Environment International 98 (2017) 46–53

Contents lists available at ScienceDirect

Environment International

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

Full length article

Glucuronide and sulfate conjugates of tetrabromobisphenol A (TBBPA): Chemical synthesis and correlation between their urinary levels and plasma TBBPA content in voluntary human donors

Ka-Lok Ho [a], Ka-Ki Yuen [a], Man-Shan Yau [a], Margaret B. Murphy [a], Yi Wan [b,1], Bonnie M.-W. Fong [c,d], Sidney Tam [c], John P. Giesy [a,b,e], Kelvin S.-Y. Leung [d], Michael H.-W. Lam [a,*]

[a] State Key Laboratory for Marine Pollution, Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, China
[b] Department of Biomedical Veterinary Sciences, Toxicology Centre, University of Saskatchewan, Canada
[c] Division of Clinical Biochemistry, Queen Mary Hospital, Hong Kong, China
[d] Department of Chemistry, Hong Kong Baptist University, Hong Kong, China
[e] Department of Zoology and Center for Integrative Toxicology, Michigan State University, USA

ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form 16 September 2016
Accepted 22 September 2016
Available online 4 October 2016

Keywords:
Tetrabromobisphenol-A
Glucuronide and sulfate conjugates
Body fluids
Human plasma
Human urine

ABSTRACT

3,3′,5,5′-Tetrabromobisphenol-A (TBBPA) is an important brominated flame retardant in epoxy, vinyl esters and polycarbonate resins. Previous studies have already shown the occurrence of its Phase II metabolites, TBBPA-glucuronide and sulfate conjugates, in human urine, after oral administration of TBBPA. The main objective of this work is to examine correlations among level of TBBPA in human blood and those of its Phase II metabolites in human urine. Four water-soluble TBBPA conjugates were synthesized, purified and characterized. An analytical protocol using solid-phase extraction and liquid chromatography–electrospray tandem mass spectrometry (SPE-LC-MS/MS) quantification was developed for the simultaneous analysis of these glucuronide and sulfate conjugates in human urine samples. TBBPA and its Phase II metabolites in paired human plasma and urine samples collected randomly from 140 voluntary donors in Hong Kong SAR, China, were determined. One or more TBBPA conjugates were detected in all of the urine samples, with concentration ranging from 0.19 to 127.24 μg g^{-1}-creatinine. TBBPA was also quantified in >85% of the plasma and urine samples. Strong correlations were observed between TBBPA content in plasma and the total amount of TBBPA-related compounds in urine.

1. Introduction

Environmental endocrine disrupting chemicals (EDCs) are those chemical species that occur in the various environmental compartments that can affect normal hormonal signaling in living organisms (Nicolopoulou-Stamati et al., 2001). Their potential risks to environmental and human health have aroused much public concerns (Norris and Carr, 2006). 3,3′,5,5′-Tetrabromobisphenol-A (TBBPA) is a synthetic chemical widely used as a brominated flame retardant, and is, therefore, commonly found in a wide variety of daily appliances (Birnbaum and Staskal, 2004, Shi et al., 2009a). Recent studies have revealed its endocrine disrupting impacts on the reproduction and the functioning of thyroid hormones of laboratory animals (Saegusa et al., 2009, van der Ven et al., 2008). Toxicological and human exposure data that we have gathered thus far warrant our concern that TBBPA may have already exerting their adverse impacts on human health (Kim and Oh, 2014). As Southern China is one of the major manufacturing hubs for consumer products in the world, there is much concern about environmental contamination and public health risk posed by EDCs in the region (Feng et al., 2012, He et al., 2010, Shi et al., 2009a, Tan et al., 2016, Tang et al., 2015, Wang et al., 2015).

At the moment, the most commonly adopted approach for the estimation of population exposure to selected environmental contaminants is the direct quantification of their levels in human tissues, mainly whole blood/serum (Cariou et al., 2008, Dirtu et al., 2008, Dirtu et al., 2010, Fujii et al., 2014a, Fujii et al., 2014b, Jakobsson et al., 2002, Kim and Oh, 2014, Nagayama et al., 2000, Shi et al., 2013b, Thomsen et al., 2001, Thomsen et al., 2002), and breast milk (Abdallah and Harrad, 2011; Carignan et al., 2012; Cariou et al., 2008; Fujii et al., 2014b; Kang et al., 2015; Lankova et al., 2013; Nakao et al., 2015; Shi et al., 2009b; Shi et al., 2013a, 2013b) from voluntary donors recruited from the population. Other less commonly used tissues for exposure estimation include adipose tissue (Cariou et al., 2008, Johnson-Restrepo et al.,

* Corresponding author at: State Key Laboratory for Marine Pollution, Department of Biology & Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China.
E-mail address: bhmhwlam@cityu.edu.hk (M.H.-W. Lam).
[1] Present address: Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing, China.

http://dx.doi.org/10.1016/j.envint.2016.09.018
0160-4120/

2008), and hair (Pellizzari et al., 1978). Nevertheless, sampling human tissues for contaminant analysis is intrusive and difficult to achieve on a large scale, especially for healthy subjects who are not hospitalized or required to go through clinical diagnostic tests. Also, this approach can only provide a snapshot view of the exposure situation, unless a long-term and extensive sampling arrangement is available, which demands huge amount of resources to sustain. Sampling of breast mike can be considered a non-invasive operation, but is very much that restricted to lactating women within a relatively narrow age distribution (Landrigan et al., 2002).

Because of their high molecular mass, excretion via urine only constitutes a minor elimination route for TBBPA and its metabolites in human (Szymańska et al., 2001, Kuester et al., 2007). Nevertheless, glucuronide and sulfate conjugated Phase II metabolites of TBBPA have been quantified in human urine (Fini et al., 2012, Schauer et al., 2006). In vitro studies on the biotransformation of TBBPA carried out on rat hepatocytes have also revealed the production of TBBPA sulfate and glucuronide conjugates (Nakagawa et al., 2007). Thus, it is deemed worthy of examining correlations of these glucuronide and sulfate conjugates of TBBPA in human urine with TBBPA exposure. In this context, we report the synthesis and purification of all the four common Phase II metabolites of TBBPA (TBBPA mono-/di-glucuronide and mono-/di-sulfate conjugates), and their use as authentic standards for the development of an analytical protocol for their determination in human urine. We have also studied the stability of these TBBPA Phase II metabolites in human urine and derived means to preserve them for quantitative determination. Finally, correlations among their levels in human urine and that of TBBPA in human blood are investigated.

2. Materials and methods

2.1. Safety precautions

Extra precaution was practiced in the handling of human blood and urine samples. Double latex gloves, facemasks and eye-protection goggles were worn all the time during their handling, spiking and transferal. All the spent samples after analysis were collected in a separated close-lipped container with proper clinical waste labels. These spent samples and all the used personal protection items were treated as clinical wastes 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

Human studies were performed in accordance with the guidelines and approval of the research ethics committee of City University of Hong Kong. Parallel human plasma and urine samples from 140 voluntary donors were collected from April to August 2012, by registered doctors and nurse, at Queens Mary Hospital, Hong Kong SAR. Of the 140 donors, 66 were male and 74 were female. The age range of these volunteers was from 18 to 96 year-old, with the mean age = 47.1 ± 18.2 year-old. These volunteers were subdivided into different age groups for comparison as following: age 18 to 25 ($n = 17$); age 26 to 35 ($n = 18$); age 36 to 45 ($n = 44$); age 46 to 55 ($n = 24$); age 56 to 65 ($n = 15$); age 66 to 80 ($n = 13$) and age > 80 ($n = 9$).

Whole blood samples were collected using the standard phlebotomy technique in vacutainer tubes containing sodium heparin anticoagulant (Vacuette, Greiner bio-one, GmbH, Austria). The blood was then centrifuged at 1500 ×*g* for 25 min. Plasma was removed from the top of the tube. Urine samples were collected in 100 mL sterilized glass bottles and stored at −80 °C, within 15 min after sampling, until being analyzed. Urine sample 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 taken for creatinine content determination. Creatinine determination was carried out by a kinetic colorimetric assay based on the modified Jaffe method (Bonsnes and Taussky, 1945) using the Roche Modular System (Roche Diagnostics, IN, USA), with an analytical range between 360 and 57,500 mmol L^{-1}.

2.3. Synthesis, purification and characterization of TBBPA glucuronides and sulfate conjugates

The general synthetic routes for the TBBPA glucuronide and sulfate conjugates are outlined in Scheme S1 of the Supporting information. Detail synthetic and purification procedures and characterization data are also given in the Supporting information.

2.4. Sample extraction and cleanup

2.4.1. TBBPA in human plasma

Literature method (Dirtu et al., 2008, Hovander et al., 2000), with slight modifications, was adopted for the extraction of TBBPA in human plasma. $^{13}C_{12}$-TBBPA (1 ng) was spiked to 1 mL of human plasma sample. The spiked plasma sample was allowed to stand at room temperature for 10 min before 50 μL of concentrated hydrochloric acid (37%), Milli-Q water (2 mL) and isopropanol (3 mL) were added. The mixture was then extracted by hexane/MTBE (3 × 5 mL, 1:1, v/v). The organic fractions were combined and partitioned with a 1% KCl solution (3 mL) followed by evaporation to dryness under a gentle steam of nitrogen. Lipid content of the plasma sample was determined gravimetrically. The residue was re-dissolved in hexane (4 mL) and partition with potassium hydroxide (2 mL, 0.5 M in 50% ethanol) to ionize the phenolic analytes. Neutral compounds were separated by hexane (2 × 4 mL). The aqueous layer was acidified by hydrochloric acid (2 mL, 0.5 M), then the phenolic compounds were extracted by hexane/MTBE (2 × 4 mL, 9:1, v/v). Phenolic fraction was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1 mL of 5% acetone in hexane. The mixture was subjected to a SPE clean-up using a Sep.-Pak Florisil cartridge previously conditioned by DCM/MeOH (6 mL, 4:1, *v*/v) and 5% acetone in hexane (6 mL) at the flow rate of 1 drop/s. The cartridge then washed by 5% acetone in hexane (6 mL). The cartridge was the dried under reduced pressure and the BPA and BPA-d_{16} were eluted by DCM/MeOH (10 mL, 4:1, v/v). The eluate was evaporated to dryness under a gentle stream of nitrogen. Analytes were reconstituted in 50 μL isooctane containing 5 ng pyrene-d_{10} as an internal standard and the phenolic analytes were derivatized by 50 μL BSTFA with 1% TMCS at 70 °C for an hour.

2.4.2. TBBPA in human urine

Literature method (Ho et al., 2016), with slight modifications, was adopted from the extraction of TBBPA from human urine. Briefly, $^{13}C_{12}$-TBBPA (1 ng) was spiked in 5 mL of human urine. The spiked sample was then allowed to stand at room temperature for 10 min. Then, 50 μL of formic acid was added, followed by extraction with ethyl acetate (3 × 5 mL). The organic fractions were combined and evaporated to dryness under a gentle steam of nitrogen. This was followed by reconstitution in 5% acetone in hexane (1 mL), and SPE clean-up with a Sep.-Pak Florisil cartridge previously conditioned by DCM/MeOH (6 mL, 4:1, v/v) and 5% acetone in hexane (6 mL) at a rate of 1 drop/s. The cartridge was then eluted by 5% acetone in hexane (6 mL). The cartridge was the dried under reduced pressure and the TBBPA and $^{13}C_{12}$-TBBPA were eluted by DCM/MeOH (10 mL, 4:1, v/v). The eluate was evaporated to dryness under a gentle stream of nitrogen. Analytes was reconstituted in 50 μL isooctane containing 5 ng pyrene-d_{10} as an internal standard and the phenolic analytes were derivatized by 50 μL BSTFA with 1% TMCS at 70 °C for an hour.