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Occurrence of estrogenic activities in second-grade surface water and ground water in the Yangtze River Delta, China

Wei Shi [a], Guanjiu Hu [b], Sulan Chen [b], Si Wei [a,*], Xi Cai [b], Bo Chen [b], Jianfang Feng [a], Xinxin Hu [a], Xinru Wang [c], Hongxia Yu [a]

[a] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210046, People's Republic of China
[b] State Environmental Protection Key Laboratory of Monitoring and Analysis for Organic Pollutants in Surface Water, Jiangsu Provincial Environmental Monitoring Center, Nanjing, People's Republic of China
[c] Key Laboratory of Reproductive Medicine & Institute of Toxicology, Nanjing Medical University, Nanjing, People's Republic of China

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ABSTRACT

Second-grade surface water and ground water are considered as the commonly used cleanest water in the Yangtze River Delta, which supplies centralized drinking water and contains rare species. However, some synthetic chemicals with estrogenic disrupting activities are detectable. Estrogenic activities in the second-grade surface water and ground water were surveyed by a green monkey kidney fibroblast (CV-1) cell line based ER reporter gene assay. Qualitative and quantitative analysis were further conducted to identify the responsible compounds. Estrogen receptor (ER) agonist activities were present in 7 out of 16 surface water and all the ground water samples. Huaihe River and Yangtze River posed the highest toxicity potential. The highest equivalent (2.2 ng E_2/L) is higher than the predicted no-effect-concentration (PNEC). Bisphenol A (BPA) contributes to greater than 50% of the total derived equivalents in surface water, and the risk potential in this region deserves more attention and further research.

1. Introduction

Growing attention has been given to the endocrine disrupting compounds (EDCs) that are known to alter the endocrine systems of humans or wildlife by mimicking or counteracting natural hormones (Benotti et al., 2008; Li et al., 2012). This situation is of concern, because extremely low concentrations of EDCs may trigger alterations, and many EDCs bio-accumulate along trophic chains which could increase their potential risk (Bellingham et al., 2009). Moreover, the question of mixture effects is raised by the simultaneous presence of numerous EDCs (Ma et al., 2007). Among these EDCs, estrogenic compounds are suspected of being responsible for the increase in disturbances of wildlife reproduction and development (Colborn et al., 1993; Sharpe and Irvine, 2004). The endocrine mimicking compounds that bind to the estrogen receptor (ER) is considered as the key factor that elicits estrogenic response in vertebrates (Colborn et al., 1993). Several potential ligands for the ER have been identified, including bisphenol A (BPA) and octylphenol (OP). These chemicals are known to exert estrogenic activity in *in vitro* and *in vivo* assays (Delfosse et al., 2012), and are also observed in river water, sediments, and fish tissue all over the world (Furuichi et al., 2004; Hashimoto et al., 2005).

The Yangtze River Delta is the most industrialized and urbanized region in China with busy transportation and crowded chemical industry parks (Chen et al., 2001). However, the inadequate investment in basic water supply and treatment infrastructure has resulted in more dangerous and polluted circumstances in the whole basin area. Daily monitoring for the aquatic environment is mainly based on the conventional indicators including chemical oxygen demand, biochemical oxygen demand, dissolved oxygen and so on. Although these water environments are up to snuff, EDCs are sill detectable including polycyclic aromatic hydrocarbons (PAHs) and alkylphenol (Shi et al., 2011). Surface water environment in China is divided into five grades according to the designated use including human contact recreation, drinking water supply, and so on (Administration) (SEPA, 2002). First-grade surface water is considered as the head stream and protection zones, while second-grade surface water supplies centralized drinking water and water for rare species. Few aquatic environments in the Yangtze River Delta are considered as head stream, and second-grade surface water as well as ground water is considered as the cleanest water environment in this region. Consequently, the occurrence of

* Corresponding author.
E-mail addresses: fanfan_67946@163.com (S. Wei), yuhx@nju.edu.cn (H. Yu).

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estrogenic chemicals and the related effects in this region should be investigated, as little research has been done regarding this.

Reporter gene assays are rapid, sensitive and reproducible *in vitro* methods, which have been used as the basis of estrogenic reporter gene assay for the screening of chemicals and environmental samples with ER agonistic properties. Previous studies have mainly focused on the estrogenic disrupting potentials of the environmental sample extracts, and little has been done for investigating the responsible contaminants (Schmitt et al., 2012). There is a growing concern for utilization of mass balance analysis for the identification of specific compounds responsible for endocrine disrupting activities. At the same time, the estrogen receptor equivalency ranges (ER-EQ_{20-80} ranges) should be included for further comparison, which may indicate the uncertainty in the equivalency estimate due to deviations from parallelism between the standard and sample dose−response curves (Shi et al., 2012).

In a long-standing research project, a large number of second-grade water and ground water samples classified as the cleanest water in the Yangtze River Delta were studied in order to assess the contaminant level of EDCs and the related effects. These water bodies were suspected to be remote, and far away from direct human impact, however, potential long-range transported pollutants also exist. The CV-1 ER assay was used to determine estrogenic disrupting effects in second-grade surface water and ground water in the Yangtze River Delta, China. The three objectives of this study were to: 1) Detect ER agonist activities in water; 2) Identify the compounds responsible for estrogenic potency of extracts by use of a combination of instrumental analysis and bioassays; 3) Examine the potential adverse effects in the second-grade surface water and ground water.

2. Materials and methods

2.1. Chemicals and reagents

The abbreviation and CAS of chemicals used in instrumental analysis are given in Table 1. Chemicals used in instrumental analysis were purchased from Sigma Chemical Co. (St. Louis, MO, USA) with the purity of over 99.5%. Chemicals used in bioassays including 3-(4,5-dimethylthiazol-2-ol)-2,5-diphenyltetrasodium bromide tetrazolium (MTT) and 17β-estradiol (E_2) with the purity of over 99% were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Site selection and field sampling

Untreated second-grade surface water and underground water in the Yangtze River Delta were targeted for this reconnaissance. Sites were chosen in areas that were known or suspected to have industrial, human and (or) animal wastewater sources in upstream or around. Each of the sampling sites could serve more than 50 thousand residents as source water. Water samples were collected in March 2009 from sixteen second-grade water areas in Huaihe River (MAOK, BINH, DAF, GEJB, GAOY, CHENGN, HUAIY), Yangtze River (QID, RUB, JIANGA, GAOC, ZHANGJG, JIANGI), Taihu Lake (DONGH, GONGH, XIAOWL). Three ground water samples (PEIZ, XUZ, PEIX) were also collection for comparison (Fig. 1).

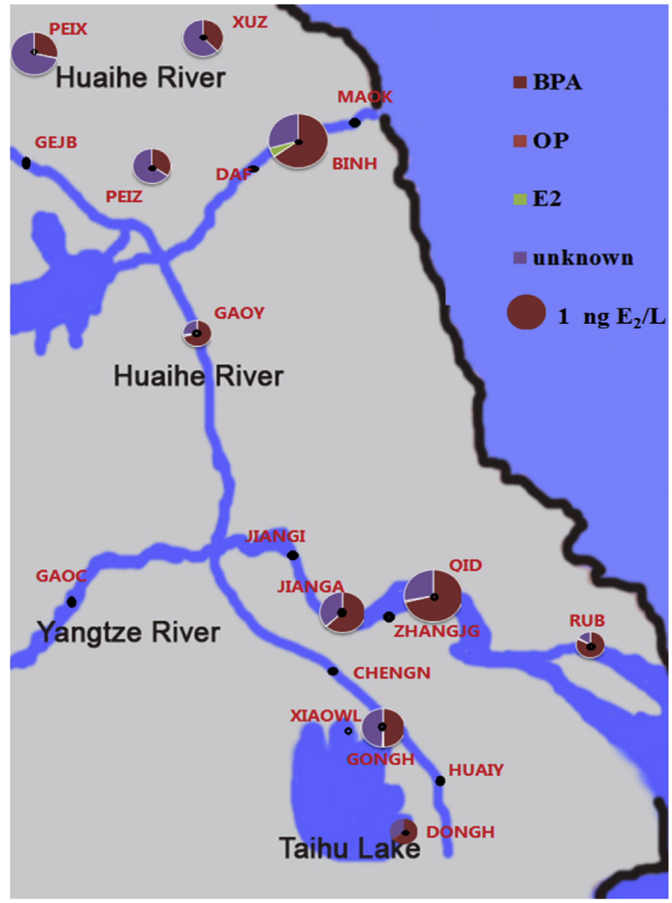

Fig. 1. Map of the chosen sampling sites (MAOK, BINH, DAF, GEJB, GAOY, CHENGN, HUAIY, QID, RUB, JIANGY, GAOC, ZHANGJG, JIANGY, DONGH, GONGH, XIAOWL, PEIZ, XUZ, PEIX) from the Yangtze River Delta.

Table 1
Abbreviation, CAS, recovery, limit of quantification (LOQ) and limit of detection (LOD) of tested chemicals.

	Abbreviation	CAS	Recovery (%)	RSD (%)	LOD (ng/L)	LOQ (ng/L)
Naphthalene	Nap	91-20-3	63%	9%	0.49	1.62
Acenaphthylene	Acy	208-96-8	81%	4%	0.53	1.78
Acenaphthene	Ace	83-32-9	93%	5%	0.16	0.55
Fluorene	Flu	86-73-7	113%	14%	0.18	0.60
Phenanthrene	Phe	85-01-8	80%	69%	0.25	0.84
Anthracene	Ant	120-12-7	86%	5%	0.13	0.43
Fluoranthene	Flt	206-44-0	96%	18%	0.16	0.54
Pyrene	Pyr	129-00-0	91%	11%	0.19	0.62
Benz[a]anthracene	B[a]A	56-55-3	94%	2%	0.26	0.85
Chrysene	Chr	218-01-9	83%	9%	0.18	0.59
Benzo[b]fluoranthene	B[b]F	50-32-8	89%	3%	0.13	0.44
Benzo[k]fluoranthene	B[k]F	207-08-9	87%	7%	0.27	0.90
Benzo[a]pyrene	B[a]P	50-32-8	87%	5%	0.54	1.81
Indeno[1,2,3-c,d]pyrene	Ind	193-39-5	83%	5%	0.20	0.68
Dibenz[a,h]anthracene	DBA	53-70-3	89%	9%	0.36	1.20
Benzo[g,h,i]perylene	B[ghi]P	191-24-2	82%	5%	0.41	1.37
Octylphenol	OP	27193-28-8	84%	6%	0.69	2.31
Bisphenol A	BPA	80-05-7	91%	2%	0.78	2.60
17β-Estradiol	E_2	79037-37-9	77%	3%	1.37	4.58

LODs: Limits of detection (S/N = 3); LOQs: Limits of quantitation (S/N = 10); Concentrations are expressed as mean ± SD ($n = 3$).