AhR-mediated activities of nine REs were evaluated at six dilutions 100%, 33%, 11%, 3.7%, 1.2% and 0.4%, equivalent to 2.5, 0.83, 0.28, 0.09, 0.03 and 0.01 μL per well. Apparent cytotoxic effects were observed at sampling Site 1, 5, 6 and 9. Among them, the REs of Site 1, 5, 9 showed cytotoxicity at concentrations of 100% (2.5 μL well^{-1}), while RE of Site 6 exhibited cytotoxicity at concentrations of 33% (0.83 μL well^{-1}) extract and greater. All raw extracts induced significant AhR-mediated potency ranged from 43.8 to 104.2 %-TCDD-max (Table 1). Eight out of nine raw extracts yielded a response greater than 50%-TCDD-max, except that of Site 9. The mean response of Sites 3, 4, 7 and 8 were greater than 80%-TCDD-max. Moreover, the influence of preexisting cytotoxic compounds on the response of luciferase remains unknown. The nonparallel nature of these dose-response curves when comparing with the standard curve renders single point estimation and makes it difficult to obtain a single TCDD-EQ value[29]. Magnitudes of bias introduced due to violation of this assumption can be estimated by comparing the EC20, EC50 and EC80 (Table 1). Generally, concentrations of TCDD-EQ based on EC20 were greater than those based on EC50 or EC80. However, unlike EC50 and EC80, EC20 values were available for all testing samples and lesser when curves were more parallel. Therefore, the values of TCDD-EQs used for further comparisons were derived from EC20.

(Insert Table 1)

The overall AhR-mediated activities induced by raw sediment extracts (359-1018 pg TCDD-EQ g^{-1} DW) were one order of magnitude greater than those reported by Xia et al[23] (17.45-114.50 pg TCDD-EQ g^{-1} DW) in Taihu Lake, while similar to the activities in sediments found at most sites of Hihe River, China (331.7-926.6 pg TCDD-EQ g^{-1} DW) and Lake Shihwa, Korea (14-868 pg TCDD-EQ g^{-1} DW)[12,30]. The group of locations with greater concentrations of TCDD-EQs included sampling sites 6 and 7, while the group of locations with lesser concentrations of TCDD-EQs included 1, 2, 3, 4, 5, 8 and 9. Considering the ecological remediation project conducted in Meiliang Bay in 2004, the results indicated that the sediment quality was generally improved from the outer waters to the source water inlet of Chongshan Drinking Water Plant (Fig. 1). Before and after the ecological cleanup project, Qiao et al[2,21] collected surface sediment samples in July 2003 and 2005, respectively and measured the bioassay-derived TCDD-EQs by use of H4IIE EROD bioassay. Those researchers found that concentrations of TCDD-EQs ranged from 17.8 to 38.5 pg g^{-1} DW in 2003 and 5.1 to 13.1 pg g^{-1} DW in 2005, which suggesting that the restoration efforts had been successful in reducing concentrations of DLS.. However, the reported concentrations of TCDD-EQs determined by use of the H4IIE EROD assay were significantly less than those determined by use of the H4IIE-*luc* bioassay in this study and that by Xia et al[23]， indicating the difficulties in comparing TCDD-EQs obtained from different bioassay methods.

3.3. AhR-mediated activity of acid-treated and fractionated extracts

Six out of nine REs were selected for further acid treatment and fractionation to elucidate potential causes of the observed TCDD-EQ. The six samples included Site 4 (greatest observed dioxin-like activity), Site 9 (least observed dioxin-like activity), Site 2 and Site 8 (approximately 80%-TCDD-max), Site 1 and Site 6 (approximately 50%-TCDD-max). Six dilutions of each of the AEs and three FEs were tested using the H4IIE-*luc* bioassay. The maximal luciferase activity induction elicited by individual FEs, AEs and REs are summarized in Fig. 3. Interestingly, the cytotoxicity effects observed in REs of Site 1, 6 and 9 were consistently detected in their AEs and non-polar fractions (F1), which indicated that the existing cytotoxic compounds tended to be acid stable, less polar and were not likely to be removed by Florisil adsorption. Elution of cytotoxic compounds in F1 was also reported previously[12]. However, reduction of cytoxicity and increase of luciferase induction were found in

sediments from inland areas of Korea, which indicated that the properties of compounds causing cytotoxicity might vary site by site[30].

The maximal response of each fraction of the selected six sediment samples was shown in Fig. 3. F1 was found to be unable to induce significant response in all the six testing samples. As mentioned previously, F1 elutes non-polar compounds containing AhR-active (dioxin-like) PCBs and a portion of PCDD/PCDFs. Based on the TEQs estimated by multiplying the concentrations of coplanar PCBs, PCDD/PCDFs and their corresponding RePs, F1 was believed to contribute negligible AhR-mediated activity (Table 2 and 3). The mid-polar (F2) and the most polar (F3) fractions were responsible for the majority of reporter gene expression in H4IIE-*luc* bioassay. F2 samples induced 52.9% to 93.8%-TCDD-max. The maximal responses in F2 exceeded the maximal responses in their corresponding REs of Site 1, 6, 8 and 9, very likely due to the elimination of non-polar cytotoxic compounds. F2 and F3 samples were estimated to contain 94–260 pg TCDD-EQ g^{-1} DW and 26–106 pg TCDD-EQ g^{-1} DW respectively. However, the overall concentrations of TCDD-EQs estimated by raw extracts were much greater than those estimated by three fractions separately. The result indicated that during fractionating, some AhR-mediated compounds might have been adsorbed onto Florisil absorbent, as well as the possibility of antagonistic interactions among compounds (Table 3)[12,31]. Although, polar dioxin-like active compounds eluted in F3 remain unknown, the presence of some polar AhR agonists in sediments has been reported previously[11,12, 22, 23].

(Insert Table 2)

(Insert Table 3)

Sulfuric acid treatment led to a significant reduction of the AhR-mediated activity (Table 3). This was very likely due to the removal of acid-liable AhR-active compounds such as PAHs[11,27]. The remaining concentrations of TCDD-EQs ranged from less than the limit of detection to 189 pg g^{-1} DW. Treatment with acid reduced concentrations of the TCDD-EQs by 47 to 100%. Villeneuve et al[27] has reported that the AhR-mediated activity caused by PAHs could be completely eliminated after a 10 hr acid treatment. Therefore, the remaining AhR-mediated activity was with respect to acid stable compounds. However, concentrations of TEQs caused by AhR-active

PAHs were small, contributing only 8.63-22.87 pg g^{-1} DW, or 7.57-20.18 pg g^{-1} DW, according to two sets of different RePs. This result suggested that PAHs were not the dominant acid-liable compounds inducing AhR-mediated activity in the complex sediment matrices[20,27]. The decrease of AhR-mediated activities in sediment samples by acid treatment was also observed in other studies, but not always[23,30]. It has been reported that acid treatment might lead to an increase of the overall AhR responses by degrading and/or breakdown the potential stressors[30].

(Insert Fig.3)

3.4. Comparison of AhR agonists

Confirmed by chemical analysis, F1 contained PCBs and a portion of the PCDD/PCDFs. F2 contained PAHs, OC pesticides and remaining PCDD/PCDFs. Besides PCDD/PCDFs and dioxin-like PCBs, nine PAH compounds, including B[*a*]P, D[*ah*]A, B[*b*]F, B[*a*]A, B[*k*]F, Chry, I[*cd*]P, Pyr and B[*ghi*]P were capable of inducing detectable AhR-mediated activity[20,27].

In this study, the average and maximum concentrations of total PAHs (∑PAHs) were 1965 ng g^{-1} DW and 2228 ng g^{-1} DW, which were slightly less than the concentrations of 2611 ng g^{-1} DW (average) and 4918 ng g^{-1} DW (maximum) reported previously for sediments collected in Meiliang Bay before restoration[15], but greater than the concentrations determined in sediments collected in 2009 (975.9 ng g^{-1} DW (average) and 1334.9 ng g^{-1} DW (maximum))[18], and much greater than that in the sediment collected in 2010 (220 ng g^{-1} DW(average) and 511 ng g^{-1} DW (maximum)[23]. The decrease in concentrations of ∑PAHs was very likely due to the effective pollution control measures undertaken over the past ten years. The most likely sources of the PAHs were tracked using ratios between individual PAH compounds described elsewhere[21,23]. Flu/Pyr ratios were approximately 1.0 at all sites and the correlation between concentrations of Flu and Pyr was significant ($r = 0.996$, $p < 0.0001$), which indicated that PAHs were generated by similar environmental polluting processes at all sampling sites. In addition, Flu/(Flu+Pry) ratios were between 0.47 and 0.52, indicating both pyrolytic origin and fossil fuels combustion (pyrogenic)[32]. The LMW/HMW ratios were relatively small (0.04-0.38), which further confirmed the importance of prolytic origin[33]. The results of PAH source apportionment in this study are similar to other studies conducted in the same area[18, 21, 23].

Congener specific PCB analysis revealed total dioxin-like PCB concentrations PCB concentrations ranged from 326.4 to 521 pg g^{-1} DW, which were one order of magnitude greater than that reported by Xia et al[23] and slightly greater than the concentrations published by Zhang et al[8]. Sampling site 6 contained the largest amount of dioxin-like PCBs among the nine sampling sites, where the highest TCDD-EQ were also observed (Table 2 and 3). The quantification of 17 PCDD/PCDFs revealed that the total PCDD/PCDFs concentrations ranged from 114.6 to 236.3 pg g^{-1} DW, close to the average PCDD/PCDFs concentration (145.8 pg g^{-1} DW) in the same study area reported previously[8]. Zhang and colleagues collected their sediment samples prior to the conduction of the ecological cleanup project, which indicated that the project was effective in reducing PAHs, but not the more persistent PCBs and PCDD/PCDFs.

Results of a potency balance analysis suggested that the AhR-active compounds quantified by instrumental analysis for the three fractions could not adequately account for bioassay-derived TCDD-EQs of the raw extracts or the fractionated extracts (Table 3). The most likely explanation for the differences between the two approaches in measuring AhR-mediated activity in sediment samples is the presence of other AhR-active compounds besides co-planar PCBs, PCDD/PCDFs and PAHs, especially in polar fraction F3[11,12,23]. Interestingly, the instrumental-derived total TEQs based on the worst-case scenario, ranging from 12.4 to 30.9 pg g^{-1} DW (Table 3), were significantly less ($p<0.05$) than the instrumental-derived TEQs (19.5 to 37.9 pg g^{-1} DW) reported elsewhere[21], suggesting an obvious decrease of AhR-mediated compounds by the ecological remediation project conducted in 2004. As mentioned above, Qiao and colleagues[2] reanalyzed the surface sediment after the completion of the remediation project and reported a decrease of AhR agonists by 42.3-80.7% using EROD bioassay. However, it is not adequate to conclude the continuously decreasing trend of the AhR-mediated activity in Meiliang Bay due to the scarce of instrumental data.

In the present study, two sets of TEFs developed by WHO and one set of RePs specifically for H4IIE-*luc* bioassay were used to compare the TEQs of PCDD/PCDFs and dioxin-like PCBs (Table 2 and Table 3). Concentrations of TEQ_{PCB} based on WHO TEFs were 22-27% less than those based on H4IIE-*luc* RePs, while concentrations of $TEQ_{PCDD/PCDF}$ with respect to WHO TEFs were 10-26% less than

those with respect to H4IIE-*luc* RePs (Table 3). For estimation of TEQ_{PAH}, two sets of RePs with respect to H4IIE-*luc* cell line were used[20,27], The TEQ_{PAH} values calculated using H4IIE-luc_{2012} RePs were 9-35% less than those estimated using H4IIE-luc_{2002} RePs (Table 3). Taking into account of the differences in cell culture and exposures, as well as the effects of synergism, TEQ_{PAH} based on H4IIE-luc_{2012} RePs might be underestimated, but still within the acceptable variation range[20].

Regardless of the uncertainties in TEQs and TCDD-EQs calculation, the instrumental-derived AhR-mediated activities were approximately 10-fold less than the bioassay-derived AhR-mediated activities from fractionated extracts (Table 3). Additionally, the maximal instrumental-derived TEQs (ΣTEQ_{great}) accounted for only 2.8-7.3% of the total TCDD-EQs from raw extracts. This suggested that the sediments in Meiliang Bay contained AhR-mediated compounds other than the dioxin-like PCBs, PAHs and PCDD/PCDFs. Similar findings were also reported in other studies[7,12,23]. Besides the incomplete quantification of AhR-active compounds, the matrix effects, synergisms, additivity, agonistic and antagonistic effects are possible explanations for the differences between TCDD-EQs and TEQs[13,19,20].

Previous studies have assessed the environmental risks posed by PCBs, PCDD/PCDFs and/or PAHs by use of instrumental analysis and/or *in vitro* bioassay[2,7,8,13,18,21,23,34-36]. Here, RePs values derived from the latest literature were reapplied to estimate and compare the TEQs for selected sediment samples reported previously (Table 4). The $\Sigma TEQ_{PCB}+TEQ_{PCDD/PCDF}$ calculated by use of RePs were generally greater than those by use of WHO TEFs, indicating the underestimation of H4IIE-*luc* related TEQs associated with dioxin-like PCBs and PCDD/PCDFs using WHO TEFs. In other words, potential balance comparisons conducted in most previous studies might not be accurate due to lack of appropriate assay-specific RePs for PCBs and PCDD/PCDFs[6,12,13,21-23]. Alternatively, TEQ_{PAH} values based on ReP values reported by Villeneuve et al[27] were almost all greater than those based on RePs developed by Larrson et al[20]. According to the worst-case scenario, ΣTEQ_{great} based on the H4IIE-*luc* related RePs obtained by Lee et al[26] and Villeneuve et al[27] were used for the comparison of instrumental-derived TEQs in different studies (Table 4). As mentioned above, it has been different to confirm continuous decreases in contamination of AhR-mediated compounds in Meiliang Bay due to the inconsistence of TEQ estimation and/or bioassay methods. For example, the TCDD-EQs published

for sediments collected in 2003 and 2005 were even less than those for sediments collected in 2010 (Table 4), which weakened the importance of the ecological remediation project. However, when incorporating the recalculation of TEQs in Table 4, a clear decreasing trend was observed in the past decade. Moreover, the results strongly supported the conclusion that the ecological project was effective in removing chronic toxicant in sediments of Meiliang Bay, Taihu Lake.

(Insert Table 4)

Acknowledgements

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Table 1. Percentages of maximum response based on TCDD standard and concentrations of TCDD-EQs (in pg g^{-1} DW) in sediments of Meiliang Bay, Taihu Lake, China

Site	%-TCDD-max	TCDD-EQs		
		EC20	EC50	EC80
1	52.5±2.3[a]	607	173	/
2	79.1±3.6	359	310	172
3	102.6±2.1	379	215	264
4	104.2±3.3	377	296	379
5	70.2±5.2[a]	546	281	261
6	57.2±3.3[a]	1018	770	/
7	88.7±5.3	751	755	617
8	80.1±9.5	453	226	185
9	43.8±3.4[a]	410	/	/

[a] %-TCDD-max values were observed at concentrations of 33% REs.

Table 2. The TEFs, RePs and concentration range of PCBs, PCDD/PCDFs and PAHs in sediments of Meiliang Bay

Chemical	MW	TEF		ReP			Concentrations (n=9)[a]		
		WHO_{1998}[25]	WHO_{2005}[25]	H4IIE-luc_{2013}[26]	H4IIE-luc_{2002}[27]	H4IIE-luc_{2012}[20]	Mean	Min	Max
PCB 77	292	0.0001	0.0001	0.00007			56.1	41.5	77.8
PCB 81	326	0.0001	0.0003	0.0034			2.0	0.8	3.4
PCB 105	326	0.0001	0.00003	0.000012			70.8	54.9	93.1
PCB 114	326	0.0005	0.00003	0.0000049			6.4	4.6	8.5
PCB 118	326	0.0001	0.00003	0.0000073			175.1	122.1	232.5
PCB 123	326	0.0001	0.00003	0.0000082			36.3	23.1	56.7
PCB 126	326	0.1	0.1	0.14			7.0	4.7	11.2
PCB 156	361	0.0005	0.00003	0.000016			15.4	10.7	23.9
PCB 157	361	0.0005	0.00003	0.000041			7.8	3.2	23.1
PCB 167	361	0.00001	0.00003	0.000000001			16.6	3.6	79.3
PCB 169	361	0.01	0.03	0.00033			0.7	0.3	1.1
PCB 189	395	0.0001	0.00003	0.000000001			1.4	0.7	2.1
2378-TCDD	322	1	1	1			0.6	0.4	1.0
12378-PeCDD	356	1	1	0.55			1.0	0.4	2.1
123478-HxCDD	391	0.1	0.1	0.12			1.2	0.5	2.6
123678-HxCDD	391	0.1	0.1	0.047			1.2	0.6	2.5
123789-HxCDD	391	0.1	0.1	0.054			1.5	0.7	2.8
1234678-HpCDD	425	0.01	0.01	0.056			11.5	6.4	23.9
OCDD	460	0.0001	0.0003	0.0005			113.5	84.7	168.2
2378-TCDF	306	0.1	0.1	0.27			2.1	1.3	2.8

12378-PeCDF	340	0.05	0.03	0.024			1.6	0.9	2.0
23478-PeCDF	340	0.5	0.3	0.5			0.9	0.4	1.6
123478-HxCDF	375	0.1	0.1	0.13			2.5	1.3	4.1
123678-HxCDF	375	0.1	0.1	0.039			1.8	0.9	2.9
234678-HxCDF	375	0.1	0.1	0.18			1.6	0.6	2.5
123789-HxCDF	375	0.1	0.1	0.11			0.5	0.3	0.7
1234678-HpCDF	409	0.01	0.01	0.011			6.1	4.7	7.4
1234789-HpCDF	409	0.01	0.01	0.041			1.0	0.3	2.6
OCDF	444	0.0001	0.0003	0.0065			7.6	5.9	10.1
B[*a*]A	228				0.0000019	0.0000006	188.3	143.8	246.2
Chry	228				0.0000023	0.0000023	242.2	127.4	483.0
B[*b*]F	252				0.0000051	0.0000125	126.5	65.5	223.1
B[*k*]F	252				0.00014	0.0000278	69.2	33.5	109.8
B[*a*]P	252				0.0000016	0.00000241	312.9	204.3	419.0
I[*cd*]P	276				0.000015	0.00000991	264.1	170.8	451.1
D[*ah*]A	278				0.0000046	0.0000471	62.5	30.2	105.9
Pyr	202				NA	0.0000013	225.5	73.1	297.0
B[*ghj*]P	276				NA	0.00000041	136.5	96.7	207.3

[a] Concentrations for PCBs and PCDD/PCDFs were in pg g^{-1} DW, while concentrations for PAHs were in ng g^{-1} DW.

Table 3. TEQs calculated with different TEF or ReP values and TCDD-EQs in FEs, AEs and REs

Sampling Site	TEQs										TCDD-EQs				
	TEQ_{PCB}			$TEQ_{PCDD/PCDF}$			TEQ_{PAH}		ΣTEQ_{small}[a]	ΣTEQ_{great}[b]	F2	F3	ΣF2+F3	AE	RE
	WHO_{1998}	WHO_{2005}	H4IIE-luc_{2013}	WHO_{1998}	WHO_{2005}	H4IIE-luc_{2013}	H4IIE-luc_{2002}	H4IIE-luc_{2012}							
1	0.78	0.76	1.03	4.18	3.97	4.64	13.19	12.08	16.8	18.9	149	26	175	77	607
2	0.64	0.62	0.83	2.61	2.47	2.95	8.63	7.57	10.7	12.4	94	106	200	189	359
3	0.89	0.87	1.18	2.89	2.70	3.29	13.21	12.05	15.6	17.7					379
4	0.59	0.58	0.77	2.28	2.18	2.70	15.82	13.57	16.3	19.3	260	61	321	169	377
5	0.75	0.73	0.99	2.39	2.25	3.07	11.13	10.03	13.0	15.2					546
6	1.20	1.17	1.59	5.74	5.41	6.39	22.87	17.25	23.8	30.9	157	75	232	181	1018
7	0.68	0.65	0.89	2.54	2.40	3.05	18.42	13.97	17.0	22.4					751
8	0.75	0.73	0.98	3.45	3.22	3.93	18.40	13.75	17.7	23.3	144	50	195	165	453
9	0.53	0.51	0.68	6.22	5.94	6.89	22.33	20.18	26.6	29.9	188	65	253	NC	410

[a]The least calculated TEQs based on WHO TEFs (2005) and RePs (H4IIE-*luc*2002)

[b]The greatest calculated TEQs based on H4IIE-*luc* RePs (H4IIE-*luc*2013 and H4IIE-*luc*2012)

Table 4. Comparison of TEQs calculated using different TEF and/or ReP values for potential dioxin-like activity in previously reported sediments

Counyty/region	Year	n	$TEQ_{PCB}+TEQ_{PCDD/PCDF}$			TEQ_{PAH}		$\Sigma TEQ_{reported}$ [a]	ΣTEQ_{high} [b]	TCDD-EQ	References
			WHO_{1998}	WHO_{2005}	H4IIE-luc_{2013}	H4IIE-luc_{2002}	H4IIE-luc_{2012}				
			Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	Min-Max	
			Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	
China											
Meiliang Bay	2002	10	1.4-8.4	1.3-7.9	1.8-9.0	NA[c]	NA	0.8-3.7	1.8-9.0	NA	[8]
Taihu Lake			3.5	3.3	4.1			2.2	4.1		
	2003	8	2.1-6.3	2.0-5.9	2.9-6.2	18.1-42.1	9.7-26.3	19.5-37.9[d]	24.3-46.8	17.8-35.8[e]	[21]
			3.4	3.2	4.1	32.4	19.8	29.4	36.4	28.3	
	2004	9	2.9-6.9	2.8-6.6	3.5-7.6	8.6-22.3	7.6-20.2	/	12.4-29.9	359-1018	This study
			4.3	4.1	5.1	16.0	13.4	/	21.1	544	
	2005	8	NA	NA	NA	NA	NA	NA	NA	5.1-13.1[e]	[2]
	2009	7	NA	NA	NA	2.6-11.8	2.3-5.5	NA	2.6-11.8	NA	[18]
						6.1	4.0		6.1		
	2010	2	ND-0.0038	ND-0.0015	ND-0.0023	0.15-1.35	0.26-0.69	0.15-1.35	0.15-1.35	17.5-76.9	[23]
			0.002	<0.001	0.001	0.8	0.5	0.8	0.8	47.2	
Serbia											
Pancevo	2004	1	146000	110000	144000	NA	NA	78000	144000	44000	[34]
South Kerea											
Hyeongsan River	2001	6	0.4-1046	0.3-1010	0.4-640	ND-83.2	ND-35.8	0.4-1130	0.4-724	0.01-1520	[7]
			224	205	154	17.7	7.5	243	172	318	
Pohang Area	2010 (Jun)	8	0.8-762	0.9-548	1.8-719	0.8-21.9	0.5-13.3	2.8-753	2.6-741	ND-800	[13]

			128	95.7	124	6.2	3.8	118	130	126	
	2010 (Aug)	8	0.4-236	0.4-167	1.2-226	0.2-18.2	0.2-10.1	1.5-238	1.4-235	ND-310	
			65.8	52.7	67.8	5.0	2.8	74.0	72.8	71.8	
Japan											
Urban area, Osaka	2003	8	193	169	243	NA	NA	190	243	NA	[35][f]
Suburban area, Osaka	2003	6	2.3	2.1	2.8	NA	NA	2.3	2.8	NA	
Urban area, Osaka	2007	16	53-44000	49-4100	69-61000	NA	NA	49-41000	69-61000	NA	[36]
(Sangamaki Waterway)			10000	9500	14000			9700	14000		
Vietnam											
Can Gio	2003	10	3.1	2.9	3.9	NA	NA	2.7	3.9	NA	[35]

[a]TEQ values reported in literatures
[b]The highest calculated TEQs based on H4IIE-*luc* RePs (H4IIE-luc_{2013} and H4IIE-luc_{2012})
[c]NA: not analyzed
[d]TEF values used in the original literature were incorrect. The authors did not convert TEFs based on molar concentration to TEFs based on mass concentration
[e]TCDD-EQs were obtained using EROD bioassay
[f]TEQ values were recalculated using the mean concentrations provided in original literature

Figure Captions

Figure 1. Sediment sampling locations in Meiliang Bay, Taihu Lake.

Figure 2. Luciferase activity induced by 2,3,7,8-TCDD in H4IIE-*luc* assay. Luciferase response in Y-axis is expressed as the percentage of luciferase activity relative to that of 30 pg well^{-1} 2,3,7,8-TCDD standard. Error bars represent the standard deviations.

Figure 3. Maximal observed luciferase activity induced by F1, F2, F3, REs and AEs in H4IIE-*luc* assay. Response in Y-axis is expressed as the percentage of luciferase response relative to that of 30 pg well^{-1} 2,3,7,8-TCDD standard. Error bars represent the standard deviations.*Obvious cytotoxicity was observed at concentrations of 100%.

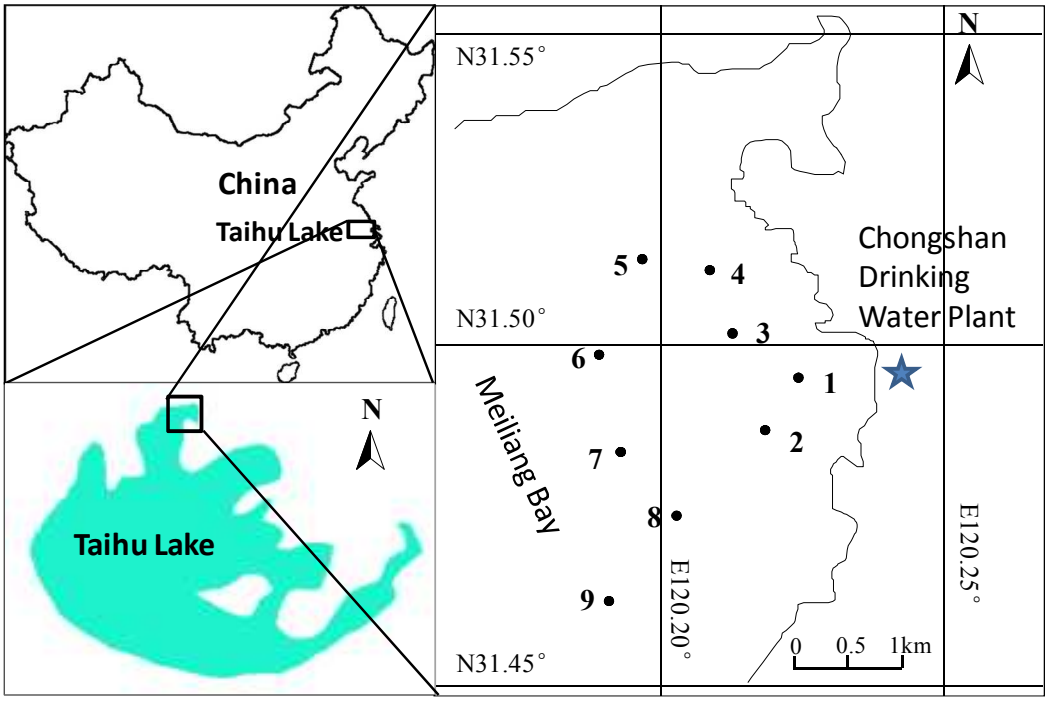

Fig. 1.

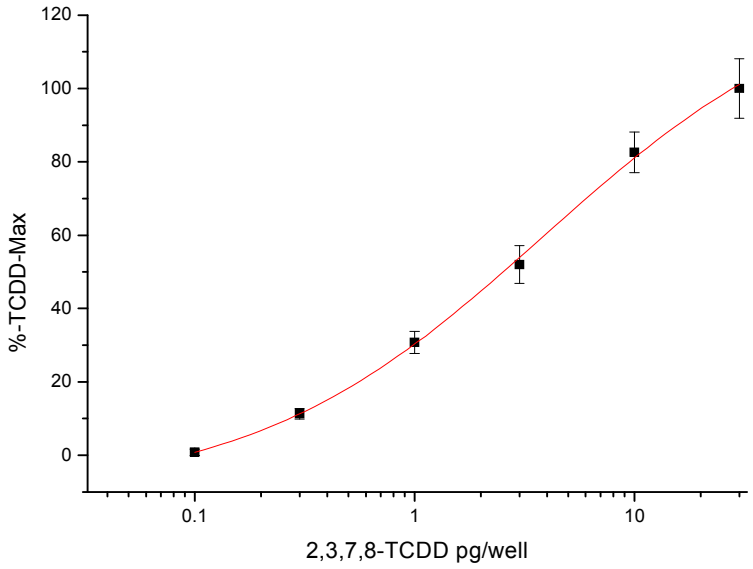

Fig. 2.

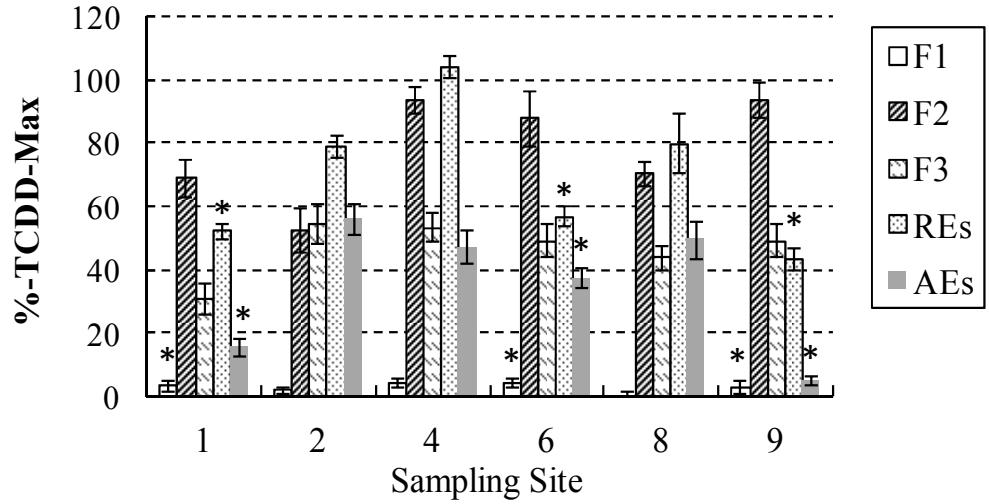

Fig. 3.