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The evaluation of endocrine disrupting effects of *tert*-butylphenols towards estrogenic receptor α, androgen receptor and thyroid hormone receptor β and aquatic toxicities towards freshwater organisms☆

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ABSTRACT

The phenolic compounds have posed public concern for potential threats to human health and ecosystem. *Tert*-butylphenols (TBPs), as one group of emerging contaminants, showed potential endocrine disrupting effects and aquatic toxicities. In the present study, we detected concentrations of 2,4-DTBP ranging from <0.001 to 0.057 μg/L (detection limit: 0.001 μg/L) in drinking water source from the Qiantang River in East China in April 2016. The endocrine disrupting effects of 2-TBP, 2,4-DTBP and 2,6-DTBP toward human estrogen receptor α (ERα), androgen receptor (AR) and thyroid hormone receptor β (TRβ) were evaluated using human recombinant two-hybrid yeast bioassay. Their aquatic toxicities were investigated with indicator organisms including *Photobacterium phosphoreum, Vibrio fischeri* and freshwater green alga *Chlamydomonas reinhardtii.* 2-TBP and 2,4-DTBP exhibited moderate antagonistic effects toward human ERα and AR in a concentration-dependent manner. 2-TBP significantly inhibited the light emission of *P. phosphoreum.* 2-TBP, 2,4-DTBP and 2,6-DTBP significantly inhibited the growth of *C. reinhardtii* and reduced the chlorophyll content. Our results suggest the potential adverse effects of TBPs on human health and aquatic organisms. The data will facilitate further risk assessment of TBPs and related contaminants.

1. Introduction

Phenolic compounds have been widely used in industries and some of them have been listed as priority pollutants by the U.S. Environmental Protection Agency and the European Community (EC, 2001; EPA, 1977). *Tert*-butylphenols (TBPs) are one group of alkylated phenolic compounds with one or more *tert*-butyls at the benzene ring. Their residues have been detected from consumer products (Dekiff et al., 2014; Jonker et al., 2016), dust (Liu et al., 2017a), surface water (Liu et al., 2016, 2017b) and effluents from waste water treatment plants (Xu et al., 2016). TBPs caused increasing public concern due to their unique environmental behavior and toxicities (Haavisto et al., 2003; Rudel et al., 2003; Ying et al., 2002).

Manufactures and suppliers of TBPs in the vicinity of drinking water source are the frequent contributors to the residues of TBPs in surface water, causing potential aquatic ecotoxicities. As the largest source of drinking water in Zhejiang Province in East China, Qiantang River has been an important commercial artery and many kind of industries are located along it (Chen et al., 2017; Lu et al.,

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2017). Till now it lacks the data on residues of TBPs in drinking water source from the Qiantang River in East China. It also remains unclear on how TBPs exhibit their aquatic toxicities toward various indicator organisms such as bioluminescent bacteria and freshwater green algae. Thus, work to monitor the residues of TBPs in Qiantang River and determine the potential aquatic toxicities after exposure to TBPs is necessary.

2-*tert*-butylphenol (2-TBP) and 2,6-di-*tert*-butylphenol (2,6-DTBP) caused cytotoxicity toward human submandibular gland carcinoma cells (Kadoma et al., 2009). 2-Alkyl- and 2,6-dialkyl-4-X-phenols induced cytotoxicity of tumor cell L1210 (Selassie et al., 2002). 2-TBP and 2,4-DTBP caused adverse effects on the liver of newborn and young rats (Hiratakoizumi et al., 2005). TBPs were also reported to exhibit endocrine disrupting effects, for example, 2-TBP was revealed as a weak inhibitor towards progesterone receptor (Li et al., 2010a), and 2-TBP, 4-TBP and 2,4-DTBP showed estrogenic disrupting effects (Akahori et al., 2008; Tollefsen and Nilsen, 2008). It is thus essential to investigate the adverse effects of TBPs toward these different nuclear receptors.

In the present study, samples were collected at 14 sites along the main stream and tributaries of Qiantang River (Fig. 1) and three TBPs were analyzed (Table S1). The endocrine disrupting effects toward human estrogenic receptor α (ERα), androgenic receptor (AR) and thyroid hormone receptor β (TRβ) were evaluated by the human recombinant yeast two-hybrid bioassay. The comprehensive toxicity of TBPs were fully investigated using two bioluminescent bacterium including *Photobacterium phosphoreum* and *Vibrio fischeri,* and freshwater green alga *Chlamydomonas reinhardtii*. The results are essential for a comprehensive evaluation of toxicity of TBPs, further facilitating the risk assessment of TBPs towards human health and aquatic organisms.

2. Materials and methods

2.1. Chemicals and reagents

2-TBP (99% purity), 2,4-DTBP (99% purity), 2,6-DTBP (99.5% purity) and 17β-Estradiol (E2, 99% purity) were purchased from J&K Chemical Ltd. (Shanghai, China). Dihydrotestosterone (DHT, 99% purity), 3,3′,5-triiodothyronine (T3, >98% purity) and dimethylsulfoxide (DMSO, 99.5% purity) were purchased from Sigma Chemical Company (St. Louis, MO, USA). The synthetic defined (SD) broths and agar minimal media (lacking tryptophan and leucine, SD/-Leu/-Trp) was obtained from Mobitec Company (Catalogue: 4823-6). All other chemicals were of analytical grade. The test chemicals were dissolved in DMSO (v/v < 0.1%) and the corresponding stock solutions were prepared with Milli-Q water (18.2 MΩ cm, Millipore, Bedford, MA, USA).

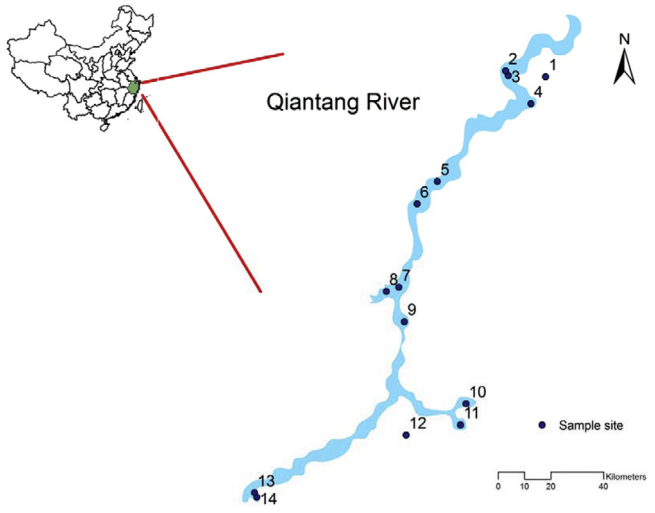

Fig. 1. Map of the sampling locations in Qiantang River. The green represented Zhejiang Province on the map of China. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Sampling and analysis

In total 42 water samples were collected from 14 sampling sites in April 2016 (Fig. 1, Table 1). The samples were transported to the laboratory under ice cold conditions and stored in the dark at 4 °C. All water samples were filtered firstly using glass fiber filters (Whatman GF/F, 0.45 μm pore size) to remove the particles. The water sample of 1 L was transferred into a separatory funnel for acidification to pH ≤ 2 by drop-wise addition of sulfuric acid solution, followed by the addition of 30 g sodium chloride and 70 mL dichloromethane. The organic phase was collected and dehydrated by anhydrous sodium sulfate. Each sample was extracted in triplicate. The organic phase was finally concentrated to 1 mL. Phenanthrene-d10 was used as the internal standard. The ultrapure water of 1 L was used as blank control. Extracts were stored at −20 °C prior to analysis.

TBPs were analyzed using gas chromatograph-mass spectrometry (Model GC-MS-QP2010 Plus, Shimadzu, Japan). TBPs were separated using an Agilent DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm) in the splitless mode. A 1 μL extract was injected to the column with the column flow rate at 1.0 mL/min with injection temperature of 270 °C. The gas chromatograph was set at 50 °C for 2 min and ramped first at 20 °C/min to 160 °C for 2 min and ramped later at 5 °C/min to 260 °C for 2 min. The quadrupole mass spectrometer was set in selected ion monitoring mode with an electron ionization source at 70 eV. The ion source temperature and transfer line temperature were set at 230 °C and 280 °C, respectively. The used carrier gas helium has high purity of 99.999%.

2.3. Quality assurance/quality control

The GC-MS system was calibrated before sample analysis. A 1 μL standard solution containing trifluorotriphenyl phosphine (DFTPP) was injected into the GC-MS to ensure the quality control criteria (Table S2). The calibration curve was constructed with standard deviation of no more than 20% relative to the internal standard. The procedural blank and field blank were performed and the detection

Table 1
Information on 14 sampling sites in Qiantang River and detected concentrations of 2,4-DTBP (μg/L) in April 2016.

Sampling site	Coordinate	2,4-DTBP
1	119°29′8″E, 29°12′15″N	-[a]
2	120°9′48.17″E, 30°12′45.29″N	–
3	120°9′32.97″E, 30°12′37″N	–
4	120°10′57.28″E, 30°4′39.4″N	–
5	119°45′48.28″E, 29°52′30.29″N	0.040
6	119°45′48.28″E, 29°48′57.13″N	0.057
7	119°32′20.91″E, 29°32′35.76″N	–
8	119°28′8.8″E, 29°32′17.96″N	0.004
9	119°32′5.61″E, 29°25′37.80″N	–
10	119°42′36.28″E, 29°6′49.35″N	–
11	119°40′40″E, 29°3′37″N	0.018
12	119°27′36.87″E, 29°11′49.99″N	–
13	118°50′23.67″E, 28°56′41.37″N	0.032
14	118°51′3″E, 28°56′24″N	–

[a] The detection limit is 0.001 μg/L. The sign - means below limit of detection.