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Urinary bromophenol glucuronide and sulfate conjugates: Potential human exposure molecular markers for polybrominated diphenyl ethers

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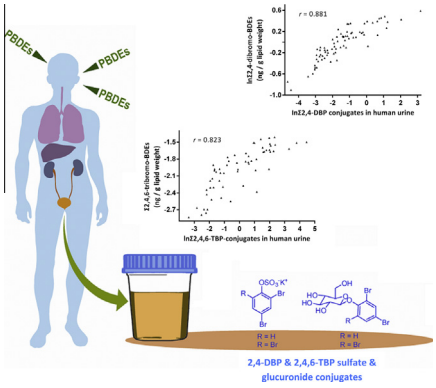
HIGHLIGHTS

- Parallel blood and urine samples were collected from 100 donors in Hong Kong.
- Levels of selected BP glucuronide and sulfate conjugates in urine were determined.
- Their levels were found to correlate well with that of PBDEs in blood plasma.
- Our results suggest that BP conjugates can be useful markers for PBDE exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

One possible source of urinary bromophenol (BP) glucuronide and sulfate conjugates in mammalian animal models and humans is polybromodiphenyl ethers (PBDEs), a group of additive flame-retardants found ubiquitously in the environment. In order to study the correlation between levels of PBDEs in human blood plasma and those of the corresponding BP-conjugates in human urine, concentrations of 17 BDE congeners, 22 OH-BDE and 13 MeO-BDE metabolites, and 3 BPs in plasma collected from 100 voluntary donors in Hong Kong were measured by gas chromatograph tandem mass spectrometry (GC–MS). Geometric mean concentration of ΣPBDEs, ΣOH-BDEs, ΣMeO-BDEs and ΣBPs in human plasma were 4.45 ng g^{-1} lw, 1.88 ng g^{-1} lw, 0.42 ng g^{-1} lw and 1.59 ng g^{-1} lw respectively. Concentrations of glucuronide and sulfate conjugates of 2,4-dibromophenol (2,4-DBP) and 2,4,6-tribromophenol (2,4,6-TBP) in paired samples of urine were determined by liquid chromatography tandem triple quadrupole mass spectrometry (LC–MS/MS). BP-conjugates were found in all of the parallel urine samples, in the range of 0.08–106.49 μg g^{-1}-creatinine. Correlations among plasma concentrations of ΣPBDEs/ΣOH-BDEs/

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Human blood plasma
Human urine

ΣMeO-BDEs/ΣBPs and BP-conjugates in urine were evaluated by multivariate regression and Pearson product correlation analyses. These urinary BP-conjugates were positively correlated with ΣPBDEs in blood plasma, but were either not or negatively correlated with other organobromine compounds in blood plasma. Stronger correlations (Pearson's r as great as 0.881) were observed between concentrations of BDE congeners having the same number and pattern of bromine substitution on their phenyl rings in blood plasma and their corresponding BP-conjugates in urine.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), a class of brominated flame retardants (BFRs), have aroused considerable public concern because of their resistance to environmental degradation, especially for lower brominated congeners, their tendency to bioaccumulate and potential adverse effects on health of humans (de Boer et al., 2000; Hooper and McDonald, 2000; Alaee et al., 2003; Guvenius et al., 2003; Henrik and Birger, 2010). In 2009, the Penta- and Octa-BDE commercial mixtures were listed as persistent organic pollutants (POPs) under the Stockholm Convention (Eljarrat and Barceló, 2011). Despite international efforts on the restriction of their production and usage, PBDEs are likely to remain in the global ecosystem for a considerable period of time because of their slow rate of degradation for lower brominated congeners, and the fact that large amounts of manufactured goods containing PBDEs are still in use (Harrad et al., 2006; Betts, 2008). Thus, the continuous monitoring of accumulation of PBDEs in humans is still important for the accurate assessment of their risk to public health at national and international levels.

The most frequently adopted approach to monitor human exposure to PBDEs is the direct quantification of selected BDE congeners and their hydroxylated (OH-BDEs) and methoxylated (MeO-BDEs) species in blood/serum (Athanasiadou et al., 2008; Turyk et al., 2008; Roosens et al., 2009; Wang et al., 2012), and human breast milk (Sudaryanto et al., 2008; Schuhmacher et al., 2009, 2013; Toms et al., 2009; Shi et al., 2013). Other human tissues such as hair, kidney, lung, liver and adipose tissues have also been used (Covaci et al., 2008; Zhao et al., 2008, 2009; Zheng et al., 2014). However, collecting human tissue samples from people for chemical/biochemical analysis and risk assessment is an intrusive operation and difficult to achieve in large-scale population-wide or national surveys. While sampling of human hair and breast milk can be considered non-intrusive processes, they face other limitations, such as the ease of exogenous contamination of hair samples (Morris et al., 2012; Barbosa et al., 2013) and the gender and age distribution restrictions of sampling breast milk (Landrigan et al., 2002). Alternatively, sampling of human urine is simple, quick and non-intrusive, making it much easier to obtain urine samples from a large number of voluntary donors within a community for large-scale surveys.

Occurrence of metabolites of selected BDE congeners in urine of mammalian animal models has already been well established in several toxicokinetic studies (Hakk and Letcher, 2003; Chen et al., 2006; Sanders et al., 2006). These metabolites are mainly glucuronide and sulfate conjugates of dibromophenols (DBPs) and tribromophenols (TBPs), probably because of their lower molecular weight (relative to their parent BDE congeners) that facilitates their renal removal. Our research team has previously reported the synthesis, purification, and characterization of glucuronide and sulfate conjugates of bromophenols (BP) and has developed an analytical protocol for their determination in human urine (Ho et al., 2012). A preliminary survey on 20 voluntary donors revealed the presence of at least one of these BP-conjugates in their urine. In this study, we examined the correlation between concentrations of PBDEs/OH-BDEs/MeO-BDEs/BPs and in blood plasma and BP-glucuronide and -sulfate conjugates in urine of humans. A total of 100 matched samples of plasma and urine were collected from volunteer donors in Hong Kong, China. The objective of this work was to evaluate whether glucuronide and sulfate conjugates of BPs in human urine are suitable molecular markers for the assessment of population exposure to PBDEs.

2. Experimental

2.1. Safety precautions

All necessary precautions were taken during the handling of samples of blood and urine. Double latex gloves, facemasks and eye-protection goggles were worn at all times during handling, spiking and transfer of samples from humans. All spent samples of urine were collected after analysis in separate capped containers with proper clinical waste labels. Both spent samples and used personal protection items were treated as clinical waste and were collected and disposed of in accordance with the "Code of Practice for the Management of Clinical Waste" issued by the Environmental Protection Department of the Hong Kong SAR Government.

2.2. Sample collection

All studies that involved human tissues and body fluids were conducted in accordance with guidelines of the Research Ethics Committee of City University of Hong Kong after proper approvals were given by the Committee. Parallel samples of human plasma and urine (n = 100; 50 from male and 50 from female donors) were collected from voluntary donors during March to July 2010 by registered doctors and nurses at Queen Mary Hospital, Hong Kong. Besides their gender and age, no other personal information of those voluntary donors was collected. The age range of the volunteers was from 16 to 93 years of age (mean ± SD: 54.9 ± 21.9 years). These donors were subdivided into different age groups for comparison: age 16–25 (n = 11); age 26–35 (n = 15); age 36–45 (n = 12); age 46–55 (n = 14); age 56–65 (n = 15); age 66–80 (n = 13) and age > 80 (n = 20).

Samples of whole blood were collected using the standard phlebotomy technique in vacutainer tubes containing sodium heparin anticoagulant (Vacuette, Greiner bio-one, GmbH, Austria). Whole blood was then centrifuged at 1500g for 25 min. Plasma was removed from the top of the tube. Morning-first urine samples were collected in 100 mL sterilized glass bottles and stored at −80 °C within 15 min of sampling until analysis. Urine from each donor was subdivided into three replicate samples before low-temperature storage. All samples were carefully labeled and documented. Upon analysis, samples were thawed, and 10 mL of each sample was retained for creatinine content determination (D'Haese et al., 1985). Creatinine determination was conducted by a kinetic colorimetric assay based on the modified Jaffe method using the Roche Modular System (Roche Diagnostics, IN, USA), with an analytical range between 360 and 57 500 mmol L^{-1}.