Environment International 42 (2012) 117–123

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Thyroid hormone disrupting activities associated with phthalate esters in water sources from Yangtze River Delta

Wei Shi [a], Feng-Xian Zhang [a], Guan-Jiu Hu [b], Ying-Qun Hao [b], Xiao-Wei Zhang [a], Hong-Ling Liu [a,*], Si Wei [a], Xin-Ru Wang [c], John P. Giesy [a,d,e,f,g,h], Hong-Xia Yu [a,*]

[a] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, 210093, PR China
[b] State Environmental Protection Key Laboratory of Monitoring and Analysis for Organic Pollutants in Surface Water, Jiangsu Provincial Environmental Monitoring Center, Nanjing, 210036, PR China
[c] Key Laboratory of Reproductive Medicine & Institute of Toxicology, Nanjing Medical University, Nanjing, 210029, PR China
[d] Department of Veterinary Biomedical Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada
[e] Department of Zoology, and Center for Integrative Toxicology, Michigan State University, East Lansing, MI, USA
[f] Zoology Department, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia
[g] Department of Biology & Chemistry, City University of Hong Kong, Kowloon, Hong Kong, SAR, China
[h] School of Biological Sciences, University of Hong Kong, Hong Kong, SAR, China

ARTICLE INFO

Available online 17 June 2011

Keywords:
Reporter gene assay
Agonist equivalents
Antagonist equivalents
Dibutyl phthalate
ATR-EQ_{20-80} range
Human risk

ABSTRACT

Thyroid hormone disrupting compounds in water sources is a concern. Thyroid hormone (TH) agonist and antagonist activities of water sources from the Yangtze River, Huaihe River, Taihu Lake and ground water in the Yangtze River Delta region were evaluated by use of a TH reporter gene assay based on the green monkey kidney fibroblast (CV-1). While weak TH receptor (TR) agonist potency was observed in only one of 15 water sources, antagonist potency was present in most of the water sources. TR antagonist equivalents could be explained by the presence of dibutyl phthalate (DBP), with concentrations ranging from 2.8×10^{1} to 1.6×10^{3} μg DBP /L (ATR-EQ_{50}s). None of the ground waters exhibited TH agonist potencies while all of the samples from Taihu Lake displayed notable TR antagonist potencies. To identify the responsible thyroid active compounds, instrumental analysis was conducted to measure a list of potential thyroid-disrupting chemicals, including organochlorine (OC) pesticides and phthalate esters. Combining the results of the instrumental analysis with those of the bioassay, DBP was determined to account for 17% to 144% of ATR-EQ_{50}s in water sources. Furthermore, ATR-EQ_{20-80} ranges for TR antagonist activities indicated that samples from locations WX-1 and WX-2 posed the greatest health concern and the associated uncertainty may warrant further investigation.

1. Introduction

Increasing attention has been given to the environmental contaminants which can disrupt the endocrine system in human and wildlife (Colborn et al., 1993). Most research has focused on chemicals that can modulate androgen and estrogen homeostasis (Kuster et al., 2010; Gracia et al., 2008; Hill et al., 2010). In contrast, less information is available regarding the compounds with thyroid disrupting activities (Jugan et al., 2009). However, several contaminants from agriculture and industry, such as pesticides and plasticizers, have been shown to exert toxic effects on thyroid gland function, which also lead to adverse effects on growth and development (Brucker-Davis, 1998; Darnerud et al., 2010) .

Thyroid hormone (TH) is a key molecule involved in regulating growth, tissue differentiation, energy metabolism, reproduction, and formation of the central nervous system (Jugan et al., 2009). Normal thyroid hormone levels are essential for mammals during development of the central nervous system and disruption of normal hormone levels can impair brain maturation, that results in permanent mental retardation (Flamant and Samarut, 2003). TH is also important for several aspects of reproduction for fish, including ovary maturation (Blanton and Specker, 2007).

Synthetic chemicals that occur in the environment, such as organochlorine (OC) pesticides, polybrominated diphenyl ethers (PBDEs) and phthalate esters are potential endocrine disruptors by modulating thyroidal system (Hofmann et al., 2009; Jugan et al., 2007; Li et al., 2010). Thyroid hormone disrupting chemicals could interfere with the thyroid receptor (TR) by agonism or antagonism. A transactivation screening method for chemicals with TR ant/agonistic properties based on CV-1 cells was used (Li et al., 2010; Shi et al., 2009). The anti/thyroid hormone effects of bisphenol A (BPA), tetrachlorobisphenol A, carbaryl, 1-naphthol, 2-naphthol, DBP, mono-n-butyl phthalate (MBP) and DEHP have been demonstrated (Shen et al., 2009; Sun et al., 2008; Sun et al.,

* Corresponding authors. Tel.: +86 25 8359 3649; fax: +86 25 8368 6761.
E-mail addresses: hlliu@nju.edu.cn (H.-L. Liu), yuhx@nju.edu.cn (H.-X. Yu).

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doi:10.1016/j.envint.2011.05.013

2009). The present study used the CV-1 cell based TR gene reporter assay to measure thyroid disrupting potentials of source water samples in the Yangtze River Delta.

Chemical pollution has recently caused public concerns about drinking water safety in the Yangtze River Delta region. The Yangtze and Huaihe River, Taihu Lake and groundwater are sources of drinking water in this region. The Yangtze River is the primary source of drinking, serving more than 50 million residents in 8 large cities in Yangtze River Delta. More than 40 chemical industrial complexes had been set up along the river during the past decade (Shen et al., 2006). The Huaihe River provides drinking water to more than 50 million people, but it is one of the most densely populated rivers in China. Due to the rapid development of industry and economy in the area, the Huaihe River has been moderately polluted since the 1980s. Thirdly, Taihu Lake is an indispensable water resource for drinking water, agriculture, aquaculture and industrial plants in the Yangtze River Delta region (Song et al., 2007). In the past decades, the extraordinary economic growth, industrialization, and urbanization, coupled with inadequate investment in basic water supply and treatment infrastructure, have resulted in more contamination of the whole basin (Wu et al., 2004). More than 20% of the Yangtze River Delta population depends on ground water for drinking water supply from either a public source or private wells. Recent studies have suggested that groundwater quality could be threatened by chemical pollution in this region (Chen et al., 2010). These concerns on source water quality in the Yangtze River Delta warrant comprehensive monitoring studies on chemical pollutants in source water.

Previous studies have reported TH-active compounds in extracts of environmental samples (Ishihara et al., 2009), however, limited information is available for the contaminants that cause the effects. PBDEs are well-known TH disrupters, but concentrations in surface water are generally small. Although no concentrations of PBDEs in Yangtze River Delta have been reported, studies have been employed in the Zhujiang River Estuary, which is much more polluted by e-waste. Concentrations ranged from 9.0 to 1.3×10^2 pg/L (Luo et al., 2008), which are insufficient to cause the observed TR antagonist potency (Li et al., 2010). We have previously reported that concentrations of phthalate esters were 100- to 1000-fold greater than those of OC pesticides, polychlorinated biphenyls and some phenols in source water in East China (Shi et al., 2011). There is a growing concern to utilize the mass balance analysis to these chemicals with high concentrations for the identification of the responsible compounds (Li et al., 2010). Deviations from parallelism between the dose–response curves of reference chemical and samples cause uncertainty in the analysis of toxic equivalency by bioassays (Villeneuve et al., 2000). In the present study, ATR-EQ_{20-80} ranges for TR antagonist activities were employed in the potency balance analysis to estimate the uncertainty associated with the bioassay approach.

The objectives of the present study were to: 1) examine the agonist and/or antagonist effects in primary water sources at the Yangtze River Delta region by the transient reporter gene assays based on the CV-1 cell line; 2) identify the responsible thyroid-active compounds by combining instrumental analysis with bioassays. 3) evaluate the uncertainty of the REP estimation by the employment of ATR-EQ_{20-80} ranges.

2. Materials and methods

2.1. Chemicals and reagents

List, purity, abbreviation and source of analytical chemicals are given in Table 1. 3-(4,5-dimethylthiazol-2-ol)-2,5-diphenyltetrasodium bromide tetrazolium (MTT) and L-3,5,3′-triiodothyronine (T_3) with the purity of over 99% were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Table 1
Sources and purities of tested chemicals.

Classes (providers)	Chemicals	Purity (%)
Organochlorine pesticides (Sigma–Aldrich)	γ-chlordane, α- chlordane, α-HCH, β-HCH, γ-HCH, δ-HCH, p,p'-DDT, o,p'-DDT, p,p'-DDD, p,p'-DDE	99.5%
Phthalate esters (Labor Dr. Ehrenstorfer-Schafers, Germany)	Dibutyl phthalate (DBP), di-2-ethylhexyl phthalate (DEHP), dimethyl phthalate (DMP), diethyl phthalate (DEP), benzyl butyl phthalate (BBP), diisodecyl phthalate (DIDP), bis(2-ethylhexyl) adipate (DEHA), di-n-octyl phthalate (DnOP), diisononyl phthalate (DINP)	>99%

2.2. Sample collection and preparation

Untreated underground and surface water as sources of drinking water in the Yangtze River Delta were studied. Sites were chosen in areas that were known or suspected to have industrial, human and (or) animal wastewater sources upstream or in the vicinity. Fifteen waterworks were selected in the Yangtze River Delta, whose drinking water daily outputs were more than 300,000 m^3. Water samples were collected in March 2009 from Yangtze River at locations LYG-1, YC-1, YC-2, XZ-1, YZ-4, Huaihe River at NT-1, NT-2, TZ-2, NJ-3, Taihu Lake at SZ-4, WX-1, WX-2 and groundwater from locations XZ-12, XZ-3, XZ-7 (Fig. 1). Samples of water (15 L) were collected in a glass vessels pre-cleaned and rinsed with methanol at each location (10 L for bioassay and 5 L for chemical analysis). The water samples were transported and stored at 4 °C pending extraction and analysis within 24 h.

Water samples were passed through Oasis cartridges (200 mg Oasis HLB glass cartridge; Waters, Milford, MA, USA) under vacuum at a flow rate of 6–8 mL/min. 2 L sample was passed through each column to avoid over filtration. A series of 5 columns were used for bioassay and 2 columns were used for instrumental analysis for each water sample. Cartridges were sequentially activated and conditioned with high-purity hexane (Merck, Darmstadt, Germany), dichloromethane (Tedia Co. Ltd, Fairfield, OH, USA), acetone (Tedia Co. Ltd, Fairfield, OH, USA) and methanol (Tedia Co. Ltd, Fairfield, OH, USA). Each cartridge was eluted stepwise as follows: 10 mL hexane, 10 mL hexane: dichloromethane (4:1), followed by 10 mL acetone: methanol (1:1, v/v). All eluates were evaporated by rotary evaporation (type TVE-1000, EYELA, Tokyo, Japan) in a thermostatic bath. Then the dehydrated extracts were blown to dryness under gentle nitrogen flow and reconstituted in 0.2 mL dichloromethane for chemical analysis. For the bioassays, extracts were blown to dryness under a gentle nitrogen flow and reconstituted in 0.2 mL of dimethyl sulfoxide (DMSO, BDH Laboratory Supplies, UK). Extracts in DMSO were diluted with appropriate culture medium to be equivalent to 12.5, 25, 50, 100 and 200 times greater the original concentration in source water before bioassays with a final solvent less than 0.5% (v/v). Blanks prepared with purified water were used to exclude endocrine disrupting toxicity during the working procedure, using the same procedure as for the environmental samples. Extracts were stored at −20 °C.

2.3. Bioassay

All media used for the assay were prepared according to the original protocol (Shi et al., 2009). Green monkey kidney fibroblast (CV-1) cells which contain no endogenous receptors were obtained from the Institute of Biochemistry and Cell Biology in Shanghai, Chinese Academy of Science. CV-1 cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Invitrogen Corporation, Carlsbad, CA, USA), 100 U/mL penicillin (Sigma) and 100 μg/mL streptomycin (Sigma, St. Louis, MO, USA) in an atmosphere containing 5% CO_2 at 37 °C. Cells were seeded into 48-well microplates and were