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Toxicological and chemical insights into representative source and drinking water in eastern China☆

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ABSTRACT

Drinking water safety is continuously threatened by the emergence of numerous toxic organic pollutants (TOPs) in environmental waters. In this study, an approach integrating *in vitro* bioassays and chemical analyses was performed to explore toxicological profiles of representative source and drinking water from waterworks of the Yangtze River (Yz), Taihu Lake (Th), and the Huaihe River (Hh) basins in eastern China. Overall, 34 of 96 TOPs were detected in all water samples, with higher concentrations in both source and drinking water samples of Hh, and pollutant profiles also differed across different river basins. Non-specific bioassays indicated that source water samples of Hh waterworks showed higher genotoxicity and mutagenicity than samples of Yz and Th. An EROD assay demonstrated dioxin-like toxicity which was detected in 5 of 7 source water samples, with toxin concentration levels ranging from 62.40 to 115.51 picograms TCDD equivalents per liter of water (eq./L). PAHs and PCBs were not the main contributors to observed dioxin-like toxicity in detected samples. All source water samples induced estrogenic activities of 8.00–129.00 nanograms 17β-estradiol eq./L, and estrogens, including 17α-ethinylestradiol and estriol, contributed 40.38–84.15% of the observed activities in examined samples. While drinking water treatments efficiently removed TOPs and their toxic effects, and estrogenic activity was still observed in drinking water samples of Hh. Altogether, this study indicated that the representative source water in eastern China, especially that found in Hh, may negatively affect human health, a finding that demonstrates an urgent requirement for advanced drinking water treatments.

1. Introduction

Drinking water safety has become a more and more serious concern with increased urbanization and industrialization. Numerous organic pollutants are continuously discharged into aquatic environments, and these compounds can then be consumed through drinking water and thus threaten human health (Kannan et al., 2005; Singh et al., 2004). Many countries have implemented strict water quality standards to ensure drinking water safety, but a tiny fraction of pollutants are listed in these regulations compared to the huge number of chemicals released into water. It was reported that more than 133 million types and 360 million tons of chemicals have been synthesized and used annually in anthropogenic activities (Louise, 2013). Some of these compounds find their way into natural waters, but less than 200 of them are monitored by regulation departments around the world (Schwarzenbach et al., 2006). Hence, it is difficult to evaluate the safety of water samples based on these limited chemical parameters. *In vitro* bioassay-based evaluation has become an effective method that supplements instrumental analyses to provide a comprehensive understanding of water quality and toxicological profiles (Lee et al., 2012; Kunz et al., 2015; Floehr et al., 2015).

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Bioassay-based evaluation has been widely applied in assessing toxicities of chemicals in aqueous samples for several decades, displaying great advantages, such as high sensitivity and time efficiency (Plewa et al., 2004; Kokkali and van Delft, 2014). However, compounds in water samples are present as mixtures, and they may produce additive, synergistic or antagonistic interactions during exposure to a test organism. This additive assessment of mixture toxicity may lead to overestimations or underestimations in terms of relative potencies (RePs) and concentrations of individual compounds (Suzuki et al., 2004). By using *in vitro* bioassays and a sample extraction method, the combined biological effects of an environmental mixture can be quantified without knowing whole profiles of chemical pollutants. Li et al. (2010) used a two-hybrid yeast assay to assess the agonistic and antagonistic thyroid receptor-mediated effects in drinking water and revealed that dibutyl phthalate was the main contributor to the effect, based on a toxic-equivalent quantity approach. Another study also detected significant androgen receptor-antagonistic potencies in drinking water sources from eastern cities of China, where diisobutyl phthalate and dibutyl phthalate were identified as major contributors to the observed effects (Hu et al., 2013). Thus, integration of bioassays and chemical analyses is an excellent tool to assess biological effects of mixtures and identify the possible constituents.

In this work, a mode of action test battery was applied to gain a comprehensive picture of the toxic potency of source and drinking water that serves millions of people near the Yangtze River (Yz), Taihu Lake (Th) and the Huaihe River (Hh) basins in eastern China. These river basins were previously reported to contain pollution consisting of various organic contaminants, including organochlorine pesticides (OCPs) (Wang et al., 2009), perfluorooctane sulfonates (PFOS) (Yu et al., 2013), polycyclic aromatic hydrocarbons (PAHs) and phthalate esters (PAEs) (Zhang et al., 2012). These detected organic compounds are known to induce different toxic activities, such as cytotoxicity, dioxin-like activity, genotoxicity and endocrine effects (Sarath Josh et al., 2014; Windal et al., 2005). Although these organic pollutants have been detected in these river estuaries for years, the potential for biological effects in exposed organisms is seldom comprehensively characterized. Hence, the neutral red (NR) assay, micronucleus test, Ames test ethoxyresorufin-*O*-deethylase (EROD) assay and ERα/AR CALUX® tests were performed to evaluate non-specific, specific and reactive toxicities of source and drinking water samples in this study. This test battery-type approach combines a number of assays across multiple categories, enabling a more comprehensive characterization of various aspects of toxicity. Moreover, a list of organic pollutants contained in the samples was also determined to help identify possible links between observed effects and biologically active chemicals.

2. Materials and methods

2.1. Sample collection and pretreatment

In October 2014, 14 source and drinking water samples were collected from 7 waterworks near Yz, Th and Hh basins in eastern China (Fig. 1). In this study, abbreviations of water sources followed by "-number", "-S" or "-D" indicate that the sample is from a waterworks, source water or drinking water, respectively. The following is a list of waterworks sampled and their locations: waterworks Yz-1 and Yz-2 were located in the Yangtze River basin, waterworks Th-1 and Th-2 were located in Taihu Lake basin and waterworks Hh-1, Hh-2 and Hh-3 were located in the Huaihe River basin. As described in Table S1, the waterworks of Hh-1, Hh-2 and Hh-3 were equipped with conventional water treatment methods including coagulation, sedimentation, sand filtration and chlorination. In the waterworks of Yz-1 and Yz-2, conventional treatments coupled with ozone-activated carbon adsorption (O_3-BAC) were applied. Ultrafiltration (UF) was added to the end of O_3-BAC in waterworks of Th-1 and Th-2, in addition to the treatment technologies present at Hh and Yz locations. Th-1 and Th-2 are the only two waterworks that collect source water from Taihu Lake, and Hh-1, Hh-2 and Hh-3 are also the three largest waterworks, which locate at the downstream Huaihe River. Yz-1 and Yz-2 are also one of the largest waterworks, which locate along the downstream Yangtze River in eastern China. Prior to sample collection, all the sampling bottles were rinsed with distilled water, ultrapure water and methanol in sequence. At each waterworks, 3 L of source or drinking water samples were collected every 2 h throughout one day and then pooled to minimize temporal variation of water quality. To prevent oxidation of the organic matter present in the samples, 0.2 g of ascorbic acid was added immediately after collection of each sample. After collection, the water samples were ice-refrigerated and transported to the laboratory within 4 h. Sample pretreatments for chemical analyses and bioassays were as described in the Supplementary Material Text S1.

2.2. Instrumental analyses

Each water sample was screened for 96 organic compounds using gas chromatography-mass spectrometry (GC-MS) or ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Compounds screened for included 16 PAHs, 15 polychlorinated biphenyls (PCBs), 16 PAEs, 22 OCPs, 19 organochlorine pesticides (OPs) and 8 environmental estrogens (EEs). In detail, PAHs, PCBs, PAEs, OCPs and OPs were all quantified by use of a TRACE ULTRA GC and ISQ MS (Thermo Fisher, USA) with a TG-5 MS capillary column (Thermo Scientific, 30 m × 0.25 mm i.d. × 0.25 μm) according to a method outlined in a previous study (Hu et al., 2013). Phenols and estrogens were measured using an ACQUITY I-Class UPLC and Xevo TQ-S micro MS (Waters, USA) with a BEH C_{18} column (1.7 μm, 2.1 × 100 mm, Waters, USA) according to a method outlined in another previous study (Loos et al., 2010) with modified parameters (Table S2). Chemicals for which samples were screened and associated information is shown in Supplementary Material Table S3, and more details about the detection methods used and quality assurance and control (QA/QC) parameters are described in Supplementary Material Text S2 and S3. Limit of quantification (LOQ), procedural and matrix recoveries of each compound is shown in Table S4.

2.3. Ethoxyresorufin-O-deethylase assay

The ethoxyresorufin-*O*-deethylase (EROD) assay was performed according to the method proposed by Keiter et al. (2008). The Rainbow Trout Liver-Waterloo 1 (RTL-W1) cells were incubated in 96-well microplates at 20 °C for 72 h according to the steps proposed by Klee et al. (2004). For each plate, 200 μL of L-15 medium was set as a negative control. As a positive control, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was selected and diluted with L-15 medium in six dilution steps (3.125–100 pM) in internal duplicates. The sample extracts were serially diluted in six replicates in eight steps (1:2), with a maximum dimethylsulfoxide (DMSO) (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) concentration of 1%. The amount of resorufin was measured using a microplate reader (Infinite M200, Tecan Austria GmbH, Grödig, Austria) at 544 nm excitation and 590 nm emission, and the protein concentration was determined at 360 nm excitation and 465 nm emission using the same microplate reader. The concentration-response curves were fitted with a nonlinear regression using GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, USA), and