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Estrogenic activity profiles and risks in surface waters and sediments of the Pearl River system in South China assessed by chemical analysis and *in vitro* bioassay†‡

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Estrogenic activity risks in the Pearl River system (Liuxi River, Zhujiang River and Shijing River) in South China were assessed by combined chemical analysis and recombinant yeast estrogen screen (YES) bioassay for surface waters and sediments collected in both dry and wet seasons. The xenoestrogens 4-*tert*-octylphenol, 4-nonylphenol and bisphenol A were detected at almost every sampling site at concentrations of several ng L^{-1} (ng g^{-1}) to tens of μg L^{-1} (μg g^{-1}) in surface waters (and sediments). The estrogens estrone and 17β-estradiol were also detected in most of the samples with concentrations from several ng L^{-1} (ng g^{-1}) to tens of ng L^{-1} (ng g^{-1}) in surface waters (and sediments). However, synthetic estrogens diethylstilbestrol and 17α-ethinylestradiol were only detected at a few sites. The 17β-estradiol equivalents (EEQ) screened by the YES bioassay were in the range of 0.23–324 ng L^{-1} in surface waters and from not detected to 101 ng g^{-1} in sediments. Shijing River displayed one to two orders of magnitude higher levels for both measured chemical concentrations and estrogenic activities than the Zhujiang River and the Liuxi River. A risk assessment for the surface waters showed high risks for the downstream reaches of the Liuxi River and the upstream to midstream reaches of the Zhujiang River and the Shijing River. Higher estrogenic risks were observed in the wet season than in the dry season for surface waters, probably due to the input of runoff and direct overflow of small urban streams during heavy rain events. Only small variations in estrogenic risk were found for the sediments between the two seasons, suggesting that sediments are a sink for these estrogenic compounds in the rivers.

Introduction

Endocrine disrupting chemicals (EDCs) have become a lasting concern in recent decades due to their potential impact on the

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endocrine systems of humans and wildlife.[1] Among EDCs, those substances with high binding affinity to estrogen receptors are thought to be estrogenic chemicals, which include some natural estrogens (*e.g.*, estrone (E1), 17β-estradiol (E2)), synthetic estrogens (*e.g.*, 17α-ethinyl estradiol (EE2), diethylstilbestrol (DES)), and some xenoestrogens (*e.g.*, 4-*tert*-octylphenol (4-*t*-OP), 4-nonylphenol (4-NP), bisphenol-A (BPA), and phthalic acid esters (PAEs)). Reports on male fish feminization[2,3] and collapse of fish population[4] were found to be strongly linked to exposure to natural or synthetic estrogens at concentrations as low as a few nanograms per liter. Significant estrogenic effects of 4-NP and BPA on female fish[5,6] were also observed after

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Environmental impact

With development of the economy, rivers in the Pearl River Delta region have received increasing inputs of treated and untreated industrial and domestic wastewater. Endocrine disrupting chemicals have become an important water quality issue in recent years. Measured estrogenic activities in surface water and sediments from the Pearl River showed high risks to aquatic organisms for some urban sections of the river. Higher estrogenic risks were observed in the wet season than in the dry season for surface waters, due to the input of runoff and direct overflow of small urban streams during heavy rain events. The results from this study suggest that measures should be taken to control the estrogenic compounds in the contributing sources.

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exposure to the xenoestrogens at concentrations on the microgram per liter level. Moreover, estrogenic effects of some EDCs on prawns and frogs have recently been reported.[7,8]

Due to the concerns of possible impacts of EDCs on aquatic organisms, investigations on these chemicals in the sewage treatment plants, receiving water, and sediments have been carried out in some regions of the world.[9–14] High detection frequencies of these EDCs are recorded in aquatic environments, with concentrations ranging from a few parts per trillion to several parts per million. In parallel with chemical analysis, *in vivo* or *in vitro* bioassays are often used to screen the estrogenic potency of the environmental samples.[15–17] Screening using bioassays could provide an integral estrogenic activity of a sample, and could also help evaluate the estrogenic contributions by certain chemicals in combination with chemical analysis. Surface water and sediments in rivers of Europe, the USA, Japan and Korea had displayed various degrees of estrogenic activities due to the presence of estrogenic substances.[12,18–20] Unfortunately, little is known about estrogenic activities in Chinese rivers, especially in the Pearl River system in South China.

The Pearl River Delta is one of the most developed areas in South China, and Guangzhou is the biggest city in the region. Rivers of the Pearl River system are important drinking water sources for many cities and towns including Guangzhou, Hong Kong and Macau. Rivers in the region have received inputs of treated and untreated industrial and domestic wastewater.[21] Recently, EDCs have become an important water quality issue in the region due to public concern about their negative impacts on the environment and human health. Limited chemical analysis has shown that some EDCs (estrone, estradiol, 4-*t*-octylphenol, 4-nonylphenol and bisphenol A) are ubiquitous in the Pearl River system, ranging from a few ng L^{-1} to several µg L^{-1}.[22,23] However, no bioassay (*in vitro* and *in vivo* test) data have been available from the Pearl River system until now. River sediments are known as important sinks for these EDCs due to partitioning processes,[24] but little is known on estrogenic effects of sediments on aquatic organisms, and how to assess sediment toxicity in terms of estrogenic effects.[25,26] Therefore, it is essential to understand the contribution of these chemicals in sediments to estrogenic activity in the water columns.

The objectives of this study were to analyze estrogenic activities in surface water and sediments from the Pearl River system (Liuxi River, Zhujiang Rivers and Shijing River) using combined chemical analysis and *in vitro* bioassay and to assess the potential risks of estrogenic activity to aquatic organisms.

Experimental section

Materials

Seven estrogenic compound standards were included in the study: 4-*t*-octylphenol (4-*t*-OP) and bisphenol A (BPA) purchased from Supelco, 4-nonylphenol (4-NP), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) from Dr Ehrenstorfer GmbH, and estrone (E1) and diethylstilbestrol (DES) obtained from Riedel-de Haën. The internal standards 4-*n*-nonylphenol (4-*n*-NP), bisphenol A-d_{16} (BPA-d_{16}) and estrone-d_4 (E1-d_4) were supplied by Dr Ehrenstorfer GmbH, Supelco and Cambridge Isotope Corporation, respectively. The stock solution for each

chemical and the internal standards were prepared in methanol at a concentration of 100 mg L^{-1}, and stored at −18 °C. The derivatization agents pentafluorobenzoyl chloride and N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) were obtained from Aldrich and Supelco, respectively. All solvents used were HPLC grade and purchased from Merck Corporation (Shanghai, China). Neutral silica gel (60–100 mesh, Qingdao, China) was Soxhlet extracted with methanol and dichloromethane for 48 h prior to use. Anhydrous sodium sulfate was baked at 400 °C and stored in sealed containers for later use. All glassware was hand-washed with detergent and tap water, rinsed with Milli-Q water, and baked at 400 °C for at least 4 h before use.

Sample collection and preparation

Surface water and sediment samples were collected from the Liuxi River, the Shijing River and the Zhujiang River of the Pearl River system on December 17–18, 2007 in the dry season and on September 10–12, 2008 in the wet season (Fig. 1). Liuxi Reservoir (S0) with little human activity was selected as the control site located upstream of the Liuxi River. Another three sites (S1–S3) were located downstream in the Liuxi River. Seven sites (S4–S10) were located in the Zhujiang River and four sites (S11–S14) were located in the Shijing River. Effluent samples were also collected from four wastewater treatment plants (W1–W4) during the dry and wet seasons.

Surface water samples in 1 L amber glass bottles were taken from 2 to 3 positions across the river section at each sampling site with the samples being collected from 0.5 m below the water surface. Four composite 1 L surface water samples were taken at each site, and 50 mL of methanol and 400 µL of 4 M H_2SO_4 were added immediately into each 1 L bottle to adjust the pH to 3.0. Surface sediment (0–10 cm) was collected using a stainless steel grab sampler from two positions of the section which were 10–20 m away from river bank. About one gram of sodium azide was added for each liter of sediment. The collected water samples were transported in coolers to the laboratory and stored in a cold room at 4 °C for a maximum of 48 h before solid phase extraction (SPE). The sediment samples were freeze-dried immediately and stored at 4 °C for later analysis. The basic physicochemical parameters of the water and sediment samples are given in the ESI (Table S1‡).

Sample extraction and purification

The procedures for surface water extraction were based on our previously reported methods.[27] Briefly, two replicate water samples were spiked with internal standards (100 µL of 1 mg L^{-1} of 4-*n*-NP, BPA-d_{16}, and E1-d_4) for chemical analysis, while another two unspiked replicate water samples were used for the bioassay. Water samples were then extracted using Waters Oasis HLB cartridges (6 cm³, 500 mg sorbents) and eluted with 7 mL of methanol and 5 mL of dichloromethane in sequence. The combined eluates were dried under a gentle nitrogen stream and reconstituted in 1 mL of methanol.

Sediment extraction was carried out using an ultrasonic extraction method. Briefly, 5 g of less polluted dry sediment samples (S0–S10) or 2 g of heavily polluted dry sediment samples (S11–S14) was weighed in to a 30-mL centrifuge tube. Similar to
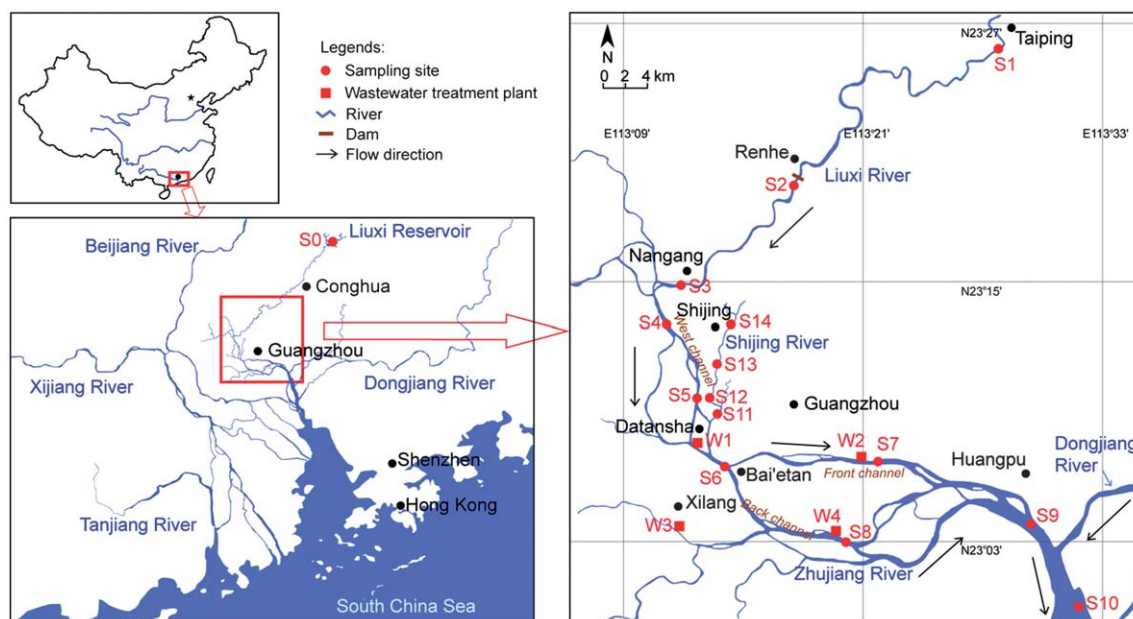
Fig. 1 Sketch map of sampling sites in the Pearl River system, South China. The control site S0 is in the Liuxi Reservoir, sites S1–S3 are in the Liuxi River, sites S4–S10 are in the Zhujiang River, and sites S11–S14 are in the Shijing River. W1–W4 are sewage treatment plants.

the spiking approach used for river water extraction, two replicate sediments were spiked with 100 ng of each internal standard for chemical analysis, and another two replicate samples were directly extracted for the bioassay. Spiked sediments were kept in a fume cabinet and covered with foil for 4 h to let the solvent evaporate completely. The sediments were then manually mixed and stored at 4 °C overnight. The samples were then extracted using 10 mL of ethyl acetate by vortex mixing thoroughly for 2 min, and ultrasonicating for 15 min. The tube was then centrifuged at 1370 g for 10 min, and the supernatant was transferred into another stock tube. The extraction step was repeated twice. The supernatants from the same sample were combined and dried under a gentle nitrogen stream and the extracts were redissolved in 1 mL of methanol.

The extracts were purified by passing through a silica gel (1 g) column, and eluted with 6 mL of *n*-hexane, 6 mL of ethyl acetate, and 6 ml of methanol in sequence. The seven selected target phenolic compounds as well as their internal standards were in the ethyl acetate phase. The ethyl acetate phase was then dried and reconstituted in 1 mL of methanol and kept at −18 °C prior to instrumental analysis and the bioassay.

Instrumental analysis and bioassay

The final extracts of surface water and sediment samples were derivatized using pentafluorobenzoyl chloride in alkaline aqueous solution to form esters to decrease the polarities of the target analytes before instrumental analysis. The derivatives were analyzed using gas chromatography-mass spectrometry under negative chemical ionization mode (GC-NCI-MS: Agilent 6890N gas chromatograph connected to an Agilent 5975B mass spectrometer with a chemical ionization source). The detailed derivatization steps and instrumental analysis procedures were described previously.[27] Some of the results were then verified

using GC-MS with electron impact mode (EI), and derivatization by MSTFA as reported in a previous study.[23]

The recombinant yeast for the yeast estrogen screen (YES) bioassay was kindly provided by J. P. Sumpter (Brunel University, Uxbridge, UK). The procedures for the YES bioassay were carried out as described by Routledge and Sumpter.[28] Briefly, two parallel extracted samples were diluted 2-fold in a series on a row of a 96-well microplate, then 10 μL of each concentration was transferred to the corresponding well on another 96-plate. The solvent in each well was allowed to dry in a laminar flow cabinet, after which 200 μL of yeast solution in growth media was added to each well, such that the well had a yeast density of 4 × 10^7 cell mL^{-1} and chlorophenol red-β-D-galactopyranoside (CPRG) concentration of 0.1 mg mL^{-1}. The microplate was sealed and packed with foil, and incubated statically at 32 °C in darkness. After 72 h incubation, the absorbance at 620 nm and 540 nm was measured on a BMG microplate reader (BMG Lab technologies, Offenburg, Germany). E2 was used as the positive control with an initial concentration of 0.2 μM, and methanol was used as the blank control. The estrogenic activity of a sample measured by the YES assay was expressed as an estradiol equivalent (EEQ).

Risk assessment

The predicted no effect concentration (PNEC) of E2 was derived from the aquatic chronic no-observed-effect-concentrations (NOECs) according to the European Commission Technical Guidance Document (TGD)[29] and the method described in the literature.[30] In total, 77 NOECs of reproduction effect data for different species (Table S4‡) were acquired to set up the species sensitivity distribution (SSD) curve, which was fitted by log-logistic modeling. Then the PNEC of E2 was calculated as the hazardous concentration for 5% of species (HC_5) value.

The potential estrogenic activity risk in surface waters of the Pearl River system was assessed according to the ranking of risk quotient (RQ), which was the ratio of EEQ at each site and the PNEC of E2. The risk assessment was carried out by following the commonly used risk ranking criteria: RQ < 0.1 means minimal risk, $0.1 \leq RQ < 1$ means median risk, and $RQ \geq 1$ means high risk.[31] The risk assessments for sediments were also carried out by converting the estrogenic activities of estrogenic chemicals into their corresponding EEQ in pore water, which were expressed as the bioavailable fractions of the EDCs in sediments. The equation used is listed as follows:

$$EEQ_{\text{pore water}}\left(\text{ng L}^{-1}\right) = \left(\sum \frac{1000 \times C_{s,i}(\text{ng g}^{-1}) \times EEF_i}{K_{oc,i}(\text{L kg}^{-1})} \right) \times \text{\%total organic carbon,}$$

$$(1)$$

where $C_{s,i}$ is the concentration of chemical i in sediment. And $K_{oc,i}$ is the organic carbon partitioning coefficient of chemical i, and the EEF_i is the estrogenic equivalent factor of chemical i. The K_{oc} values and the EEFs are listed in Table S5 and S6, respectively.‡ The risk of estrogenic activity in pore water was then assessed using the same method for surface water.

Results

Levels of estrogenic chemicals

The concentrations of four estrogens (E1, E2, EE2 and DES) and three xenoestrogens (4-t-OP, 4-NP and BPA) in surface water and sediments of the Pearl River system (Liuxi River, Zhujiang River and Shijing River) are presented in Table 1. The seven target estrogenic chemicals were detected frequently in the three rivers. In surface water, E1 had the highest detection frequency and the maximum concentration among the four estrogens, up to 100% and 78.7 ng L^{-1}, respectively. E2 was occasionally detected in the

three rivers, with the maximum concentration of 7.72 ng L^{-1}. EE2 and DES were only detected in the Shijing River, with maximum concentrations up to 53.5 ng L^{-1} and 1.66 ng L^{-1}, respectively. In sediments, the four estrogens showed reduced detection frequencies than those in surface water, and DES was only found occasionally in sediments of the Zhujiang River. The detected maximum concentrations for E1, E2, EE2 and DES in sediments were 38.0 ng g^{-1}, 4.12 ng g^{-1}, 21.2 ng g^{-1} and 10.6 ng g^{-1}, respectively. The sites with the maximum concentrations for estrogens were located in the Shijing River.

Three xenoestrogens (4-t-OP, 4-NP and BPA) had obviously higher detection frequencies than estrogens in surface water and sediments. The detection frequencies were almost 100%, except that 4-t-OP and BPA were below the limit of quantification (LOQ) in some sediment samples from the Liuxi River. All of the maximum concentrations for 4-t-OP, 4-NP and BPA were located in the Shijing River, in surface water and sediments, up to 3150 ng L^{-1}, 20080 ng L^{-1} and 1390 ng L^{-1}, respectively, in surface water, and up to 979 ng g^{-1}, 28830 ng g^{-1} and 296 ng g^{-1}, respectively, in sediments. Of the seven EDCs, the mean concentrations in the Shijing River were one to two orders of magnitude higher than those in the Zhujiang River and the Liuxi River. Also, the water quality parameters for the Shijing River (Table S1‡) indicate that the water quality of the Shijing River was much worse than that of the Zhujiang River or the Liuxi River.

Compared with the three rivers, the control site, Liuxi Reservoir, with little human activity, is the cleanest site among these sampling sites. No estrogens were detected in both the surface water or sediment of the reservoir. The xenoestrogens, 4-t-OP, 4-NP and BPA, were detected at much lower concentrations, ranging from below the LOQ to 3.03 ng L^{-1} for 4-t-OP, from 26.6 to 123 ng L^{-1} for 4-NP, and from below the LOQ to 4.53 ng L^{-1} for BPA in the surface water. The concentrations of xenoestrogens were also lower in reservoir sediments, with concentrations below the LOQ for 4-t-OP and BPA, and from 15.9 ng g^{-1} to 23.1 ng g^{-1} for 4-NP.

Table 1 Concentration distributions (ranges, mean and median values, and detection frequencies) of seven selected endocrine disrupting chemicals by chemical analysis and EEQs (estradiol equivalents) measured by the YES (recombinant yeast estrogen screen) bioassay in the Pearl River system

| Compound | Liuxi River[a] | | | | Zhujiang River | | | | Shijing River | | | |
	Range	Mean	Median	Freq[b] (%)	Range	Mean	Median	Freq (%)	Range	Mean	Median	Freq (%)
Water (ng L^{-1})												
Estrone	ND[c]–3.11	2.14	2.86	92	0.91–7.83	2.73	1.93	100	5.00–78.7	35.2	33.4	100
17β-estradiol	ND–2.11	1.64	1.72	33	ND–1.80	1.34	1.28	39	ND–7.72	3.67	2.83	56
17α-ethinylestradiol	ND				ND				1.43–53.5	18.1	10.3	100
Diethylstilbestrol	ND				ND				ND–1.66	0.97	0.80	38
4-t-octylphenol	2.38–16.85	7.79	5.68	100	3.08–27.1	10.6	7.74	100	33.1–3150	433	159	100
4-nonylphenol	64.8–1550	474	293	100	184–2900	869	295	100	1780–20080	7800	6170	100
Bisphenol A	7.72–311	93.4	50.6	100	51.8–319	169	155	100	308–1390	865	930	100
EEQ	0.23–2.50	0.82	0.46	100	0.30–7.54	1.90	1.18	100	17.5–324	88.2	71.5	100
Sediments (ng g^{-1})												
Estrone	ND–12.8	9.04	9.01	33	<LOQ–30.6	11.8	9.19	75	5.54–38.0	19.1	20.2	100
17β-estradiol	ND–1.53	1.45	1.45	17	ND–4.12	2.41	2.38	25	ND			
17α-ethinylestradiol	ND				ND				ND–21.2	11.8	11.0	57
Diethylstilbestrol	ND				ND–2.24	1.92	2.09	14	ND–10.6	7.33	6.82	29
4-t-octylphenol	<LOQ[d]–30.4	15.2	14.3	67	1.80–51.2	19.6	16.0	100	103–979	385	309	100
4-nonylphenol	11.4–3750	1570	1160	100	533–5500	3020	2850	100	13240–28830	20400	17560	100
Bisphenol A	<LOQ–76.6	34.9	29.8	83	18.3–148	72.7	71.4	100	133–296	213	216	100
EEQ	ND–7.24	3.87	3.50	50	ND–14.0	4.66	3.38	86	9.80–101	36.9	29.6	100

[a] Mean value, median value and frequency are calculated based on those with concentrations higher than the limit of quantification. [b] Freq, the detection frequency. [c] ND, not determined. [d] LOQ, limit of quantification.

We also analyzed the estrogens and xenoestrogens in four sewage treatment plant effluents. E1 was detected 100% in all of the effluents, with a maximum concentration of 54.4 ng L^{-1}. E2 and EE2 were only detected in one effluent in the wet season with concentrations of 2.90 and 8.83 ng L^{-1}, respectively. Three xenoestrogens, 4-t-OP, 4-NP and BPA, were found in all of the effluents, with maximum concentrations up to 36.9 ng L^{-1}, 3820 ng L^{-1} and 256 ng L^{-1}, respectively. The mean concentrations of the seven EDCs in effluents were ranked between those in the Zhujiang River and the Shijing River, indicating that the Shijing River and effluents were the potential pollution sources for the Zhujiang River.

Estrogenic activities

In addition to chemical analysis, estrogenic activities in the surface water and sediments of the Pearl River system were also screened using the YES bioassay. The EEQs, including range, mean, median and detection frequency, are also listed in Table 1. At the control site with less human sewage discharge, no estrogenic activity was detected in both the surface water and sediments from the Liuxi Reservoir. This was in agreement with the undetectable or lower concentrations of the seven selected estrogenic compounds at this site.

Along the Liuxi River, weak estrogenic activities were found in the three sampling sites. The mean EEQs were 0.82 ng L^{-1} in the surface water and 3.87 ng g^{-1} in sediment, respectively. The site (S3) with the maximum EEQ was located in the downstream section of the Liuxi River, where it received direct discharge of domestic sewage from residents near the river.[32] The estrogenic activities in the Zhujiang River were slightly higher than those in the Liuxi River. In the Zhujiang River, the mean EEQs were 1.90 ng L^{-1} in the surface water and 4.66 ng g^{-1} in sediment. The sites with the highest EEQs in the surface water and sediments of the Zhujiang River were located in the upstream to midstream reaches near the city center of metropolitan Guangzhou. As for the estrogenic activities in the Shijing River, the EEQs were much higher than those of the Zhujiang River and the Liuxi River. The mean EEQs were 88.2 ng L^{-1} in surface water and 36.9 ng g^{-1} in sediment, respectively. The maximum EEQs were 324 ng L^{-1} in surface water and 101 ng g^{-1} in sediment, respectively. The mean EEQs of the Shijing River were also one to two orders of magnitude higher than those in the other two rivers, showing similar trends to the data from chemical analysis.

Correlations between chemical analysis and bioassay

In order to correlate the measured EEQs by bioassay and the concentration data from chemical analysis, the chemical concentrations were expressed as calculated EEQs, which were calculated using the relative potencies and environmental concentrations of the seven target compounds based on the addition model[33] (see ESI‡). Good linear correlations (R^2 = 0.9168 and $p < 0.0001$ for surface water samples; R^2 = 0.9391 and $p < 0.0001$ for sediment samples) were found between the measured EEQs and calculated EEQs as shown in Fig. 2, indicating a high degree of consistency between estrogenic activity and the presence of estrogenic chemicals.

However, from the slopes of the modeling equations, we could see that the measured EEQs from the YES assay were about 1.75-fold higher than the calculated EEQs using chemical analysis data for surface water samples (slope = 0.57), which might indicate that some unknown compounds could have contributed to the estrogenic activities in surface water. While the measured EEQs for sediment samples were almost equal to the calculated EEQs (slope = 0.93), suggesting that the estrogenic activities in the sediments were mainly attributed to the seven selected chemicals.

Temporal variations of EDCs and estrogenic activities

The statistical method of one-way analysis of variance (ANOVA) was used to assess the seasonal differences. For the three xenoestrogens in surface water, significant seasonal differences of the concentrations for 4-NP were observed in the Liuxi River ($F = 6.66$, $p = 0.03$), the Zhujiang River ($F = 16.5$, $p = 0.0004$) and the Shijing River ($F = 7.64$, $p = 0.015$). Significant seasonal differences for 4-t-OP were also found in the Luixi River ($F = 7.85$, $p = 0.02$) and the Zhujiang River ($F = 4.28$, $p = 0.048$). The concentrations of 4-t-OP and 4-NP in the wet season were higher than those in the dry season. However, no significant seasonal difference for BPA was observed. For the four natural and synthetic estrogens in surface water, the only significant seasonal differences were observed for E1 ($F = 6.89$, $p = 0.02$), E2 ($F = 0.12$, $p = 0.001$) and EE2 ($F = 12.9$, $p = 0.003$) in the Shijing River. The mean concentration for EE2 in the wet season was higher than that in the dry season in the Shijing River, while the mean concentrations for E1 and E2 in the wet season were lower than those in the dry season of the Shijing River. The
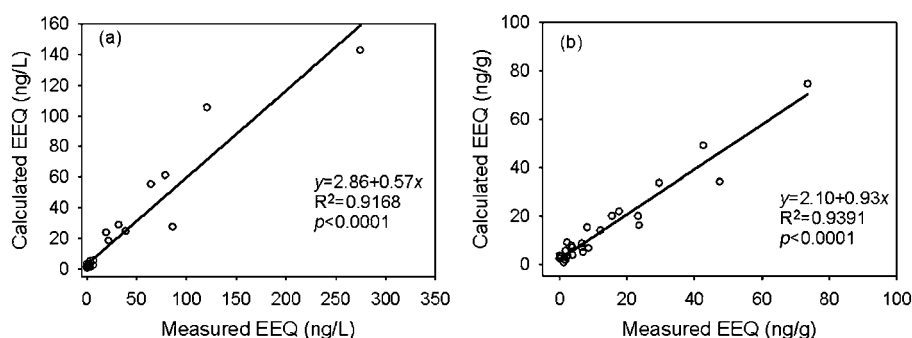

Fig. 2 Relationships of measured EEQs *versus* calculated EEQs for (a) surface water, and (b) sediment in the dry season (December 17–18, 2007) and the wet season (September 12–13, 2008). EEQ: 17β-estradiol equivalent.

concentrations of DES also displayed an obvious seasonal difference in the Shijing River since DES was only detected in surface water from the wet season.

In the sediments of the three rivers, seasonal differences for most chemicals were not as obvious as those in surface water. Among the three xenoestrogens, significant seasonal differences for 4-*t*-OP were observed in the three rivers (Liuxi River: $F = 5.23$, $p = 0.05$; Zhujiang River: $F = 10.45$, $p = 0.003$; Shijing River: $F = 6.60$, $p = 0.025$), and BPA only showed significant seasonal difference in the Zhujiang River ($F = 7.05$, $p = 0.013$). No significant seasonal difference for 4-NP was found in sediments. For the four estrogens, only the concentrations for E1 showed a significant seasonal difference in the Shijing River ($F = 9.88$, $p = 0.008$).

Statistical analysis also showed significant seasonal differences for estrogenic activities in the surface water of the Zhujiang River ($F = 12.99$, $p = 0.0013$) and in sediments of the Liuxi River ($F = 18.22$, $p = 0.013$). Mean values of EEQs in the wet season were higher than those in the dry season in surface water of the Zhujiang River and in sediments of the Liuxi River.

Discussion

Comparison with other regions of the world

EDCs have been detected at various levels in surface water and sediment in some regions of the world (Table 2). Lower concentrations of xenoestrogens (4-*t*-OP, 4-NP and BPA) and estrogens (E1, E2 and EE2) were reported in surface water from Germany,[37,38] while slightly higher concentrations for 4-*t*-OP and 4-NP detected in surface water from Canada[34] and Japan.[9] Levels of EDCs in the Liuxi River and the Zhujiang River from the present study fall within the concentration ranges of the rivers of Germany, Canada and Japan. In the USA, significant differences between the concentrations for xenoestrogens and estrogens were found between small streams and large rivers.[10,14] In the Mississippi River, the maximum concentrations for 4-NP and E1 were only several hundreds of ng L^{-1} and a few ng L^{-1},

respectively,[14] while in small streams, the maximum concentrations were up to tens of thousands of ng L^{-1} for 4-NP, and a few hundred ng L^{-1} for E1.[10] Significant differences also existed in the Pearl River system. Much higher levels of the EDCs were found in the Shijing River when compared to those found in the Zhujiang River and the Liuxi River (Table 1).

In sediments, the reported concentrations for xenoestrogen ranged from ND to several parts per million, while most of the estrogens were not detected or at a few parts per billion levels.[11,12,38–41] Obviously, the concentrations of the EDCs in sediments of the Pearl River system (especially the Shijing River) were generally higher than those in other regions of the world. The maximum concentrations in the sediments of the Shijing River were 10–40-fold higher than the reported maximum concentrations in sediments of other regions.

The reported ranges of estrogenic activities by bioassay in surface water from Korea, Japan, Portugal, the UK and the USA were from below the limit of detection to several ng L^{-1} EEQ,[12,24,42–45] which were similar to the range in the Liuxi River and the Zhujiang River (Table 3). The estrogenic activities in the Rhine River in Germany were slightly higher than the above regions, with an average EEQ of 19.42 ± 2.8 ng L^{-1}.[19] Compared with these regions, the estrogenic activities in the Shijing River were generally higher than any other reported data, with the maximum EEQ up to several hundred ng L^{-1}. Data on estrogenic activities in sediments are scarce in the literature. Previously reported EEQs in sediments near to sewage treatment plant outfalls were at the few ng g^{-1} level in Tokyo Bay, Japan[12] and at the pg g^{-1} level in the Ouse River, UK.[24] The EEQs in the sediments of the Pearl River system ranged from ND to 101 ng g^{-1}, and the maximum EEQ in the Shijing River were higher than those found in other regions. The high estrogenic activities in the surface water and sediments of the Shijing River were consistent with the results from the chemical analysis of the seven estrogenic compounds.

The Liuxi River and the Zhujiang River are important drinking water sources for Guangzhou city and the surrounding towns. The water quality of the two rivers was classified as level "II" and level "III" (slightly contaminated water) according to

Table 2 Distributions of some endocrine disrupting chemicals in surface waters and sediments in different regions of the world

| Location | Concentration[a] | | | | | | |
	4-*t*-OP	4-NP	BPA	E1	E2	EE2	Reference No.
Surface water (ng L^{-1})							
USA		ND[b] – 40000 (800)	ND–12000 (140)	ND–112 (27)	ND–93 (9)	ND–831 (73)	10
USA		ND–147.2		ND–4.7	ND–4.5	ND	14
Canada	< LOD[c]–84	< LOD–920	<LOQ	ND	ND		34,35
Spain				2.5–21.7	ND	ND	36
Germany	0.8–54 (3.8)	6.7–134 (23)	0.5–14 (3.8)	0.1–4.1 (0.4)	0.15–3.6 (0.3)	0.1–5.1 (0.4)	37
Germany	<0.5–3.3 (1.9)	13–53 (28)	3.8–30 (20.5)	1.6	ND	ND	38
Japan	10–180	51–1080					9
Korea				1.7–5 (3.6)	ND	ND	13
China	2.38–3150 (10.2)	64.8–20080 (521)	4.35–1390 (163)	0.62–78.7 (3.03)	ND–7.72 (1.58)	ND–53.4 (10.3)	This study
Sediments (ng g^{-1})							
USA	ND–12.5	ND–22.9	ND–5.0				39
USA	<LOD–8.2	122–3200		<0.03–0.6	0.16–0.45 (0.3)		11
Germany	2.4–25 (8.3)	27–428 (67)	10–379 (58)	<0.2	<0.2	<0.2	39
Japan		ND–46 (31)	ND–22.0	(10.3)	(4.8)		12,40,41
China	<LOD – 979 (21.2)	11.4–28830 (3460)	<LOD – 296 (76.5)	ND–38.0 (11.2)	ND–4.12 (1.68)	ND–21.2 (11.0)	This study

[a] Concentration range, with median value in parentheses. 4-*t*-OP: 4-*t*-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol A; E1: estrone; E2: 17β-estradiol; EE2: 17α-ethinylestradiol. [b] ND, not detected. [c] LOD, the limit of detection.

Table 3 Distributions of estrogenic activities in surface waters and sediments in different regions of the world

Location	EEQ[a]	Bioassay method[d]	Reference No.
Surface water (ng L^{-1} EEQ)			
Elkhorn River, Nebraska, USA[b]	(0.465)	E-SCREEN	42
Flemish Rivers, France	2.75–81.4	YES	18
Rhine River, Germany[b]	(19.42 ± 2.8)	YES	19
Ouse River, UK	<0.04	YES	24
Rivers, Portugal	0.10–1.7	YES	43
Tokyo Bay, Japan	0.70–4.01	MVLN	12
Kumho river, Korea	ND[c]–7.43	E-SCREEN	44
Youngsan River, Korea	0.021–1.918	E-SCREEN	45
The Pearl River system, China	0.23–324 (1.53)	YES	This study
Sediments (ng g^{-1} EEQ)			
Southern California Bight, USA	1–90	VTG	11
Ouse River, UK	0.0213–0.0299	YES	24
Tokyo Bay, Japan	2.07–12.1	MVLN	12
The Pearl River system, China	ND–101 (6.81)	YES	This study

[a] EEQ, estradiol equivalent. Range with median value in parentheses. [b] Mean value in parentheses. [c] ND, not detected. [d] E-SCREEN, MCF-7 (human estrogen receptor-positive breast cancer cell line) cell proliferation assay; YES, recombinant yeast estrogen screen; MVLN, pERE-luciferase reporter assay; VTG, vitellogenin assay.

the water quality parameters (chemical oxygen demand, NH$_3$-nitrogen, total phosphorus and dissolved oxygen, *etc.*) of the Chinese national standards.[46] The Shijing River receives direct discharge of untreated wastewater from the nearby towns along the river.[32] The annual input of the wastewater into the river was estimated to be more than 100 million tonnes, which was mainly domestic wastewater. The water quality was classified as level "V" (most polluted water).[46,47] As a tributary river of the Pearl River system, the Shijing River has become a source of micropollutants, including EDCs, to the Zhujiang River.[48–50] Despite the fact that these micropollutants from the Shijing River are diluted in the Zhujiang River because of its large water flow from upstream and mixing by tidal waves (Table S7‡), some could partition into the sediments and become a sink for these estrogenic substances.[24] These contaminants in the sediments could be a potential pollution source to surface water when these compounds are released from the sediments through desorption processes.

Risk assessment of estrogenic activities in the Pearl River system

The PNEC for E2 was 1.5 ng L^{-1}, which was derived from the HC_5 value (the hazardous concentration for 5% of species) of the SSD curve using 77 *in vivo* NOECs (Fig. S1‡). The risk of estrogenic activities to aquatic organisms was then assessed according to the rank of RQs, which was the ratio of measured environmental concentration (MEC) of estrogenic activity in a certain site and PNEC of E2.

In surface water, the MEC corresponding to estrogenic activity could be the measured EEQ or the calculated EEQ from chemical analysis and bioassay methods, respectively. However, from the modeling equation between measured EEQs and calculated EEQs in surface water (Fig. 2(a)), we can see that the calculated EEQ from the addition of seven selected estrogenic chemicals only reflects part of the estrogenic effect of a certain sample. Hence the measured EEQ by bioassay was used as the MEC of estrogenic activity when the risk assessment was performed for surface water. Based on the RQ criteria, the

estrogenic risks of surface water in the Liuxi River, the Zhujiang River and the Shijing River were illustrated in Fig. 3(a) for the two seasonal samplings. In the Pearl River system, the upstream reaches in both seasons and the downstream reaches in the wet season for the Liuxi River showed minimal or medium risks. Only one site (S5) in the dry season in the upstream section of the Zhujiang River displayed high risk, while the area with high risks expanded to include midstream sections of the Zhujiang River and downstream sections of the Liuxi River in the wet season. For the estrogenic risks in the Shijing River, the whole reach had high risks whether in the dry or wet season; however, the RQs in the wet season were also higher than in the dry season. Higher risks for surface water were found in the wet season than in the dry season, suggesting that runoff from nearby towns and roads of Guangzhou are very likely to carry estrogenic compounds during the wet season. This phenomenon was also found in an investigation into the distribution of polycyclic aromatic hydrocarbons (PAHs) in this area.[51]

Since the measured EEQs of sediments were based on organic solvent extraction, risk potentials using these data may be overestimated since the estrogenic compounds in the sediments are not fully bioavailable.[17,52,53] The present study developed a sediment risk assessment approach by using pore water EEQs calculated from chemical concentration data based on equilibrium partitioning theory, and the same aquatic toxicity data as used for surface water. Since the estrogenic activity is mainly attributed to the seven selected estrogenic chemicals in sediments (Fig. 2(b)), it is reasonable that the pore water EEQ calculated from chemical concentration data be used as the MEC. The RQs in the sediments of the Pearl River system were then illustrated in Fig. 3(b). The whole of the Shijing River, downstream sections of the Liuxi River, and the upstream and midstream reaches of the Zhujiang River displayed high estrogenic risks, while the other river reaches showed minimal or medium estrogenic risks. Estrogenic risks from the sediments of the Pearl River system had little variation between the dry and wet seasons. The sediments were an important sink of these estrogenic compounds in the Pearl River system, a finding consistent with a previous study in
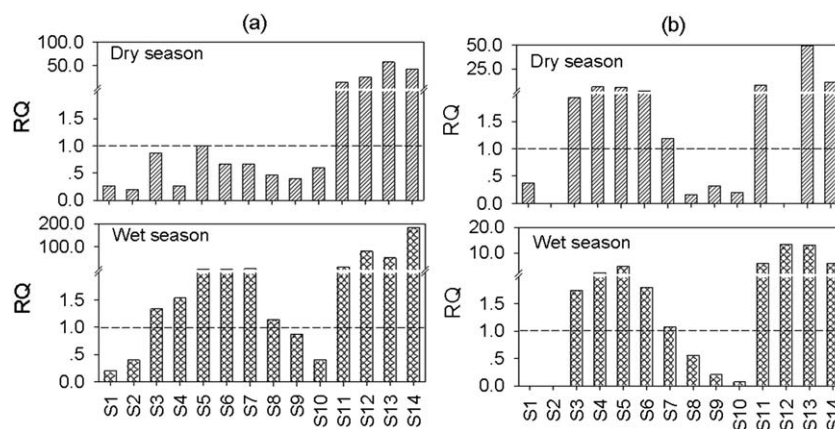
Fig. 3 Risk quotients (RQs) of estrogenic activities in (a) surface waters and (b) sediments of the Pearl River system in the dry season (December 17–18, 2007) and the wet season (September 12–13, 2008).

another region;[24] therefore, the sediments could be a good indicator of estrogenic contamination in the rivers.

In general, those river sections with high estrogenic risks are urban streams or reaches near the central areas of Guangzhou city. There are more than 200 urban streams along the Zhujiang River. These small streams are dammed most of the time, but direct overflow into the Zhujiang River often occurs during heavy rain events. Hence, these streams as well as effluents constitute the pollution sources for the Zhujiang River and the Liuxi River. As the Zhujiang River and the Liuxi River are the most important drinking water sources for Guangzhou city and nearby towns, as well as the habitat for many fish species, proper measures should be taken to control estrogenic contamination sources along the river sections with high estrogenic risks.

Conclusions

Estrogenic contamination patterns were assessed in the Pearl River system (Liuxi River, Zhujiang River and Shijing River) by chemical analysis and *in vitro* (YES) bioassay of surface water and sediment in dry and wet seasons. Higher concentrations of seven selected estrogenic compounds were found in the surface waters and sediments in the Shijing River than in the Zhujiang River and the Liuxi River, which were consistent with the measured EEQs in the same surface waters and sediments in those rivers. The modeling of the measured EEQs and calculated EEQs displayed good linear correlations, but also revealed that the seven selected chemicals only accounted for part of the estrogenic activity in surface water, whilst accounting for almost all of the estrogenic activity in sediments. High estrogenic risks to aquatic organisms could be found in the whole of the Shijing River, the upstream and midstream reaches of the Zhujiang River, and downstream sections of the Liuxi River. Higher risks are expected in wet seasons mainly owing to the input of runoff and overflow of urban streams. Little variations in the estrogenic risks from the river sediments were found between the two seasons. Further studies are needed to investigate potential biological effects to aquatic organisms in the river sections with high estrogenic risks.

References

1 T. Colborn, F. S. V. Saal and A. M. Soto, *Environ. Health Perspect.*, 1993, **101**, 378–384.
2 C. Desbrow, E. J. Routledge, G. C. Brighty, J. P. Sumpter and M. Waldock, *Environ. Sci. Technol.*, 1998, **32**, 1549–1558.
3 K. L. Thorpe, T. H. Hutchinson, M. J. Hetheridge, J. P. Sumpter and C. R. Tyler, *Environ. Toxicol. Chem.*, 2000, **19**, 2812–2820.
4 K. A. Kidd, P. J. Blanchfield, K. H. Mills, V. P. Palace, R. E. Evans, J. M. Lazorchak and R. W. Flick, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 8897–8901.
5 J. P. Giesy, S. L. Pierens, E. M. Snyder, S. Miles-Richardson, V. J. Kramer, S. A. Snyder, K. M. Nichols and D. A. Villeneuve, *Environ. Toxicol. Chem.*, 2000, **19**, 1368–1377.
6 P. Sohoni, C. R. Tyler, K. Hurd, J. Caunter, M. Hetheridge, T. Williams, C. Woods, M. Evans, R. Toy, M. Gargas and J. P. Sumpter, *Environ. Sci. Technol.*, 2001, **35**, 2917–2925.
7 M. Hecker, W. J. Kim, J. W. Park, M. B. Murphy, D. Villeneuve, K. K. Coady, P. D. Jones, K. R. Solomon, G. van der Kraak, J. A. Carr, E. E. Smith, L. du Preez, R. J. Kendall and J. P. Giesy, *Aquat. Toxicol.*, 2005, **72**, 383–396.
8 I. Yano and R. Hoshino, *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.*, 2006, **144**, 18–23.
9 T. Isobe, H. Nishiyama, A. Nakashima and H. Takada, *Environ. Sci. Technol.*, 2001, **35**, 1041–1049.
10 D. W. Kolpin, E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber and H. T. Buxton, *Environ. Sci. Technol.*, 2002, **36**, 1202–1211.
11 D. Schlenk, Y. Sapozhnikova, M. A. Irwin, L. T. Xie, W. Hwang, S. Reddy, B. J. Brownawell, J. Armstrong, M. Kelly, D. E. Montagne, E. P. Kolodziej, D. Sedlak and S. Snyder, *Environ. Toxicol. Chem.*, 2005, **24**, 2820–2826.
12 S. Hashimoto, A. Horiuchi, T. Yoshimoto, M. Nakao, H. Omura, Y. Kato, H. Tanaka, K. Kannan and J. P. Giesy, *Arch. Environ. Contam. Toxicol.*, 2005, **48**, 209–216.
13 S. D. Kim, J. Cho, I. S. Kim, B. J. Vanderford and S. A. Snyder, *Water Res.*, 2007, **41**, 1013–1021.

14 S. Y. Zhang, Q. A. Zhang, S. Darisaw, O. Ehie and G. D. Wang, *Chemosphere*, 2007, **66**, 1057–1069.

15 E. L. M. Vermeirssen, R. Burki, C. Joris, A. Peter, H. Segner, M. J. F. Suter and P. Burkhardt-Holm, *Environ. Toxicol. Chem.*, 2005, **24**, 2226–2233.

16 L. Salste, P. Leskinen, M. Virta and L. Kronberg, *Sci. Total Environ.*, 2007, **378**, 343–351.

17 L. Viganò, E. Benfenati, A. van Cauwenberge, J. K. Eidem, C. Erratico, A. Goksøyr, W. Kloas, S. Maggioni, A. Mandich and R. Urbatzk, *Chemosphere*, 2008, **73**, 1078–1089.

18 H. E. Witters, C. Vangenechten and P. Berckmans, *Water Sci. Technol.*, 2001, **43**, 117–123.

19 S. Pawlowski, T. A. Ternes, M. Bonerz, A. C. Rastall, L. Erdinger and T. Braunbeck, *Toxicol. in Vitro*, 2004, **18**, 129–138.

20 B. L. L. Tan, D. W. Hawker, J. F. Muller, F. D. L. Leusch, L. A. Tremblay and H. F. Chapman, *Environ. Int.*, 2007, **33**, 654–669.

21 Y. T. Li, F. B. Li, T. B. Zhang, G. Y. Yang, J. J. Chen and H. F. Wan, *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.*, 2007, **42**, 1979–1987.

22 B. Chen, B. X. Mai, J. C. Duan, X. J. Luo, Q. S. Yang, G. Y. Sheng and J. M. Fu, *Mar. Pollut. Bull.*, 2005, **50**, 993–997.

23 X. Z. Peng, Y. J. Yu, C. M. Tang, J. H. Tan, Q. X. Huang and Z. D. Wang, *Sci. Total Environ.*, 2008, **397**, 158–166.

24 M. Peck, R. W. Gibson, A. Kortenkamp and E. M. Hill, *Environ. Toxicol. Chem.*, 2004, **23**, 945–952.

25 B. Sundelin, C. Ryk and G. Malmberg, *Environ. Toxicol.*, 2000, **15**, 518–526.

26 M. Duft, C. Schmitt, J. Bachmann, C. Brandelik, U. Schulte-Oehlmann and J. Oehlmann, *Ecotoxicology*, 2007, **16**, 169–182.

27 J. L. Zhao, G. G. Ying, L. Wang, J. F. Yang, X. B. Yang, L. H. Yang and X. Li, *Sci. Total Environ.*, 2009, **407**, 962–974.

28 E. J. Routledge and J. P. Sumpter, *Environ. Toxicol. Chem.*, 1996, **15**, 241–248.

29 E.C., *Technical Guidance Document on Risk Assessment in support of Commissions Directive 93/67/EEC on Risk Assessment for new notified substances and Commissions Regulation (EC) No. 1488/94 on risk assessment for existing substances*, Ispra, Italy, European Chemicals Bureau, 1996.

30 D. J. Caldwell, F. Mastrocco, T. H. Hutchinson, R. Lange, D. Heijerick, C. Janssen, P. D. Anderson and J. P. Sumpter, *Environ. Sci. Technol.*, 2008, **42**, 7046–7054.

31 M. D. Hernando, M. Mezcua, A. R. Fernandez-Alba and D. Barcelo, *Talanta*, 2006, **69**, 334–342.

32 Q. Li, X. P. Sun, X. H. Chen, J. Liu, H. X. Yu and Z. Tao, *Acta Sci. Nat. Univ. Sunyatseni*, 2008, **47**, 131–135.

33 K. L. Thorpe, M. Gross-Sorokin, I. Johnson, G. Brighty and C. R. Tyler, *Environ. Health Perspect.*, 2006, **114**, 90–97.

34 D. T. Bennie, C. A. Sullivan, H. B. Lee, T. E. Peart and R. J. Maguire, *Sci. Total Environ.*, 1997, **193**, 263–275.

35 G. R. Boyd, H. Reemtsma, D. A. Grimm and S. Mitra, *Sci. Total Environ.*, 2003, **311**, 135–149.

36 S. Rodriguez-Mozaz, M. J. L. de Alda and D. Barcelo, *J. Chromatogr., A*, 2004, **1045**, 85–92.

37 H. M. Kuch and K. Ballschmiter, *Environ. Sci. Technol.*, 2001, **35**, 3201–3206.

38 B. Stachel, U. Ehrhorn, O. P. Heemken, P. Lepom, H. Reincke, G. Sawal and N. Theobald, *Environ. Pollut.*, 2003, **124**, 497–507.

39 J. D. Stuart, C. P. Capulong, K. D. Launer and X. J. Pan, *J. Chromatogr., A*, 2005, **1079**, 136–145.

40 H. Kawahata, H. Ohta, M. Inoue and A. Suzuki, *Chemosphere*, 2004, **55**, 1519–1527.

41 Y. Kitada, H. Kawahata, A. Suzuki and T. Oomori, *Chemosphere*, 2008, **71**, 2082–2090.

42 A. M. Soto, J. M. Calabro, N. V. Prechtl, A. Y. Yau, E. F. Orlando, A. Daxenberger, A. S. Kolok, L. J. Guillette, I. le Bizec, I. G. Lange and C. Sonnenschein, *Environ. Health Perspect.*, 2004, **112**, 346–352.

43 L. Quirós, R. Céspedes, S. Lacorte, P. Viana, D. Raldúa, D. Barceló and B. Piña, *Environ. Toxicol. Chem.*, 2005, **24**, 389–395.

44 S. M. Oh, S. Y. Choung, Y. Y. Sheen and K. H. Chung, *Sci. Total Environ.*, 2000, **263**, 161–169.

45 S. M. Oh, K. Park and K. H. Chung, *Sci. Total Environ.*, 2006, **354**, 252–264.

46 China EPA, *Environmental quality standards for surface water (in Chinese)*, GB 3838–2002, National Environmental Protection Agency of China, Beijing, 2002.

47 J. H. Luo, *Chongqing Environ. Sci.*, 2002, **24**, 70–72.

48 J. F. Yang, G. G. Ying, J. L. Zhao, R. Tao, H. C. Su and F. Chen, *Sci. Total Environ.*, 2010, **408**, 3424–3432.

49 J. L. Zhao, G. G. Ying, Y. S. Liu, F. Chen, J. F. Yang and L. Wang, *J. Hazard. Mater.*, 2010, **179**, 215–222.

50 J. L. Zhao, G. G. Ying, Y. S. Liu, F. Chen, J. F. Yang, L. Wang, X. B. Yang, J. L. Stauber and M. St. J. Warne, *Environ. Toxicol. Chem.*, 2010, **29**, 1377–1384.

51 J. Z. Wang, Y. F. Guan, H. G. Ni, X. L. Luo and E. Y. Zeng, *Environ. Sci. Technol.*, 2007, **41**, 5614–5619.

52 G. Pojana, A. Gomiero, N. Jonkers and A. Marcomini, *Environ. Int.*, 2007, **33**, 929–936.

53 G. G. Ying and R. S. Kookana, *Environ. Sci. Technol.*, 2003, **37**, 1256–1260.