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## Determination of estrogens and estrogenic activities in water from three rivers in Tianjin, China

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### Abstract

Studies on estrogenic disrupting compounds (EDCs) occurrence and identification of main responsible compounds in river water discharged into the sea are of significance. In the present research, we screened estrogenic activities of 10 river water samples from 3 main rivers discharged into Bohai Sea in Tianjin using a recombinant two-hybrid yeast assay and chemical analysis by gas chromatography-mass spectrometry. All sample extracts induced significant estrogenic activity, with 17 $\beta$ -estradiol equivalents (EEQ) of raw water ranging from 5.72 to 59.06 ng/L. Six most concerned EDCs in the river water samples including estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol, estriol, diethylstilbestrol and estradiol valerate were determined, with their concentrations up to 50.70, 31.40, 24.40, 37.20, 2.56, and 8.47 ng/L, respectively. Through causality analysis by comparing the EEQ values of yeast assay and chemical analysis, 17 $\alpha$ -ethinylestradiol and 17 $\beta$ -estradiol were identified as the main contributors to the estrogenic effects of the river samples, accounting for the whole estrogenic activities (62.99% to 185.66%), and estrogen antagonistic compounds might presented in the heavy polluted water samples. The proposed approach using both chemical analysis and bioassay could be used for identification and evaluation of the estrogenic activity of EDCs in river water.

**Key words:** river water; endocrine disrupting compounds (EDCs); yeast assay; bioassay

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### Introduction

There is growing concern that a large number of industrial, agricultural and medical chemicals can cause endocrine disrupting effects in wild animals and humans (Sumpter, 1997; Tashiro et al., 2003). Of the endocrine disrupting chemicals (EDCs), estrogenic compounds which are biologically active at very low concentrations may be the major endocrine disruptors responsible for the increase in disturbances of wildlife reproduction and development (Sharpe and Irvine, 2004). Laboratory studies with fish provided evidence that feminization responses in several species of fish could be found at environmentally relevant concentrations (ng/L) of human estrogens (Zha et al., 2008)

The appearance of artificial estrogens in marine ecosystem and their endocrine disrupting effects on the wild

marine fish species have been caused increasing concern (Scott et al., 2006). Many male marine species such as flounder, grey mullet and tuna in estuarine, coast or in the open sea have been found with unexpectedly high concentrations of vitellogenin, a protein associated with reproducing females, in male fish (Sumpter and Jobling, 1995; Allen et al., 1999; Hara et al., 2001; Ohkubo et al., 2003). Discharge of raw sewage has been identified as one of the most important sources of human estrogens in the marine environment (Saravanabhavan et al., 2009).

Recent years, pollution in Bohai Sea becomes more serious in China. The Bohai Sea is a shallow and semi-enclosed water basin. The water exchange between Bohai Bay and Bohai Sea is weak, which leads to poor self-clean capacity and high accumulation of pollutants. As the main water-receiving body, the Bohai Sea which receives both industrial and domestic wastewater discharges has been greatly influenced by sewage discharge from Tianjin, Beijing and Hebei Province (Song et al., 2006). Therefore, it is necessary to trace EDCs in river water discharged into

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the sea so as to protect the marine ecosystem.

To detect and identify the substances responsible for the observed estrogenic activities in environmental samples and trace their sources, the bioassay combined with gas (or liquid) chromatography-mass spectrometry was proved to be an effectively method (GS-MS or LC-MS) (Reineke et al., 2002; Matthiessen et al., 2006; Jonkers et al., 2010). Of the bioassays, *in vivo* experiments for investigating estrogenic effects are usually expensive and time-consuming, while *in vitro* bioassays are relatively rapid, inexpensive, and easy to perform to give first evidence of the occurrence of the total estrogenic contaminants in environmental samples (Campbell et al., 2006). Among the *in vitro* bioassays, the yeast estrogen screen has been successfully applied in determining estrogenic activity of chemicals or environmental samples (Vermeirssen et al., 2005). For example, 17 $\beta$ -estradiol, estrone and ethinyl-estradiol were identified as the main contributors to the estrogenic effects of the environment samples in the United Kingdom using *in vitro* yeast system and bioassay directed chemical analysis method (Desbrow et al., 1998).

Considering that the estrogenic activity in river waters discharged into the Bohai Sea as well as the responsible compounds have been hardly studied, in the present study, we have applied a combination of chemical analysis and a two-hybrid yeast assay to assess the estrogenic activity in water from three rivers in Tianjin which discharged into the Bohai Sea and estimated the contribution of the estrogens on the total estrogenic activities of the river samples. The three rivers flowing through Tianjin city are very important channels to carry sewage from Tianjin into the Bohai Sea. Six estrogens including estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol, estriol, diethylstilbestrol and estradiol valerate (Lu et al., 2010), which were most frequently discovered EDCs in water bodies of China, were selected. To identify the main contributors to the estrogenic activities of the rivers, 17 $\beta$ -estradiol equivalent (EEQ) approach was introduced in the present study (Ra et al., 2011). Therefore, the aim of the present work was to screen the level of estrogenic activities in three rivers in Tianjin and identify the specific compounds responsible for the estrogenic activities, so as to provide useful information to protect the marine ecosystem of the Bohai Sea.

## 1 Materials and methods

### 1.1 Chemicals and materials

The purity of target compounds estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol, estriol, diethylstilbestrol,  $\beta$ -estradiol-17-valerate, and solvent dimethyl sulfoxide were higher than 98% (Sigma Chemical Co., St. Louis, USA). The derivatization reagent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% of trimethylchlorosilane (TCMS) and trimethylsilylimidazole (TMSI) and internal standard

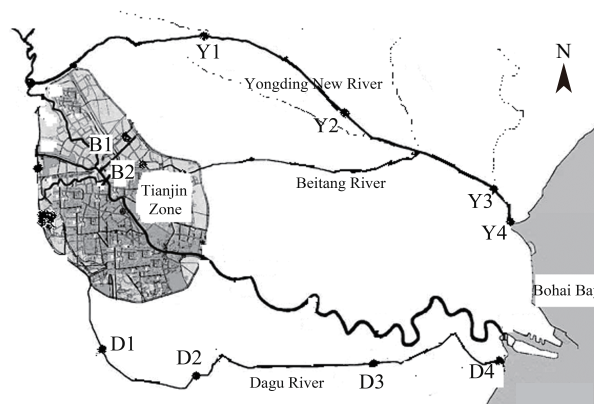
pyrene-d10 were purchased from Supelco (Supelco /Sigma Chemical Co., St. Louis, USA). HPLC grade hexane, dichloromethane, methanol and tert-butyl methyl ether were purchased from Fisher Scientific (Fair Lawn, USA). The cartridges used for solid phase extraction were Oasis hydrophilic lipophilic balance (HLB) cartridges (500 mg, 6 mL, N-vinylpyrrolidone-m-divinylbenzene copolymer) which were obtained from Waters Corporation (USA). Glass fiber filters were purchased from Millipore (0.45  $\mu$ m, USA) and pyrolyzed at 450°C for 4 hr prior to use.

### 1.2 Sample preparation

Samples were taken from the Yongding New River, Beitang River and Dagou River in Tianjin area, northern China in June 2007 (rainy season). Industrial, domestic and agricultural wastewaters from Tianjin area are directly or indirectly discharged into the Yongding New River, Beitang River and Dagou River which carry the water to the Bohai Sea (Song et al., 2006). Samples were conducted along the rivers according to the location of chemical factories (D3), drainage outlets (B1, B2, D2), bridges (Y2), river confluence reaches (D1, Y1, Y3), and entrances of rivers (D4, Y4) (Fig. 1). Throughout the survey a global positioning system was used to locate the sampling locations.

The water samples (2 L for bioassay and 2 L for chemical analysis) were collected in pre-cleaned amber glass bottles. Appropriate amount of methanol (2 mL/L, V/V) was added in each sample immediately to prevent bacterial degradation. Samples were immediately sealed and stored at 4°C until treatment. All samples were treated within 48 hr.

Water samples and procedure blank (Milli-Q water, 18.2  $\Omega$ /cm) were filtered with polytetrafluoroethylene fiberglass filters (0.45  $\mu$ m, Millipore, USA) to remove insoluble materials and adjusted pH to 2–3 with hydrochloric acid. A 500 mg Oasis HLB cartridges (Waters, USA) were used for solid phase extraction, and conditioned with 5 mL of tert-



**Fig. 1** Sampling sites. D3: chemical factories; B1, B2, D2: drainage outlets; Y2: bridges; D1, Y1, Y3: river confluence reaches; D4, Y4: entrances of rivers.



butyl methyl ether, 5 mL of methanol and 5 mL of Milli-Q water prior to use. The 4 L sample was filtered through the cartridges under vacuum at a flow rate of 6 mL/min, and then kept under vacuum aspiration for 5 min to dry out. After that, the HLB cartridges were then rinsed sequentially with 5 mL of methanol/Milli-Q water (1:3, V/V), 5 mL of Milli-Q water and 5 mL of methanol/ammonia/ultra-pure water (10:2:88, V/V/V). After drying by vacuum pressure, the analytes were eluted with 10 mL of methanol/tert-butyl methyl ether (1/9, V/V). The extracts were then combined and filtered by anhydrous sodium sulphate to remove water and evaporated to dryness in a rotary evaporator (R-200, Buchi, France) at 40°C to 2 mL. The dehydrated extract was blown to dryness under gentle nitrogen flow and reconstituted in 100  $\mu$ L of dimethyl sulfoxide used for bioassay and 100  $\mu$ L for chemical analysis immediately. Finally extracts were stored at –20°C in glass vials.

### 1.3 Yeast assay

The bioassay was conducted using ER yeast assay as described by Li et al. (2010). All assays were conducted in four times. Each assay group included the sample, the procedural blank (extracts of Milli-Q water), the positive control (17 $\beta$ -estradiol), and the negative control (dimethyl sulfoxide). Five microlitre of serial dilutions of test samples were combined with 995  $\mu$ L of medium containing  $5 \times 10^3$  yeast cells/mL. The  $\beta$ -galactosidase activity was calculated according to the following equations:

$$u = C_s/t \times V \times D \times ODs \quad (1)$$

$$C_s = 10^{-6}(A_s - A_B)/\epsilon d \quad (2)$$

where,  $u$  is  $\beta$ -galactosidase activity,  $t$  is incubation duration of the enzyme reaction,  $V$  is volume of the test culture,  $D$  is dilution factor,  $OD_s$  is  $OD_{600}$  of test culture,  $A_s$  is  $OD_{420}$  of the enzyme reaction supernatant of the sample,  $A_B$  is  $OD_{420}$  of the enzyme reaction supernatant of the blank,  $\epsilon$  is  $\epsilon$  for o-nitrophenol in the enzyme assay reaction mix, and  $d$  is diameter of the cuvette (Routledge and Sumpter, 1996; Gaido et al., 1997).

Test cultures (200  $\mu$ L) were transferred into each well of the 96-well plate and incubated at 30°C with vigorous orbital shaking (800 r/min) on a titer plate shaker (Heidolph TITRAMAX 1000, Hamburg, Germany) for 2 hr, then the cell density of the culture was measured at 600 nm wavelength (TECAN GENios A-5002, Salzburg, Austria). Then a 50- $\mu$ L test culture was transferred to a new 96-well plate. After addition of 120  $\mu$ L of Z-buffer (16.1 g/L  $Na_2HPO_4 \cdot 7H_2O$ ; 5.5 g/L  $NaH_2PO_4 \cdot H_2O$ ; 0.75 g/L KCl; 0.246 g/L  $MgSO_4 \cdot 7H_2O$ ) and 20  $\mu$ L chloroform, the solution were carefully mixed and preincubated for 5 min at 30°C. The enzyme reaction was started by adding 40  $\mu$ L o-nitrophenyl- $\beta$ -D-galactopyranoside (13.3 mmol/L, dissolved in Z-buffer), then incubated at 30°C with vigorous orbital shaking (800 r/min) on a titer plate shaker for 60

min. The reactions were terminated by the addition of 100  $\mu$ L  $Na_2CO_3$  (1 mol/L). After centrifugation at 12,000  $\times g$  for 15 min (Sigma Laborzentrifugen 2K15, Osterode, Germany), 200  $\mu$ L of the supernatant was transferred into a new 96-well plate and  $OD_{420\text{ nm}}$  was determined. To exclude the false results caused by cytotoxicity, the cell viability was determined spectrophotometrically as a change of cell density ( $OD_{600\text{ nm}}$ ) in the assay medium.

### 1.4 Instrumental analysis

Details of the derivatization and analytical conditions of target compounds are described elsewhere (Lei et al., 2009). In brief, the residues of water samples were redissolved in 0.4 mL of hexane that contained 50  $\mu$ L of the derivatization mixture BSTFA/TCMS/TMSI (bis(trimethyl)-trifluoroacetamide/trimethylchlorosilane/trimethylsilylimidazole) (99:1:0.5, V/V/V) and 50 ng/mL of pyrene-d10. After the derivatization was performed at 60°C for 2 hr, they were cooled at room temperature and stored at 4°C.

The instrumental analysis was performed within two days. The targets in the samples were detected by an Agilent 6890 gas chromatograph equipped with Agilent MSD 5973 mass spectrometer. System control and data acquisition were achieved with ChemStation Software. The DB-5 capillary column of 30 m  $\times$  0.25 mm  $\times$  i.d. 0.25  $\mu$ m DB-5 was applied. Mass spectrum was operated in selected ion monitoring mode for quantitative analysis. Sample injection (1  $\mu$ L) was in splitless mode.

### 1.5 Quality assurance and quality control

To avoid contamination during the sampling and preparation processes, all laboratory materials were made of either glass or polytetrafluoroethene. All glassware were cleaned by soaking in 10% nitric acid overnight and chromic acid solution for 30 min, then washed three times by double-distilled water, and burned in a muffle furnace at 450°C for at least 4 hr.

For bioassay, the  $\beta$ -galactosidase activity of the sample was examined and compared with that of the control. Significant dose-response relationships were obtained by testing samples at serial dilutions.

For chemical analysis, all data generated from the chemical analysis were subject to strict quality control procedures. A solvent blank, a standard and a procedure blank were processed in sequence along with each set of samples to be analyzed. Relative recoveries of the six estrogenic compounds were higher than 72% for the river samples at the spiked concentration of 10 ng/L. The limit of detection and limit of quantitation for river water were 0.10–0.65 ng/L and 0.20–1.30 ng/L, respectively. Relative standard deviations were 5%–15%. Details of the quality control of chemical analysis are described by Lei et al. (2009).

## 1.6 Causality analysis

The  $EEQ_{bio}$  values were calculated according to the dose-response curve of standard chemical, 17 $\beta$ -estradiol. The  $EEQ_{cal}$  values were calculated using following equation:

$$EEQ_{cal} = \sum EEQ_i = \sum (C_i \times RP_i) \quad (3)$$

where,  $EEQ_i$  represented the EEQ value of estrogens  $i$ .  $C_i$  was the relative potency of estrogens  $i$ ;  $RP_i$  represented the relative potency of estrogens  $i$  which was obtained from the ratio between  $EC_{50}$  of 17 $\beta$ -estradiol and that of other target chemicals. The RP values of estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol, estriol, diethylstilbestrol and estradiol valerate were 0.09, 1, 1.73, 0.02, 0.02 and 0.0001, respectively (Liu et al., 2009). Then, the percentile contribution of the estrogens ( $EEQ_i$ ) in the  $EEQ_{bio}$  was calculated.

## 2 Results and discussion

### 2.1 Estrogenic activities in river waters

All extracts of river waters induce significant estrogenic activities. The estrogenic activities of river samples were then calibrated regarding to 17 $\beta$ -estradiol, the concentration dependent curve was reported by Li et al. (2010) (Table 1). The  $EEQ_{bio}$  values ranged from 5.72 to 59.06 ng/L. The highest  $EEQ_{bio}$  value has been found in sampling sites Y3, which is in Yongding New River.

The *in vitro* estrogenic activities of the river waters were extraordinarily higher than European countries and Japan, where the estrogenic activities ranged from 0.30–4.50 ng/L in France, 0.3–7.0 ng/L in Switzerland and 0.7–4.01 ng/L in Japan (Cargöuet et al., 2004; Vermeirssen et al., 2005; Hashimoto et al., 2005).

### 2.2 Concentrations of estrogenic compounds in river waters

The presence of the estrogens in river waters varied spatially (Table 1). Among 10 samples, estrone prevailed in all samples with concentrations ranged from 0.64 to 50.7 ng/L. Other estrogens could be detected partly. The concentrations of 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol, estriol, diethylstilbestrol and estradiol valerate ranged from 1.87 to 11.50 ng/L, from 1.55 to 24.40 ng/L, from 3.20 to 37.20 ng/L, from 0.67 to 2.56 ng/L and from 1.06 to 8.47 ng/L respectively. In general, concentrations of diethylstilbestrol and estradiol valerate were much lower than others. The highest concentrations of the estrogens also have been found in sampling sites Y3, which is consistent with the bioassay results.

In Table 2, the concentration ranges for selected estrogens in river waters were compared to those of previous works studied in river waters and surface waters. Diethylstilbestrol and estradiol valerate which were hardly studied by others were not involved. In Yangtze River in China, concentrations of estrone, 17 $\beta$ -estradiol and estriol were up to 3.80, 0.97 and 5.79 ng/L, respectively (Lu et al., 2010). The concentrations of estrone and 17 $\beta$ -estradiol found in Tama River in Japan were up to 19.7 and 5.1 ng/L, respectively (Kawaguchi et al., 2004). In the work of Shen et al. (2001), concentrations of 17 $\beta$ -estradiol and 17 $\alpha$ -ethinylestradiol were found in Lake Taihu in China up to 15.5 and 30.8 ng/L, respectively. In the work of Chen et al. (2007), concentrations of estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol and estriol were found in Dan-shui River up to 66.2, 33.9, 27.4 and 73.6 ng/L, respectively, similar to the present study.

### 2.3 Risk assessment and causality analysis

The  $EEQ_{cal}$  value was calculated based on the concept of concentration addition from chemical analysis, repre-

**Table 1**  $EEQ_{bio}$  and  $EEQ_{cal}$  values and concentrations of six estrogen compounds in river waters

Site		Estrone (ng/L)	17 $\beta$ -Estradiol (ng/L)	17 $\alpha$ -Ethinylestradiol (ng/L)	Estriol (ng/L)	Diethylstilbestrol (ng/L)	Estradiol valerate (ng/L)	$EEQ_{bio}$ (ng/L)	$EEQ_{cal}$ (ng/L)
Yongding	River confluence reaches (Y1)	5.20	2.06	1.55	3.2	0.92	nd	7.53	5.29
New River	Bridges (Y2)	0.6	nd	nd	nd	nd	nd	5.72	0.06
	River confluence reaches (Y3)	50.7	31.40	19.80	37.2	2.56	8.47	59.06	71.01
	Entrances of rivers (Y4)	9.7	1.87	10.10	5.96	nd	nd	18.52	20.34
Beitang River	Drainage outlets (B1)	10.5	2.82	3.24	3.15	0.67	1.06	14.21	9.45
	Drainage outlets (B2)	15.2	3.04	1.64	13.1	0.82	1.56	12.23	7.52
Dagu River	River confluence reaches (D1)	49.8	11.50	24.4	14.2	2.12	7.66	35.45	58.52
	Drainage outlets (D2)	15.4	10.80	1.89	7.18	2.15	2.30	18.09	15.64
	Chemical factory (D3)	24.2	10.90	17.40	8.26	1.07	3.62	22.08	43.37
	Entrances of rivers (D4)	12.6	6.66	6.29	3.69	1.40	nd	18.52	18.78

$EEQ_{bio}$  values of river waters derived from yeast assay; nd: below limit of detection.

Data are expressed as mean value ( $n = 3$ ).

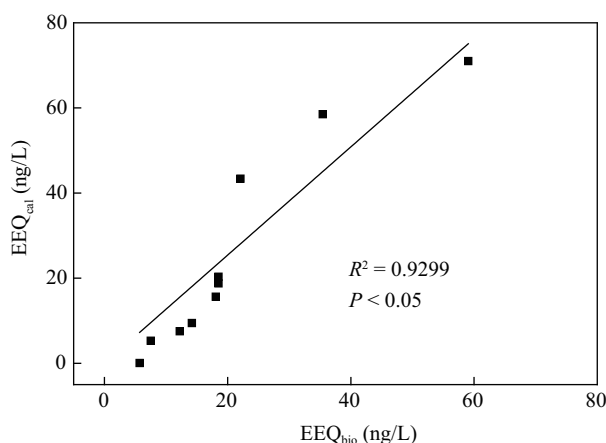
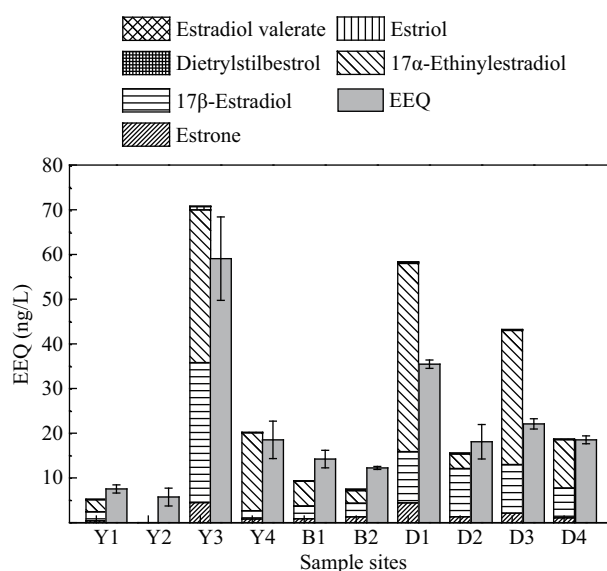
**Table 2** Comparison of estrogenic compounds concentrations in water with other studies

Location	Estrone	17 $\beta$ -Estradiol	17 $\alpha$ -Ethinylestradiol	Estril	Reference
Germany	0.1–4.1	0.15–3.6	0.1–5.1	–	Kuch and Ballschmiter, 2001
France	nd–30	nd–1.5	nd–2	nd–1.8	Labadie and Budzinski, 2005
Portugal	nd	nd	nd	nd	Ribeiro et al., 2008
The Netherlands	0.1–3.4	0.3–5.5	0.1–4.3	–	Belfroid et al., 1999
Austria	nd–4.6	nd–1.2	nd–0.33	nd–1.9	Hohenblum et al., 2004
Japan	8.7–19.7	nd	0.1–5.1	–	Kawaguchi et al., 2004
Nanjing, China	nd–3.80	nd–0.97	nd	nd–5.79	Lu et al., 2010
Jiangsu, China	–	5.5–15.5	5.7–30.8	nd	Shen et al., 2001
Shandong, China	14–180	nd–134	7–24	4–94	Zhou et al., 2011
Beijing, China	nd–2.3	–	5.0–127.9	nd–13.9	Sun et al., 2009
Beijing, China	1.1	0.2	nd	nd	Hu et al., 2005
Liaoning, China	nd–55.8	nd–7.4	nd	–	Wang et al., 2011
Taiwan, China	22.4–66.2	1.4–33.9	7.53–27.4	12.4–73.6	Chen et al., 2007
Tianjin, China	0.64–50.7	nd–31.4	nd–24.4	nd–37.2	This study

–: not available; nd: not detected.

senting the sum of estrogenic activities of six selected estrogens in the present study. Significant correlation between  $EEQ_{bio}$  and  $EEQ_{cal}$  was observed (**Fig. 2**). For the samples with high concentration of estrogens, the  $EEQ_{cal}$  values were higher than the corresponding  $EEQ_{bio}$  values. The disagreement between  $EEQ_{bio}$  and  $EEQ_{cal}$  might be due to the presence of estrogen antagonistic compounds in the heavy polluted water samples.

$EEQ$  values of the six estrogens to the whole estrogenic activity were compared with the corresponding  $EEQ_{bio}$  values to investigate their individual contributions. The ranges of contribution rates of estrone, 17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol, estril, diethylstilbestrol and estradiol valerate were 1.01%–12.64%, 10.10%–59.71%, 23.20%–136.30%, 0.40%–2.14%, 0.00%–0.23% and 0.00%–0.002% of  $EEQ_{bio}$  value, respectively, not including the site Y2 at which only estrone was detected. 17 $\alpha$ -ethinylestradiol and 17 $\beta$ -estradiol together accounted for 78.14%–95.09% of  $EEQ_{cal}$ , and 62.99%–185.66% of  $EEQ_{bio}$ , while other compounds also showed some minor contributions (**Fig. 3**). It could be speculated that 17 $\alpha$ -ethinylestradiol and 17 $\beta$ -estradiol represented the major contributors to the whole estrogenic activity ( $EEQ_{bio}$ )

**Fig. 2** Plots of the  $EEQ_{bio}$  values versus  $EEQ_{cal}$  values.**Fig. 3** Calculated  $EEQ$  values of estrogens versus  $EEQ_{bio}$  value.

of the river waters. It is necessary to consider the two compounds in further environmental regulation.

17 $\alpha$ -Ethinylestradiol is a synthetic steroid estrogen used in almost combined oral contraceptive pills and 17 $\beta$ -estradiol is a natural estrogen (Stanback, 1997). A main reason of large presence of 17 $\alpha$ -ethinylestradiol and 17 $\beta$ -estradiol in Chinese water bodies might be that China has the world's largest population and is the world's largest contraceptive pills consumer (Stanback, 1997). The high concentration of 17 $\alpha$ -ethinylestradiol and 17 $\beta$ -estradiol in the three rivers maybe relate to that the three drainage rivers flowing through industry zone, farmlands and small towns in Tianjin receive most of untreated industrial, agricultural and domestic wastewater (Tianjin Environmental Protection Bureau, 2001; Liu et al., 2007). The Beitang River flows through chemical industry zones, villages, and farmlands along the rivers. The Dagu River receives effluents from four main municipal wastewater treatment plants and wastewater from agriculture along its way to the Bohai Bay. The Yongding New River is a natural water with a



collection area of 83,000 km<sup>2</sup> and also receives discharged contaminants because of rapid economic development in recent years (Tianjin Environmental Protection Bureau, 2001). From the 1960s to the 1980s, large amounts of wastewater for irrigating and pesticides were applied in the farmlands of Tianjin (Tianjin Environmental Protection Bureau, 1996). The estrogenic potency was observed in the soil of Tianjin with a mean value of 2.5 ng EEQ/kg soil (dry weight) (Xiao et al., 2006).

When large amount of 17 $\alpha$ -ethinylestradiol and 17 $\beta$ -estradiol enter into environment, it may cause male reproductive dysfunction to wildlife (Wang et al., 2008). Studies in Canadian lakes treated with 17 $\alpha$ -ethinylestradiol led to extinction of fathead minnows (*Pimephales promelas*) within two years (Kidd et al. 2007). Considering that 1 ng/L of 17 $\beta$ -estradiol in the water may disrupt freshwater life (Young et al., 2002), and steroidal estrogens may be the primary cause of fish feminization (Sumpter and Johnson, 2005). It is possible that the estrogens in the three rivers which transport and discharge the wastewater into the Bohai Sea might affect marine organisms inhabiting those waters by disrupting their normal hormonal functions.

### 3 Conclusions

This study showed that estrogenic activities could be observed in the three drainage rivers in Tianjin. 17 $\alpha$ -Ethinylestradiol and 17 $\beta$ -estradiol out of six estrogens were found to be the main contributors to the estrogenic activities in most river waters, and estrogen antagonistic compounds might present in the heavy polluted water samples. The results of the present work could reveal potential risk of marine organisms in Bohai Sea.

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### References

- Allen Y, Matthiessen P, Scott A P, Haworth S, Feist S, Thain J E, 1999. The extent of oestrogenic contamination in the UK estuarine and marine environments further surveys of flounder. *Science of the Total Environment*, 233(1-3): 5–20.
- Belfroid A C, Van der Horst A, Vethaak A D, Schäfer A J, Rijs G B J, Wegener J et al., 1999. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. *Science of the Total Environment*, 225(1-2): 101–108.
- Campbell C, Borglin S E, Green F B, Grayson A, Wozel E, Stringfellow W T, 2006. Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: A review. *Chemosphere*, 65(8): 1265–1280.
- Cargöuet M, Perdiz D, Mouatassim-Souali A, Tamisier-Karolak S, Levi Y, 2004. Assessment of river contamination by estrogenic compounds in Paris area (France). *Science of the Total Environment*, 324(1-3): 55–66.
- Chen C Y, Wen T Y, Wang G S, Cheng H W, Lin Y H, Lien G W, 2007. Determining estrogenic steroids in Taipei waters and removal in drinking water treatment using high-flow solid-phase extraction and liquid chromatography/tandem mass spectrometry. *Science of The Total Environment*, 378(3): 352–365.
- Desbrow C, Routledge E J, Brighty G C, Sumpter J P, Waldock M, 1998. Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and *in vitro* biological screening. *Environmental Science and Technology*, 32(11): 1549–1558.
- Gaido K W, Leonard L S, Lovell S, Gould J C, Babai D, Portier C J et al., 1997. Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. *Toxicology and Applied Pharmacology*, 143(1): 205–212.
- Hara A, Matsubara H, Soyano K, 2001. Endocrine and sexual disruption in wild grey mullet. In: UK Japan Research Cooperation on Endocrine Disrupting Chemicals (OECC. Japan, ed.). Ministry of the Environment of Japan, Tokyo. 42–46.
- Hashimoto S, Horiuchi A, Yoshimoto T, Nakao M, Omura H, Kato Y et al., 2005. Horizontal and vertical distribution of estrogenic activities in sediments and waters from Tokyo bay, Japan. *Archives of Environmental Contamination and Toxicology*, 48(2): 209–216.
- Hohenblum P, Gans O, Moche W, Scharf S, Lorbeer G, 2004. Monitoring of selected estrogenic hormones and industrial chemicals in groundwaters and surface waters in Austria. *Science of the Total Environment*, 333(1-3): 185–193.
- Hu J Y, Zhang H F, Chang H, 2005. Improved method for analyzing estrogens in water by liquid chromatography-electrospray mass spectrometry. *Journal of Chromatography A*, 1070(1-2): 221–224.
- Jonkers N, Sousa A, Galante-Oliveira S, Barroso C M, Kohler H-P E, Giger W, 2010. Occurrence and sources of selected phenolic endocrine disruptors in Ria de Aveiro, Portugal. *Environmental Science and Pollution Research*, 17(4): 834–843.
- Kawaguchi M, Ishii Y, Sakui N, Okanouchi N, Ito R, Inoue K et al., 2004. Stir bar sorptive extraction with *in situ* derivatization and thermal desorption-gas chromatography-mass spectrometry in the multi-shot mode for determination of estrogens in river water samples. *Journal of Chromatography A*, 1049(1-2): 1–8.
- Kidd K A, Blanchfield P J, Mills K H, Palace V P, Evans R E, Lazorchak J M et al., 2007. Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences of the United States of America*, 104(21): 8897–8901.
- Kuch H M, Ballschmiter K, 2001. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. *Environmental Science and Technology*,

- 35(15): 3201–3206.
- Labadie P, Budzinski H, 2005. Determination of steroidal hormone profiles along the Jalle d'Eysines River (near Bordeaux, France). *Environmental Science & Technology*, 39(14): 5113–5120.
- Lei B L, Huang S B, Zhou Y Q, Wang D H, Wang Z J, 2009. Levels of six estrogens in water and sediment from three rivers in Tianjin area, China. *Chemosphere*, 76(1): 36–42.
- Li J, Ma M, Wang Z J, 2010. *In vitro* profiling of endocrine disrupting effects of phenols. *Toxicology in Vitro*, 24(1): 201–207.
- Liu H X, Zhang Q H, Wang Y W, Cai Z W, Jiang G B, 2007. Occurrence of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls pollution in sediments from the Haihe River and Dagou Drainage River in Tianjin City, China. *Chemosphere*, 68(9): 1772–1778.
- Liu Y, Li N, Ma M, Rao K F, Wang Z J, 2009. Comparative study of phenolic compounds estrogenic and anti-estrogenic activities. *China Environmental Science*, 29(8): 873–878.
- Lu G H, Song W T, Wang C, Yan Z H, 2010. Assessment of *in vivo* estrogenic response and the identification of environmental estrogens in the Yangtze River (Nanjing section). *Chemosphere*, 80(9): 982–990.
- Matthiessen P, Arnold D, Johnson A C, Pepper T J, Pottinger T G et al., 2006. Contamination of headwater streams in the United Kingdom by oestrogenic hormones from livestock farms. *Science of the Total Environment*, 367(2-3): 616–630.
- Ohkubo N, Mochida K, Adachi S, Hara A, Hotta K, Nakamura Y et al., 2003. Estrogenic activity in coastal areas around Japan evaluated by measuring male serum vitellogenins in Japanese common goby *Acanthogobius flavimanus*. *Fisheries Science*, 69(6): 1135–1145.
- Ra J S, Lee S H, Lee J, Kim H Y, Lim B J, Kim S H et al., 2011. Occurrence of estrogenic chemicals in South Korean surface waters and municipal wastewaters. *Journal of Environmental Monitoring*, 13(1): 101–109.
- Reineke N, Bester K, Hühnerfuss H, Jastorff B, Weigel S, 2002. Bioassay-directed chemical analysis of River Elbe surface water including large volume extractions and high performance fractionation. *Chemosphere*, 47(7): 717–723.
- Ribeiro C, Pardal M Â, Martinho F, Margalho R, Tiritan M E, Rocha E et al., 2008. Distribution of endocrine disruptors in the Mondego River estuary, Portugal. *Environmental Monitoring and Assessment*, 149(1-4): 183–193.
- Routledge E J, Sumpter J P, 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environmental Toxicology and Chemistry*, 15(3): 241–248.
- Saravanabhavan G, Helleur R, Hellou J, 2009. GC-MS/MS measurement of natural and synthetic estrogens in receiving waters and mussels close to a raw sewage ocean outfall. *Chemosphere*, 76(8): 1156–1162.
- Scott A P, Katsiadaki I, Witthames P R, Hylland K, Davies I M, McIntosh A D et al., 2006. Vitellogenin in the blood plasma of male cod (*Gadus morhua*): A sign of oestrogenic endocrine disruption in the open sea? *Marine Environmental Research*, 61(2): 149–170.
- Sharpe R M, Irvine D S, 2004. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? *British Medical Journal*, 328(7437): 447–451.
- Shen J H, Gutendorf B, Vahl H H, Shen L, Westendorf J, 2001. Toxicological profile of pollutants in surface water from an area in Taihu Lake. Yangtze Delta. *Toxicology*, 166(1-2): 71–78.
- Song M Y, Xu Y, Jiang Q T, Lam P K S, O'Toole D K, Giesy J P et al., 2006. Measurement of estrogenic activity in sediments from Haihe and Dagou River, China. *Environment International*, 32(5): 676–681.
- Stanback J, 1997. Perspectives on Local Manufacture of Contraceptives in Developing Countries. Family Health International, Arlington, VA, USA.
- Sumpter J P, 1997. Environmental control of fish reproduction: a different perspective. *Fish Physiology and Biochemistry*, 17(1-6): 25–31.
- Sumpter J P, Jobling S, 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environmental Health Perspectives*, 103(7): 173–178.
- Sumpter J P, Johnson A C, 2005. Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment. *Environmental Science & Technology*, 39(12): 4321–4332.
- Sun L, Yong W, Chu X G, Lin J M, 2009. Simultaneous determination of 15 steroidal oral contraceptives in water using solid-phase disk extraction followed by high performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, 1216(28): 5416–5423.
- Tashiro Y, Takemura A, Fujii H, Takahira K, Nakanishi Y, 2003. Livestock wastes as a source of estrogens and their effects on wildlife of Manko tidal flat, Okinawa. *Marine Pollution Bulletin*, 47(1-6): 143–147.
- Tianjin Environmental Protection Bureau, 1996. Environmental Quality Statement (1986–1990). Tianjin Environmental Protection Bureau, Tianjin, China.
- Tianjin Environmental Protection Bureau, 2001. Environmental Quality Report of Tianjin in 1996–2000. Tianjin, China.
- Vermeirssen E L M, Burki R, Joris C, Peter A, Segner H, Suter M J F et al., 2005. Characterization of the estrogenicity of swiss midland rivers using a recombinant yeast bioassay and plasma vitellogenin concentrations in feral male brown trout. *Environmental Toxicology and Chemistry*, 24(9): 2226–2233.
- Wang L, Ying G G, Zhao J L, Liu S, Yang B, Zhou L J et al., 2011. Assessing estrogenic activity in surface water and sediment of the Liao River system in northeast China using combined chemical and biological tools. *Environmental Pollution*, 159(1): 148–156.
- Wang S, Huang W, Fang G Z, Zhang Y, Qiao H, 2008. Analysis of steroidal estrogen residues in food and environmental samples. *International Journal of Environmental Analytical Chemistry*, 88(1): 1–25.
- Xiao R Y, Wang Z J, Wang C X, Yu G, 2006. Soil screening for identifying ecological risk stressors using a battery of *in vitro* cell bioassays. *Chemosphere*, 64(1): 71–78.
- Young W F, Whitehouse P, Johnson I, Sorokin N, 2002. Proposed Predicted-No-Effect-Concentrations (PNECs) for Natural and Synthetic Steroid Oestrogens in Surface Waters. Environment Agency R&D Technical Report P2-T04/1. England and Wales Environment Agency Bristol. 172.

- Zha J M, Sun L W, Zhou Y Q, Spear P A, Ma M, Wang Z J, 2008. Assessment of 17 $\alpha$ -ethinylestradiol effects and underlying mechanisms in a continuous, multigeneration exposure of the Chinese rare minnow (*Gobiocypris rarus*). *Toxicology and Applied Pharmacology*, 226(3): 298–308.
- Zhou X, Lian Z R, Wang J T, Tan L J, Zhao Z C, 2011. Distribution of estrogens along Licun River in Qingdao, China. *Procedia Environmental Sciences*, 10: 1876–1880.

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