The distributions, removals and estrogenic effects of selected endocrine disrupting chemicals in two drinking water factories in China

Hong-Chang Zhang, Ting Xu, Xia-lin Hu, Wei-hai Pang and Da-Qiang Yin

ABSTRACT

The distributions and effects of 31 selected endocrine disrupting chemicals (EDCs) in two drinking water factories were analyzed in this study. The distributions of EDCs were analyzed by solid phase extraction (SPE) combined with liquid chromatography tandem mass spectrometry (LC-MS/MS). The concentrations of these EDCs were from lower than the LOD (limit of detection) to 23.13 ng L^{-1} in the samples; most of them were lower than 1 ng L^{-1}. The highest concentration (23.13 $\pm$ 1.45 ng L^{-1}) was detected in the raw water. Twenty-six chemicals were found in the raw water and only five in the finished water of drinking water factory A, while 25 chemicals were detected in the raw water and two in the finished water of drinking water factory B. The results indicate that most of the EDCs can be removed by the water treatment process. In the advanced treatment process, the ozonation processes have the highest removal efficiency. Separate analyses in May and September show similar results. Apart from the chemical analysis, yeast strain transformed when the estrogen receptor α (ERα) gene was employed to test the estrogenic effects of the water samples. Due to the low concentrations of these EDCs, no significant estrogenic effects were found from the samples.

Key words | drinking water, drinking water factories, endocrine disrupting compounds, estrogenic effect

Hong-Chang Zhang
Ting Xu
Xia-lin Hu
Da-Qiang Yin (corresponding author)
Key Laboratory of Yangtze River Water Environment, Ministry of Education,
College of Environmental Science and Engineering,
Tongji University,
No. 1239, Siping Road,
Yangpu District, Shanghai 200092,
China
E-mail: *yindq@tongji.edu.cn*

Hong-Chang Zhang
Shanghai Academy of Environmental Sciences,
No. 508, Qinzhou Road,
Xuhui District, Shanghai 200233,
China

Wei-hai Pang
Da-Qiang Yin
State Key Laboratory of Pollution Control and Resources Reuse,
College of Environmental Science and Engineering,
Tongji University,
No. 1239, Siping Road, Yangpu District,
Shanghai 200092,
China

INTRODUCTION

Endocrine disrupting chemicals (EDCs) have generated a considerable amount of attention in the past two decades (Hotchkiss *et al*. 2008). Some studies showed that EDCs can disturb the endocrine system of animals and induce many severe problems. What's more severe is that these EDCs can threaten human beings' health and induce many diseases (Tsutsumi 2005). As a result, the occurrence and effects of EDCs have become hot topics, and many analytical methods and effect assay methods have been established for different EDCs.

The distributions of EDCs in wastewater treatment plants (WWTPs) have been well documented throughout the world. Paraskevi *et al*. found nonylphenol (NP), octylphenols (OP) and bisphenol A (BPA) in municipal and industrial WWTPs in Northern Greece (Pothitou & Voutsa 2008). The environmental fate studies showed the EDCs in the surface water usually come from domestic, industrial and livestock wastewater (Voutsa *et al*. 2006; Zuccato *et al*. 2006) and many kinds of EDCs can be detected from different kinds of surface water. Chang *et al*. detected androgens, glucocorticoids, progestogens, and estrogens in the river water of Beijing (Chang *et al*. 2009). Nowadays, many drinking water factories are employing surface water as source water, so the EDCs in the source water may not be well removed and may then get into the drinking water. Benotti *et al*. found estrone (E1), 17β-estradiol (E2), BPA, NP, progesterone and testosterone in raw water and finished water in 19 US drinking water factories (Benotti *et al*. 2009). These EDCs in the drinking water factories may threaten the health of

doi: 10.2166/wh.2012.121

people, especially those EDCs which can get into the finished water. At present, both conventional treatment processes and advanced treatment processes are utilized in drinking water factories in China. But there are few studies on the distribution and removal of these EDCs in different drinking water factories.

Apart from the distributions of the EDCs in water bodies, some studies also tested the estrogenic effects of EDCs in surface water by many methods. The recombinant yeast system transformed with estrogen receptor (ER) was one of the widely used methods and it has been used to test the estrogenic effects of chemicals and environmental samples (Chen *et al.* 1997; Rehmann *et al.* 1999). In some studies, researchers combined analytical methods and effect assay to analyze the EDCs in water samples. Bicchi *et al.* analyzed the concentrations and effects of some EDCs in one WWTP and found estrogenic effects in some samples (Bicchi *et al.* 2009). In another study, Liscio *et al.* found the EEQ (Estrogen Equivalents) values derived from the bioassay showed a positive correlation with the EEQ values calculated from chemical analyses data (Liscio *et al.* 2009). These studies indicated that the combined application of an analytical method and effects analysis can interpret more about the water quality. The effects assays of the samples are more intuitive and persuasive than the chemical analysis alone. So, in this study, the chemical analysis and the effects assay were combined to investigate the water samples from the drinking water factories.

In this study, two drinking water factories, one with a conventional treatment process and the other with an advanced treatment process, were chosen, and water samples from each process were separately analyzed. Thirty-one EDCs including seven estrogens, eight androgens, six progestogens, five adrenocortical hormones and five industrial compounds were analyzed and the effects of the samples were also assayed with a recombinant yeast system. The objective of this study is to analyze the distributions and effects of the selected EDCs in the different treatment processes of two drinking water factories, to find the key processes for the removal of the EDCs and provide more information for the process improvement of drinking water treatment.

MATERIALS AND METHODS

Materials

All the test compounds used in the chemical analysis and five internal standards including Estrone-D2, Diethylstilbestrol-D8, Testosterone-D3, Progesterone-D9 and Norgestrel-D6 were purchased from Sigma-Aldrich (St Louis, MO, USA) and Dr. Ehrenstorfer (Augsburg, Germany). Milli-Q water was obtained from a Millipore system (Billerica, MA, USA). Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Glass fiber filters (GF/F) were supplied by Whatman (Middlesex, UK). Mixed-mode cationic exchange (MXC) extraction cartridges (500 mg, 6 mL) were purchased from Anpelclean (Shanghai, China). Ammonia water and ammonium acetate were purchased from Sigma-Aldrich (St Louis, MO, USA). The amino acids used in the synthetic dropout (SD) media were obtained from Amresco (Solon, USA). Information on each of the chemicals is presented in Table S1 (available online at http://www.iwaponline.com/jwh/011/121.pdf).

Drinking water factories and sample collection

In this study, two typical drinking water factories, one employing the conventional treatment process and the other employing an advanced treatment process, were chosen. These two drinking water factories share the same source water from upstream of Huangpu River. Drinking water factory A employs the conventional treatment process, which treats Raw water (A1) with Coagulation/flocculation (A2) – Clarification (A3) – Filtration (A4) – Chlorination (A5) sequentially. Drinking water factory B uses an advanced treatment process, which treats Raw water (B1) with Pre-ozonation (B2) – Tube settling (B3) – Filtration (B4) Post-ozonation (B5) – Granular activated carbon adsorption (B6) Ultraviolet disinfection (B7) sequentially. Figure 1 shows the processes of each drinking water factory. The water samples (10 L per site) were collected with a clean stainless steel sampler from each process of the two drinking water factories, and the sampler and pre-cleaned brown bottles were washed three times with the target water sample

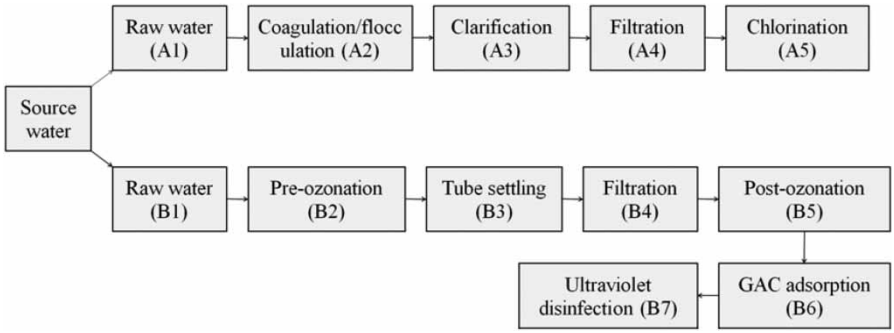

Figure 1 | The processes in the two drinking water factories.

before collection. The water samples were stored in brown bottles surrounded by broken ice. After that, the bottles were carried back to the lab, stored at low temperature (4 °C) and prepared for the analysis. In the 10 L water samples, 4 L was used to analyze the distribution of EDCs and 5 L was used to test the effects of the water samples on the recombinant yeast system. Sample collections were conducted twice, in May and September 2010.

Chemical analysis

The distributions of EDCs were analyzed by the analytical method developed in our former study (Zhang *et al.* 2011). In brief, 1 L water samples were filtered through pre-ashed 0.7 μm GF/F and then spiked with internal standards. After that, solid phase extraction (SPE) utilizing MCX SPE cartridges was performed for the isolation and concentration of target EDCs. Samples were loaded through the activated MCX cartridges (500 mg, 6 mL). After all the samples were filtered, the cartridges were dried under nitrogen and cleaned sequentially with 6 mL of 0.1 M HCl, 6 mL of pure water and 6 mL of methanol/water solution (1:1, v/v); the EDCs were eluted with 9 mL of 0.6 M ammonia/methanol solution. The extracts were dried under a gentle stream of nitrogen and redissolved in 1 mL methanol. Then the solutions were filtered through a 0.22 μm filter unit (Millex, Billerica, MA, USA) and prepared for analyzing with liquid chromatography tandem mass spectrometry (LC-MS/MS) with the corresponding mobile phase. For the water samples which were used to test the effects, preparation was nearly the same, except no internal standards were added in, and the extracts of 5 L samples were dried under a gentle stream of nitrogen and redissolved in 1 mL DMSO (dimethyl sulfoxide) for the effects assay.

Effects assay

Yeast strain transformed with the ERα gene was a gift of Dr Zijian Wang (State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences). It was stably transformed with the human ERα gene and the LacZ gene. The LacZ gene encoding the enzyme β-galactosidase was used as reporter. The two-hybrid yeast assays were performed according to Li *et al.* (2010a). In brief, the yeasts were cultured in SD/-Leu/-Trp (the SD medium contains all essential amino acids except for leucine and tryptophan) overnight, and diluted with medium to an OD600 (Optical Density at 600 nm) of 0.75 with a spectrophotometer (UV-2102C, Unico, Shanghai). One positive control (E2, Androgen or T3) and a negative control (DMSO) were used to test the assay. For each sample, 5 μL of sample was added to 995 μL medium. After being fully mixed, 200 μL of the test cultures were transferred into wells of a 96-well plate and incubated at 30 °C, for 2 h; in this period, a constant shaking (300 rpm) was supplied by a shaker (PST-60HL-4, Biosan, Latvia). After the incubation, the cell density was measured at 600 nm with a spectrophotometer. Then, 150 μL of test culture was removed and 50 μL was left in the wells. With the addition of 120 μL Z-buffer(16.1 g L^{-1} $Na_2HPO_4{\cdot}7H_2O$; 5.5 g L^{-1} $NaH_2PO_4{\cdot}H_2O$; 0.75 g L^{-1} KCl; 0.246 g L^{-1} $MgSO_4{\cdot}7H_2O$) and 20 μL chloroform, the assays were pre-incubated for 10 min at 30 °C with a rotation speed of 750 rpm. After the

pre-incubation, 40 μL *o*-nitrophenyl-β-D-galactopyranoside (13.3 mmol L^{-1}, dissolved in Z-buffer) was added to initiate the enzyme reactions. After that, the reactions were carried out for 60 min at 30 °C with a rotation speed of 750 rpm. After that, the reactions were terminated by the addition of 100 μL Na_2CO_3 (1 mol L^{-1}). A volume of 200 μL of the test cultures was transferred into a new 96-well plate and the OD420 (Optical Density at 420 nm) was determined. The *β*-galactosidase activity (U) was calculated according to the following equation:

$$U = Cs/t \cdot V \cdot D \cdot ODs \text{ and } Cs = 10^{-6}(A_S - A_B)/e \cdot d \quad (1)$$

where U is *β*-galactosidase activity; Cs is concentration of *o*-nitrophenol in the enzyme assay reaction mix; t is incubation duration of the enzyme reaction; V is volume of the test culture; D is dilution factor; ODs is OD600 of the test culture; A_S is OD420 of the enzyme reaction supernatant of the sample; A_B is OD420 of the enzyme reaction supernatant of the blank; e is for *o*-nitrophenol in the enzyme assay reaction mix; and d is diameter of the cuvette (Li *et al.* 2010a).

In the chemical analysis, the samples were analyzed in three parallel samples; the results show the mean ± standard deviation (SD). For the effects assay, each sample was tested in eight parallel samples and mean values were used.

RESULTS AND DISCUSSION

The occurrence of EDCs in the two drinking water factories

The analytical method we developed was successfully employed in the test for target chemicals in the drinking water factories. The recoveries of these chemicals in the two surveys were from 82 to 103%, and the SD values were within 14%. The results indicate that this method can perform well in the detection of target chemicals in the two drinking water factories.

In the May survey, 26 chemicals were detected in the raw water of drinking water factory A, while in the finished water only five of them were detected, and only BPA had a concentration higher than 1 ng L^{-1} (1.12 ng L^{-1}). For drinking water factory B, 25 chemicals were detected in the raw water, while in the finished water only two industrial chemicals (OP and BPA) were detected.

In the 31 chemicals which were tested for in this study, dienoestrol (Dieno), testosterone propionate (Tes-pro), 4-*t*-nonyl phenol (NP), nandrolone phenylpropionate (Nan-phen) and epitestosterone (Epite) were not detected in any samples, while estradiol benzoate (E2-ben), testosterone (TES), boldenone (Bold), hydroxyprogesterone (Hydrop) and norethisterone (Noreth) were only detected in the raw water samples at very low concentrations and were not detected in the subsequent samples (Table 1) from the two drinking water factories. A similar situation was found for the September samples. Nan-phen, Tes-pro, Hydrop and NP were not detected in any samples, and E2-ben, Bold and Epite were only found in several samples in low concentrations (Table S2, available online at http://www.iwaponline.com/jwh/011/121.pdf).

For all the detected chemicals, the concentrations were very low; in the May survey, only nine chemicals had a maximum detected concentration higher than 1 ng L^{-1}. These chemicals were mainly industrial chemicals, estrogens, and adrenocortical hormones. The three highest concentrations were 23.13 ng L^{-1} (OP), 19.49 ng L^{-1} (bisphenol F (BPF)) and 7.44 ng L^{-1} (BPA) in the May survey. Similar results were found in the September survey, where 12 chemicals had a maximum detected concentration higher than 1 ng L^{-1}, and the highest concentrations were 20.42 ng L^{-1} (OP), 16.82 ng L^{-1} (BPF) and 8.01 ng L^{-1} (BPA). The results indicate that the industrial chemicals were still the primary EDCs in the drinking water factories. The highest concentrations were all detected in the raw water. In many other studies, these chemicals were detected in many sources of water and raw water of drinking water factories. Kuch *et al.* detected low concentrations of NP, E1 and E2 in the water source of drinking water (Kuch & Ballschmiter 2001). Magi *et al.* found BPA (17.0–56.4 g L^{-1}), NP (2.4–9.9 g L^{-1}) and E1 (3.8 g L^{-1}) in the raw water of drinking water factories; the concentrations were similar to our detection (Magi *et al.* 2010). Similar levels of NP and E2 were also detected in Brazil (Moreira *et al.* 2009) and Canada (Yu *et al.* 2007).

The androgens and progesterones were present in a relatively low concentration, lower than 1 ng L^{-1}, and many of them were not detected in any samples. That may be

Table 1 | The concentrations of the tested chemicals in the two waterworks in May 2010 (ng L^{-1})

Chemicals	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	B6	B7	Recoveries (%)
E1	0.99 ± 0.16	0.48 ± 0.14	0.37 ± 0.14	ND	ND	0.74 ± 0.09	0.46 ± 0.01	0.38 ± 0.03	0.29 ± 0.01	ND	ND	ND	82 ± 10
E2	2.76 ± 0.87	1.70 ± 0.10	1.06 ± 0.05	0.40 ± 0.07	ND	4.12 ± 0.43	1.78 ± 0.24	1.15 ± 0.05	1.05 ± 0.04	ND	ND	ND	83 ± 11
E3	0.70 ± 0.15	ND	ND	ND	ND	0.72 ± 0.10	ND	ND	ND	ND	ND	ND	85 ± 14
DES	1.07 ± 0.08	0.60 ± 0.04	0.48 ± 0.01	0.16 ± 0.02	ND	0.73 ± 0.13	0.39 ± 0.09	0.28 ± 0.07	0.20 ± 0.02	ND	ND	ND	90 ± 10
Dieno	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	90 ± 7
Hexe	1.22 ± 0.18	0.76 ± 0.11	0.43 ± 0.11	ND	ND	0.71 ± 0.06	ND	ND	ND	ND	ND	ND	88 ± 7
E2-ben	0.05 ± 0.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	91 ± 10
Nortes	0.32 ± 0.07	ND	ND	ND	ND	0.33 ± 0.00	ND	ND	ND	ND	ND	ND	94 ± 10
Tren	0.65 ± 0.30	0.37 ± 0.02	0.23 ± 0.09	ND	ND	0.98 ± 0.03	0.51 ± 0.14	0.51 ± 0.05	0.47 ± 0.03	0.02 ± 0.00	ND	ND	95 ± 11
TES	0.08 ± 0.01	0.03 ± 0.01	ND	ND	ND	0.09 ± 0.01	0.06 ± 0.03	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.01	ND	ND	89 ± 10
Me-tes	0.20 ± 0.02	0.11 ± 0.01	0.06 ± 0.01	ND	ND	0.15 ± 0.04	0.08 ± 0.00	ND	ND	ND	ND	ND	86 ± 8
Nan-phen	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	88 ± 11
Tes-pro	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	91 ± 9
Bold	0.05 ± 0.00	ND	ND	ND	ND	0.08 ± 0.02	ND	ND	ND	ND	ND	ND	93 ± 11
Epite	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	86 ± 12
Noreth	0.46 ± 0.03	ND	ND	ND	ND	0.48 ± 0.01	ND	ND	ND	ND	ND	ND	85 ± 11
Norges	0.55 ± 0.05	0.25 ± 0.04	0.21 ± 0.01	ND	ND	0.65 ± 0.02	0.30 ± 0.06	0.24 ± 0.03	0.24 ± 0.04	ND	ND	ND	90 ± 13
Me-pro	0.47 ± 0.10	0.35 ± 0.01	0.12 ± 0.03	0.09 ± 0.00	ND	0.12 ± 0.02	0.07 ± 0.00	0.64 ± 0.02	0.05 ± 0.00	ND	ND	ND	89 ± 11
Proges	0.03 ± 0.00	ND	ND	ND	ND	0.06 ± 0.04	0.02 ± 0.00	ND	ND	ND	ND	ND	86 ± 11
Me-ace	0.77 ± 0.19	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.05 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	ND	ND	ND	87 ± 11
Hydrop	0.05 ± 0.01	ND	ND	ND	ND	0.06 ± 0.00	0.02 ± 0.00	ND	ND	ND	ND	ND	91 ± 9
Predn	1.62 ± 0.27	1.04 ± 0.03	0.65 ± 0.12	0.40 ± 0.04	ND	1.44 ± 0.04	0.61 ± 0.06	0.56 ± 0.03	0.45 ± 0.03	ND	ND	ND	85 ± 10
Corti	1.11 ± 0.09	0.70 ± 0.18	0.44 ± 0.02	0.24 ± 0.01	ND	0.81 ± 0.12	0.37 ± 0.03	0.33 ± 0.02	0.28 ± 0.02	ND	ND	ND	94 ± 11
Dexa	0.74 ± 0.05	0.47 ± 0.01	0.25 ± 0.02	0.12 ± 0.02	0.05 ± 0.01	0.17 ± 0.01	0.07 ± 0.01	0.07 ± 0.03	0.06 ± 0.00	ND	ND	ND	85 ± 8
Prednl	0.65 ± 0.12	0.57 ± 0.02	0.39 ± 0.12	0.16 ± 0.09	ND	0.45 ± 0.09	0.26 ± 0.03	0.18 ± 0.02	0.14 ± 0.02	ND	ND	ND	100 ± 10
Me-prednl	0.78 ± 0.11	0.55 ± 0.03	0.46 ± 0.03	0.37 ± 0.02	ND	0.60 ± 0.17	0.22 ± 0.02	0.15 ± 0.06	0.14 ± 0.01	ND	ND	ND	82 ± 10
BPS	1.36 ± 0.19	0.56 ± 0.06	0.48 ± 0.06	0.35 ± 0.08	ND	0.98 ± 0.20	0.69 ± 0.14	0.59 ± 0.09	0.49 ± 0.04	ND	ND	ND	96 ± 15
NP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	99 ± 9
OP	23.13 ± 1.45	11.66 ± 1.70	7.19 ± 1.65	2.72 ± 0.52	0.62 ± 0.03	11.72 ± 0.60	5.63 ± 0.67	5.32 ± 0.59	4.21 ± 0.16	1.17 ± 0.06	0.64 ± 0.07	0.39 ± 0.04	91 ± 10
BPA	7.44 ± 0.46	4.56 ± 0.28	4.06 ± 0.08	3.62 ± 0.32	1.12 ± 0.07	8.26 ± 0.33	4.14 ± 0.31	3.49 ± 0.14	3.36 ± 0.16	1.43 ± 0.08	1.10 ± 0.03	0.56 ± 0.04	95 ± 12
BPF	19.49 ± 1.48	12.51 ± 1.01	8.96 ± 0.19	2.95 ± 0.39	0.32 ± 0.03	20.94 ± 3.95	12.39 ± 1.30	8.83 ± 0.40	6.79 ± 0.08	1.50 ± 0.23	0.38 ± 0.04	ND	86 ± 7

ND: Not detected.

The names of the compounds are abbreviated for convenience according to Table S1 (available online at http://www.iwaponline.com/jwh/011/121.pdf).

For treatment steps see the text of Figure 1.

because these chemicals were mainly pharmaceuticals which were used to treat the endocrine diseases of people, and the doses were very low. Hence the chemicals which can get into the wastewater with excretion, and finally get into the environment water, were much less. So, they were usually absent in the environment water or present in extremely low concentrations. There were only a few studies on the occurrence of these chemicals in surface water and drinking water. Benotti *et al.* got similar results to us; they found testosterone in the source water of two of 19 drinking water factories with a highest concentration of 1.1 ng L^{-1}. Also, they detected progesterone in the source water of four drinking water factories with a highest concentration of 2.2 ng L^{-1} (Benotti *et al.* 2009).

Not much difference was found between our two surveys; the detected chemicals and concentrations show similar results. This may be because May and September were both within the wet season, and hence the hydrological condition did not change much between the two surveys.

The removal of EDCs from the drinking water factories

In the May survey only five chemicals were detected in the finished water (in low concentrations) of drinking water factory A, and only BPA had a concentration higher than 1 ng L^{-1} (1.12 ng L^{-1}). For drinking water factory B, only two industrial chemicals (OP and BPA) were detected in the finished water and both of them were lower than 1 ng L^{-1} in May. A similar result was detected in the September survey. The results indicate that the conventional treatment process and the advanced treatment process can both remove most of the target EDCs, and the advanced treatment process performs better than the conventional treatment process. Benotti *et al.* investigated the occurrence of some selected EDCs in the 19 drinking water factories employing different treatment processes. The results showed that most EDCs can be removed by the treatment processes, and only a few of them can be detected in the finished water at rather low concentrations (Benotti *et al.* 2009). In another study of drinking water factories with conventional treatment processes, the results showed a substantial removal of EDCs through the conventional process; however, there were several EDCs left in the finished water at a low concentration, such as BPA and NP (Stackelberg *et al.* 2007).

For drinking water factory A, a big portion of the EDCs were removed from the water sample by the coagulation/flocculation (A2) process. E3, E2-ben, Nortes, Bold, Noreth, Proges and Hydrop were nearly all removed because of their low initial concentrations. Therefore, the concentrations after sedimentation were lower than their limits of detection (LODs). For drinking water factory B, the first process was pre-ozonation, and this step removed a big portion of the EDCs. Nearly all of the E3, Hexe, E2-ben, Me-tes, Nortes, Bold, Noreth, Proges and Hydrop were removed, which may be because of their low initial concentrations.

To evaluate and compare the ability to remove EDCs, the removal efficiencies of five classes of EDCs in each process were calculated and the results are shown in Figure 2 and Figure 3.

From Figure 2 we can see that the removal efficiencies of estrogens and androgens are 100%, and the removal

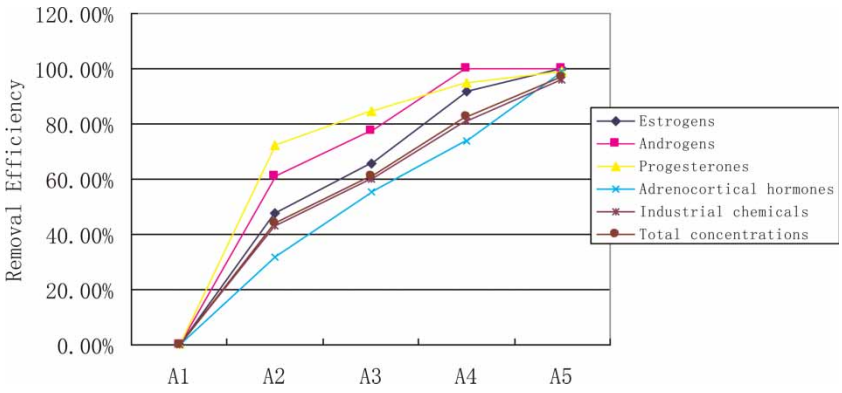

Figure 2 | The efficiencies of removal of EDCs in drinking water factory A (May 2010).

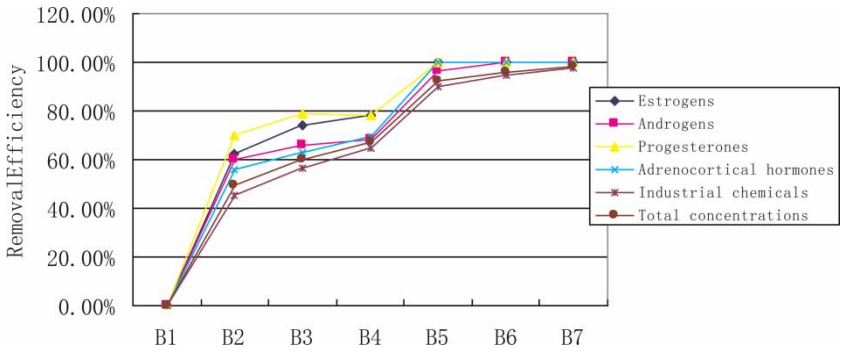

Figure 3 | The efficiencies of removal of EDCs in drinking water factory B (May 2010).

efficiencies of the other three classes are also higher than 95%. Of all the processes, the coagulation/flocculation process in drinking water factory A has good removal efficiencies. A total of 72.53% of progesterones were removed in this process, and the removal efficiencies of androgens and estrogens were 60.77 and 47.86%. In this step, flocculant (aluminum sulfate) was added into the water sample and the suspended particles were flocculated and removed from the water phase. The floc may adsorb the EDCs and take them into the sediments and result in the removal of the EDCs from the water phase. A laboratory study had shown that the conventional water treatment process had very low removal efficiencies for EDCs (Choi *et al.* 2006). But there were other studies which indicated that the low efficiency of coagulation/flocculation may be due to the lack of the suspended sediment particulate materials in the artificial water, and the removal could be improved by the particulate matter initially present in the water (Westerhoff *et al.* 2005). The suspended sediment particulate materials in raw water may improve the removal of EDCs. In the subsequent clarification and filtration steps, the remaining EDCs were removed little by little, and by the last step, chlorination, most of the EDCs were removed from the water phase and only several were left at a very low concentration.

The removal efficiencies in Figure 3 show that drinking water factory B has better removal efficiencies than drinking water factory A. The removal efficiencies of estrogens, androgens, progesterones and adrenocortical hormones were 100% in factory B. Of all these processes, the pre-ozonation (B2) has a good performance; more than 60% of estrogens, androgens and progesterones were removed by this process, and the removal efficiencies of adrenocortical hormones and industrial chemicals were 55.91 and 45.47% respectively. In contrast, tube settling (B3) and filtration (B4) can only remove a small part of the chemicals. The post-ozonation (B5) also removed a big part of the chemicals and, after this process, more than 92.5% of initial chemicals were removed from the water. Among them, estrogens, androgens and adrenocortical hormones were totally removed. The high efficiency of ozonation may result from the oxidation of ozone, which converts the EDCs into other forms. Some researchers found that, in ozone treatment, ozone (O_3) attacks the organic contaminants either by direct reaction (as molecular O_3) or through the formation of free radicals, such as the hydroxyl radical (·OH), and results in the removal of EDCs (Ning *et al.* 2007a, b). Esplugas *et al.* found that advanced oxidation processes with ozone had a good efficiency in the removal of EDCs from water (Esplugas *et al.* 2007). Snyder *et al.* testified that, in a drinking water experiment, the majority of target EDCs were removed by greater than 90% on O_3 exposure (Snyder *et al.* 2006). In this study, tube settling (B3) and filtration (B4) processes also remove some of the EDCs. It is reported that granular activated carbon adsorption (GAC, B6) has a good efficiency for the removal of several EDCs (Rahman *et al.* 2009a, b), but the good efficiency was not present in this study. That may be because most of the EDCs have been removed from the water phase and the remaining concentrations are extremely low.

The effects of the water samples

By the two-hybrid yeast assays, the estrogenic effects of the water samples in May were analyzed. For all the water

Table 2 | The EC50 of estrogenic effect of selected chemicals

Chemicals	DES	E1	E2	E3	Hexe	OP	BPA	BPS	BPF
EC_{50}	9.59×10^{-7}	2.17×10^{-7}	9.57×10^{-8}	5.10×10^{-6}	1.62×10^{-7}	9.78×10^{-5}	6.80×10^{-6}	ND	2.70×10^{-3}
Max	1.07×10^{-9}	9.90×10^{-10}	4.12×10^{-9}	7.20×10^{-10}	1.22×10^{-9}	2.31×10^{-8}	8.26×10^{-9}	1.36×10^{-9}	2.09×10^{-8}
EC_{50}/Max	8.96×10^{2}	2.19×10^{2}	2.32×10^{1}	7.08×10^{3}	1.33×10^{2}	4.23×10^{3}	8.23×10^{2}	$>1.00\times10^{3}$	1.29×10^{5}

The names of the compounds are abbreviated for convenience according to Table S1 (available online at http://www.iwaponline.com/jwh/011/121.pdf).
Max means the maximum concentrations of the chemicals in the surveys.

samples, no estrogenic effects were detected because of the low concentrations of these EDCs in the water samples.

To test the absence of estrogenic effects of all these water samples, the estrogenic effects of some chemicals present in the water samples were analyzed by the two-hybrid yeast assay and their EC50 values are presented in Table 2. From the results we can see that most of the chemicals have EC50 values higher than 100 ng L^{-1}, which is too high to reach in the environmental water samples. Meanwhile, the maximum concentrations of each chemical detected from the two drinking water factories are also presented in Table 2. Compared to the maximum concentrations of the chemicals in the water samples, the EC50 values were at least 23 times the maximum concentration; most of them were 200 times higher than the maximum concentrations. Especially for BPF, the EC50 values were 1.29×10^{5} times the highest concentration in these water samples. So, it is reasonable that the effects were difficult to detect.

In another study, fish were used to evaluate the effects of EDCs in raw water and treated water in France and no significant effect was observed (Cargouet *et al.* 2007). In China, Li *et al.* used the two-hybrid yeast assay employed in this study to screen for agonistic thyroid receptor (TR) mediated effects in drinking waters and found no significant effects (Li *et al.* 2010b). The significant effects were only detected from waste water with a high concentration of EDCs (Inoue *et al.* 2009; Liu *et al.* 2009).

For many of the EDCs, there is no drinking water standard or health advice (Stackelberg *et al.* 2004); even for some with a standard, it is difficult to judge their safety with a complex mixture in the water. So, it is hard to judge whether a concentration level in drinking water is safe or not. Therefore, effects assay was needed to test the safety of water. Though the effects assay employed in this study did not show effects, some more appropriate methods should be developed and modified to test the effects of water and ensure the safety of drinking water.

CONCLUSION

The concentrations of all 31 tested chemicals were very low in all the samples and the highest concentrations were from the industrial chemicals in the raw water. Only five of 31 chemicals were detected in the finished water and these were in ultra-low concentrations.

The conventional treatment process can remove most of the EDCs and the advanced treatment process performs better. The removal efficiencies of five classes of EDCs were higher than 95% in drinking water factory A and 97% in drinking water factory B. The coagulation/flocculation process and ozonation process have the highest efficiencies for the removal of EDCs in each of the drinking water factories.

No significant effect was detected by the yeast assay, which may be because of the low concentrations of the EDCs in the water samples. The effects assay may provide a better insurance of the safety of drinking water.

ACKNOWLEDGEMENTS

The authors appreciate the assistance of Dr Zijian Wang (State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences). This work was partially supported by the International Science & Technology Cooperation Program of China (2010DFA91800), the National Major Science and

Technology Projects: Water Pollution Control and Management (2012ZX7430-004), and the National Natural Science Foundation of China (51278353).

REFERENCES

Benotti, M. J., Trenholm, R. A., Vanderford, B. J., Holady, J. C., Stanford, B. D. & Snyder, S. A. 2009 Pharmaceuticals and endocrine disrupting compounds in US drinking water. *Environ. Sci. Technol.* **43**, 597–603.

Bicchi, C., Schiliro, T., Pignata, C., Fea, E., Cordero, C., Canale, F. & Gill, G. 2009 Analysis of environmental endocrine disrupting chemicals using the E-screen method and stir bar sorptive extraction in wastewater treatment plant effluents. *Sci. Total Environ.* **407**, 1842–1851.

Cargouet, M., Perdiz, D. & Levi, Y. 2007 Evaluation of the estrogenic potential of river and treated waters in the Paris area (France) using *in vivo* and *in vitro* assays. *Ecotoxicol. Environ. Safety* **67**, 149–156.

Chang, H., Wan, Y. & Hu, J. 2009 Determination and Source Apportionment of Five Classes of Steroid Hormones in Urban Rivers. *Environ. Sci. Technol.* **43**, 7691–7698.

Chen, C. W., Hurd, C., Vorojeikina, D. P., Arnold, S. F. & Notides, A. C. 1997 Transcriptional activation of the human estrogen receptor by DDT isomers and metabolites in yeast and MCF-7 cells. *Biochem. Pharm.* **53**, 1161–1172.

Choi, K. J., Kim, S. G., Kim, C. W. & Park, J. K. 2006 Removal efficiencies of endocrine disrupting chemicals by coagulation/flocculation, ozonation, powdered/granular activated carbon adsorption, and chlorination. *Korean J. Chem. Eng.* **23**, 399–408.

Esplugas, S., Bila, D. M., Krause, L. G. T. & Dezotti, M. 2007 Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents. *J. Hazard. Mater.* **149**, 631–642.

Hotchkiss, A. K., Rider, C. V., Blystone, C. R., Wilson, V.S., Hartig, P. C., Ankley, G. T., Foster, P. M., Gray, C. L. & Gray, L. E. 2008 Fifteen years after 'Wingspread' – environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. *Toxicol. Sci.* **105**, 235–259.

Inoue, D., Nakama, K., Matsui, H., Sei, K. & Ike, M. 2009 Detection of agonistic activities against five human nuclear receptors in river environments of Japan using a yeast two-hybrid assay. *Bull. Environ. Contam. Toxicol.* **82**, 399–404.

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. *Environ. Sci. Technol.* **35**, 3201–3206.

Li, J., Wang, Z. J., Ma, M. & Peng, X. Z. 2010a Analysis of environmental endocrine disrupting activities using recombinant yeast assay in wastewater treatment plant effluents. *Bull. Environ. Contam. Toxicol.* **84**, 529–535.

Li, N., Wang, D. H., Zhou, Y. Q., Ma, M., Li, J. A. & Wang, Z. J. 2010b Dibutyl-phthalate contributes to the thyroid receptor antagonistic activity in drinking water processes. *Environ. Sci. Technol.* **44**, 6863–6868.

Liscio, C., Magi, E., Di Carro, M., Suter, M. J. F. & Vermeirssen, E. L. M. 2009 Combining passive samplers and biomonitors to evaluate endocrine disrupting compounds in a wastewater treatment plant by LC/MS/MS and bioassay analyses. *Environ. Pollut.* **157**, 2716–2721.

Liu, Z. H., Ito, M., Kanjo, Y. & Yamamoto, A. 2009 Profile and removal of endocrine disrupting chemicals by using an ER/AR competitive ligand binding assay and chemical analyses. *J. Environ. Sci-China* **21**, 900–906.

Magi, E., Scapolla, C., Di Carro, M. & Liscio, C. 2010 Determination of endocrine-disrupting compounds in drinking waters by fast liquid chromatography-tandem mass spectrometry. *J. Mass. Spectrom.* **45**, 1003–1011.

Moreira, D. S., Aquino, S. F., Afonso, R. J. C. F., Santos, E. P. P. C. & de Padua, V. L. 2009 Occurrence of endocrine disrupting compounds in water sources of Belo Horizonte Metropolitan Area, Brazil. *Environ. Technol.* **30**, 1041–1049.

Ning, B., Graham, N. J. D. & Zhang, Y. P. 2007a Degradation of octylphenol and nonylphenol by ozone – Part I: Direct reaction. *Chemosphere* **68**, 1163–1172.

Ning, B., Graham, N. J. D. & Zhang, Y. P. 2007b Degradation of octylphenol and nonylphenol by ozone– Part II: Indirect reaction. *Chemosphere* **68**, 1173–1179.

Pothitou, P. & Voutsa, D. 2008 Endocrine disrupting compounds in municipal and industrial wastewater treatment plants in Northern Greece. *Chemosphere* **73**, 1716–1723.

Rahman, M. F., Yanful, E. K. & Jasim, S. Y. 2009a Occurrences of endocrine disrupting compounds and pharmaceuticals in the aquatic environment and their removal from drinking water: challenges in the context of the developing world. *Desalination* **248**, 578–585.

Rahman, M. F., Yanful, E. K. & Jasim, S. Y. 2009b Endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) in the aquatic environment: implications for the drinking water industry and global environmental health. *J. Water Health* **7**, 224–243.

Rehmann, K., Schramm, K. W. & Kettrup, A. A. 1999 Applicability of a yeast estrogen screen for the detection of oestrogen-like activities in environmental samples. *Chemosphere* **38**, 3303–3312.

Snyder, S. A., Wert, E. C., Rexing, D. J., Zegers, R. E. & Drury, D. D. 2006 Ozone oxidation of endocrine disruptors and pharmaceuticals in surface water and wastewater. *Ozone Sci. Eng.* **28**, 445–460.

Stackelberg, P. E., Furlong, E. T., Meyer, M. T., Zaugg, S. D., Henderson, A. K. & Reissman, D. B. 2004 Persistence of

pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water treatment plant. *Sci. Total Environ.* **329**, 99–113.

Stackelberg, P. E., Gibs, J., Furlong, E. T., Meyer, M. T., Zaugg, S. D. & Lippincott, R. L. 2007 Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. *Sci. Total Environ.* **377**, 255–272.

Tsutsumi, O. 2005 Assessment of human contamination of estrogenic endocrine-disrupting chemicals and their risk for human reproduction. *J. Steroid. Biochem.* **93**, 325–330.

Voutsa, D., Hartmann, P., Schaffner, C. & Giger, W. 2006 Benzotriazoles, alkylphenols and bisphenol A in municipal wastewaters and in the Glatt River, Switzerland. *Environ. Sci. Pollut. Res.* **13**, 333–341.

Westerhoff, P., Yoon, Y., Snyder, S. & Wert, E. 2005 Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. *Environ. Sci. Technol.* **39**, 6649–6663.

Yu, Z. R., Peldszus, S. & Huck, P. M. 2007 Optimizing gas chromatographic-mass-spectrometric analysis of selected pharmaceuticals and endocrine-disrupting substances in water using factorial experimental design. *J. Chromatogr. A.* **1148**, 65–77.

Zhang, H. C., Yu, X. J., Yang, W. C., Peng, J. F., Xu, T. & Yin, D. Q. 2011 MCX based solid phase extraction combined with liquid chromatography tandem mass spectrometry for the simultaneous determination of 31 endocrine-disrupting compounds in surface water of Shanghai. *J. Chromatogr. B.* **879**, 2998–3004.

Zuccato, E., Castiglioni, S., Fanelli, R., Reitano, G., Bagnati, R., Chiabrando, C., Francesco Pomati, F., Rossetti, C. & Calamari, D. 2006 Pharmaceuticals in the environment in Italy: causes, occurrence, effects and control. *Environ. Sci. Pollut. Res.* **13**, 15–21.

First received 24 July 2012; accepted in revised form 14 October 2012. Available online 7 November 2012