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Urinary bisphenol analogues and triclosan in children from south China and implications for human exposure☆

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ABSTRACT

Bisphenols and triclosan (TCS) are widely used in consumer products. However, knowledge on human exposure to these anthropogenic chemicals has remained limited in China, especially for children. In this study, concentrations of seven bisphenols and TCS were determined in 283 urine samples collected from South China children aged between 3 and 11 years old. Bisphenol A (BPA), bisphenol S (BPS) and TCS were frequently detected in urine samples, with a detection rate of 93%, 89%, and 95%, respectively. Urinary concentrations of Σ_7BPs (the sum concentrations of the seven bisphenols) ranged from 0.43 to 31.5 μg/L, with a median value of 0.91 μg/L, while TCS concentrations ranged from < limit of quantification to 21.9 μg/L (median: 0.21 μg/L). BPA was the predominant analogue (median: 0.35 μg/L), accounting for 49.8% of Σ_7BPs. The urinary BPA concentrations in children from Guangzhou were significantly greater than those from Shenzhen. Correlation analysis suggested that multiple exposure sources to South China children likely existed for BPA, BPS, and TCS. Age, but not gender, was negatively associated with urinary residues of BPA and BPS ($p < 0.05$) and positively with TCS concentrations ($p < 0.05$). The estimated daily intake of Σ_7BPs (23.9 ng/kg bw/day) or TCS (5.63 ng/kg bw/day) was below the tolerant reference dose of BPA, indicating no considerable health hazard to South China children.

1. Introduction

As one of the most important man-made chemicals, bisphenol A (BPA) has been widely applied to consumer and industrial products, with a global production of approximately 5.5 million tons per year (Zhang et al., 2013, 2016). Because of the extensive use and leaching from host products, BPA has become a ubiquitous pollutant and been frequently detected in foodstuffs and various environmental matrices, including air, water, sewage sludge, and house dust (Kleywegt et al., 2011; Li et al., 2010; Liao et al., 2012a, 2013), as well as in human body fluids (Geens et al., 2015; Ye et al., 2008; Zhang et al., 2013). Toxicological studies demonstrated that BPA is a potential endocrine disruptor and associated with various adverse health effects, for instance, behavioral and reproductive abnormalities, disruption of thyroid hormone activity, estrogenic and anti-androgenic effects, obesity-accelerating effects, as well as chronic diseases, especially for teenagers who are exposed during a critical period of growth (Braun et al., 2011; Lang et al., 2008; Nahar et al., 2012). High urinary BPA concentrations may be related to cardiovascular diseases, diabetes and liver enzyme abnormality in humans (EFSA., 2014; Wang et al., 2012).

Considering the potential harm of BPA to animals and humans, regulations have been taken to restrict its use. However, alternatives such as bisphenol S (BPS), bisphenol F (BPF), bisphenol AP (BPAP), and bisphenol AF (BPAF) were developed and applied in various industrial products (Liao et al., 2012b, 2014a; Kitamura et al., 2005; Konno et al., 2004). Recent studies have increasingly detected these bisphenol analogues in environmental media and human bodies (Liao et al., 2012a; 2012c; Xue et al., 2017). Studies showed that some of these BPA alternatives have similar estrogenic activity and potential toxicity when compared with BPA (Okuda et al., 2011; Yoshihara et al., 2004). Some of them are even more

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resistant to biodegradation than BPA (Ike et al., 2006). Thus, human exposure to these BPA alternatives and associated effects merit more investigations.

Triclosan (TCS), known as a broad-spectrum antimicrobial, is widely used in household, health care and personal care products, as well as in clothes (Zhu et al., 2016). The annual commercial usage of TCS in China was about 100 tons in 2011, and the output has been increasing with year (Zhang et al., 2015). The large-scale use of TCS has resulted in its wide occurrence in sewage, biosolids, and sediment (Ahn et al., 2016). It is also frequently detected in humans and suspected to have an anti-androgenic activity in human breast cancer cells (Gee et al., 2008). As a result, TCS has been banned from the application as a food additive in food-grade plastic materials by the European Commission since 2010 (EC, 2010). Recently the United States (U.S.) Food and Drug Administration prohibited the addition of TCS to over-the-counter antibacterial hand and body washes (FDA, 2016).

Human exposure to bisphenol analogues and TCS is mainly via ingestion, inhalation, and dermal absorption (CDC, 2009). Urine is a useful matrix for the investigation of human exposure to a variety of chemicals because it reflects integrated exposure from different sources and pathways (Geens et al., 2015). Children are usually more vulnerable to toxic chemicals than the general populations (Wang et al., 2012). It was reported that a growing number of children has suffered from endocrine-related diseases caused by BPA and phthalates (Ryan et al., 2013). The Pearl River Delta is one of the most economically developed and urbanized regions in China, where heavy use of a large variety of anthropogenic chemicals including bisphenol analogues and TCS has been documented (Li et al., 2013). However, data on human (particularly children) exposure to bisphenol analogues and TCS and potential health effects remained scarce in this region.

Therefore, the main aims of the present study were to: (1) investigate the urinary concentrations and compositional profiles of seven bisphenol analogues and TCS in children aged between 3 and 11 years old from South China; (2) understand the potential sources of these chemicals; and (3) estimate the daily intake of bisphenol analogues and TCS by children. To our knowledge, this is the first study investigating urinary bisphenol analogues and TCS in South China children.

2. Materials and methods

2.1. Chemicals and reagents

BPA (97% purity), BPS (98% purity), BPF (98% purity), bisphenol P (BPP, 99% purity), bisphenol Z (BPZ, 98% purity), BPAF (97% purity), bisphenol AP (BPAP, 99% purity), $^{13}C_{12}$-labeled BPA (98% purity) and $^{13}C_4$-4-methylumbelliferone were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). TCS and d_3-triclosan (98% purity) were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and CDN Isotopes (Quebec, Canada), respectively. 4-methylumbelliferone glucuronide, 4-methylumbelliferyl sulfate and β-glucuronidase/arylsulfatase from *Helix pomatia* were purchased from Sigma (St. Louis. MO, USA). High performance liquid chromatography (HPLC) grade methanol was purchased from Merck (Darmstadt, Germany). Hydrochloric acid, acetic acid, sodium acetate, ammonium acetate, and mono-potassium phosphate were of analytical grade (Fisher Scientific, Houston, TX, USA). Water was obtained by using a Millipore water purification system (Millipore Co., Ltd., Billerica, MA, USA). Solid phase extraction (SPE) cartridges (Bond Elut C_{18}, 500 mg/6 mL) were purchased from Agilent (Santa Clara, CA, USA).

2.2. Sample collection

A total of 283 children from Shenzhen and Guangzhou were recruited and urine samples were collected in September 2015. Of these, 213 children (male: n = 122, female: n = 91) were from 8 to 11 years old and from different primary schools covering all six administrative districts in Shenzhen. Another 70 children (male: n = 40, female: n = 30) were 3–7 years old and were from a kindergarten located in Guangzhou. Before sample collection, each participant completed a questionnaire under the direction of his or her parents or teachers. The questionnaire covered personal information including age, sex, weight and height. Detailed demographic information is summarized in Table S1, and the sampling locations are shown in Fig. S1.

Approximately 50 mL of first-voided morning urine was collected in a glass bottle pre-cleaned with 0.1 M hydrochloric acid followed by Milli-Q water. The glass bottle was covered with a Teflon lid and sent to the laboratory with dry ice. Samples were stored at −20 °C until analysis.

2.3. Sample preparation

Urine samples were prepared according to a previously published procedure with slight modifications (Ren et al., 2016). Briefly, a 4 mL aliquot of urine was transferred into a glass tube. After spiked with 10 ng $^{13}C_{12}$-BPA and d_3-TCS as the internal standards, the urine sample was added with 3 mL of 0.1 M acetate buffer (pH = 5) and 20 μL of β-glucuronidase/arylsulfatase successively, and then incubated by shaking at 37 °C for 16 h. The hydrolyzed sample was loaded onto a C_{18} SPE cartridge pre-conditioned with 5 mL of methanol, 5 mL of pure water and 10 mL of 25 mM KH_2PO_4 buffer. A total of 3.0 mL of 25 mM KH_2PO_4 buffer and 5.0 mL of pure water was used to elute matrix interference from the cartridge. After complete dryness under vacuum, the cartridge was eluted with 8 mL of methanol. The eluate was concentrated to 400 μL under nitrogen gas, filtered through a 0.22 μm filter membrane (Anpel, Shanghai, China) and stored at −20 °C until instrumental analysis.

2.4. Instrumental analysis

Target chemicals were analyzed using a 20A HPLC system (Shimadzu, Japan) coupled to a Q-Trap 5500 tandem mass spectrometer (MS/MS; Applied Biosystems, Foster City, CA, USA). Chromatographic separation of the analytes was performed on an Atlantis C_{18} column (2.1 × 150 mm, 5 μm, Waters, Ireland), accompanied by mobile phases of methanol and 10 mM ammonium acetate in water. The linear gradient elution began with 30% methanol (held for 4 min), increased from 30% to 100% methanol in 4 min (held for 4 min), and finally dropped from 100% to 30% methanol in 0.5 min and held for 4 min. The injection volume was 10 μL and the column temperature was set at 40 °C. The flow rate was set at 0.3 mL/min. The mass spectrometer was performed with negative electron spray ionization (ESI) mode. The source temperature was set as 450 °C and the ionization voltage was −4500 V. The MS/MS was operated in multiple reaction monitoring (MRM) mode with a dwell time of 20 ms. The optimized mass spectroscopic parameters, such as precursor ion (Q1), product ion (Q3), collision energies (CE), declustering potential (DP), entrance potential (EP) and collision cell exit potential (CXP) for each analyte and corresponding internal standard, are summarized in Table S2.

2.5. Quality assurance and quality control

To avoid potential background contamination, all glassware