Chemosphere 112 (2014) 481–486

Contents lists available at ScienceDirect

Chemosphere

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

Short Communication

Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China

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HIGHLIGHTS

- A LC–MS/MS method was developed for determining seven bisphenols in human urine.
- BPS, BPF, BPA and BPAF were detected in urine samples of participants.
- This is the first report of the occurrence of BPF and BPAF in individuals.

ARTICLE INFO

Article history:
Received 25 February 2014
Received in revised form 30 April 2014
Accepted 4 May 2014

Handling Editor: A. Gies

Keywords:
Bisphenol analogues
Biomonitoring
Urine
Liquid chromatography
Mass spectrometry

ABSTRACT

The use of bisphenol A (BPA) has been restricted in many countries because of its potential health effects. As a result of these restrictions, a group of bisphenol analogues that are structurally similar to BPA have been developed as the alternatives for industrial applications. However, latest researches indicated that these chemicals have similar endocrine-disrupting effects as BPA in humans. Moreover, only a limited number of studies have attempted to monitor the exposure level in humans of other bisphenol analogues. In the present study, the concentrations of seven bisphenols, including bisphenol S (BPS), bisphenol F (BPF), bisphenol B (BPB), BPA, bisphenol AF (BPAF), tetrachlorobisphenol A (TCBPA) and tetrabromobisphenol A (TBBPA), in human urine samples were measured by liquid chromatography coupled to mass spectrometry (LC–MS/MS) following the enzymatic hydrolysis of glucuronidase/arylsulfatase and liquid–liquid extraction (LLE). Under the optimised conditions, high recoveries (81.6–116.8%) were obtained for all the analytes, and the relative standard deviations (RSD, %) were less than 16.4% ($n = 6$). The isotopic internal standard calibration curves for each of the target compounds exhibited excellent linearity ($r^2 > 0.99$) and the limit of quantification (LOQ) for the analytes in urine ranged from 0.024 to 0.310 ng mL^{-1}. The method was applied to investigate the urinary levels of these seven bisphenols in a cohort of residents living near a BPAF manufacturing plant in south China. BPS, BPF, BPA and BPAF were detected in urine samples at concentrations ranging from <LOQ to a few ng mL^{-1}, whereas BPB, TCBPA and TBBPA were not detected. This is the first study to report the occurrence of BPF and BPAF in human urine samples. The availability of rapid and simple analytical methods may be highly useful for the future biomonitoring of these compounds.

1. Introduction

Bisphenols (BPs) are a group of chemical compounds that consist of two phenolic rings joined together through a bridging carbon or other chemical structures (Chen et al., 2002). Bisphenol A (BPA) is the most significant endocrine disruptor of this group and is mainly used in the manufacturing of polycarbonate plastics and epoxy resins; approximately 8 billion pounds of BPA are produced annually (Welshons et al., 2006; Vandenberg et al., 2010). In addition to BPA, other bisphenols are also used in a variety of industrial applications: e.g., bisphenol S (BPS) is widely used in thermal receipt papers (Liao et al., 2012a, 2012b); bisphenol F (BPF) can be used in the production of epoxy resins and polycarbonates for lining large food containers (Fromme et al., 2002); bisphenol AF (BPAF) is mainly used as a crosslinker in the synthesis of specialty fluoroelastomers (National Toxicology program, 2008); and bisphenol B (BPB) is used by the chemical industry for the manufacturing of phenolic resins (Cunha and Fernandes, 2010; Cunha et al., 2011).

As a result of the widespread exposure to BPA and potential health risks to humans, restrictions and legislation for the use of

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http://dx.doi.org/10.1016/j.chemosphere.2014.05.004
0045-6535/

BPA have been suggested worldwidely (European Union, 2011; U.S. Food and Drug Administration, 2013). Recently, the European Food Safety Authority (2014) identified likely adverse effects on the liver and kidneys of animals and effects on the mammary gland as being linked to exposure to BPA. Therefore, it has been recommended that the current tolerable daily intake (TDI) of BPA be lowered from its current level of 50 μg kg^{-1} bw d^{-1} to 5 μg kg^{-1} bw d^{-1}. The production and consumption of BPs other than BPA, especially those of BPS and BPF, have tended to increase (Danzl et al., 2009). Several studies have reported the occurrence of BPS, BPF, BPB and BPAF in dust (Liao et al., 2012c), water and sediment (Song et al., 2012; Liao et al., 2012d). Meanwhile, BPF and BPS have been detected in foodstuffs and soft drinks (Grumetto et al., 2008; Gallart-Ayala et al., 2011; Cacho et al., 2012; Liao and Kannan, 2013). It seems that these BPs will become widespread environmental pollutants and food contaminants in the near future, in addition to BPA.

The presence of BPs has received increased attention lately because of their potential toxicity, especially their estrogenic activity, and several studies have been recently published (Kitamura et al., 2005; Audebert et al., 2011; Okuda et al., 2011; Feng et al., 2012; Grignard et al., 2012). Human biomonitoring is an effective way to provide baseline information concerning levels of exposure to environmental chemicals and can help in conducting human health risk assessments. A number of biomonitoring studies have attempted to quantify human exposure to BPA (Matsumoto et al., 2003; Calafat et al., 2005; Dekant and Völkel, 2008; Völkel et al., 2008; He et al., 2009). In these studies, human exposure to BPA generally has been estimated by the measurement of total BPA, that is, unconjugated BPA (free BPA) plus the BPA derived from conjugated metabolites, such as BPA-glucuronide, after enzymolysis of human urine samples. In various cases, the blood and urine levels of BPA measured in humans were determined to be associated with human diseases such as gonadotrophic hormone disruptions, endometrial hyperplasia and recurrent miscarriage (Hanaoka et al., 2002; Vandenberg et al., 2007). In contrast with BPA, few studies have been conducted for the biomonitoring of other BPs in humans. Liao et al. (2012b) analysed the presence of total BPS (free plus conjugated) in urine by LC–MS/MS, which was detected in 81% of the urine samples analysed at concentrations ranging from below the limit of quantification (LOQ) to 21 ng mL^{-1}. Cunha and Fernandes (2010) developed a novel method combining dispersive liquid–liquid microextraction and heart-cutting multidimensional GC–MS for the determination of BPB in human urine samples. To our knowledge, no studies have attempted to measure BPF and BPAF levels in humans for biomonitoring.

As mentioned above, in order to obtain more information concerning the levels of BPs in individuals and provide useful data for human risk assessments, we evaluated the feasibility of determining the concentrations of seven bisphenols (BPS, BPF, BPA, BPB, BPAF, tetrachlorobisphenol A (TCBPA) and tetrabromobisphenol A (TBBPA)) simultaneously in human urine samples. In addition, the validated method was used to investigate the urinary levels of these seven bisphenol analogues in individuals residing near a BPAF manufacturing plant in south China, in an area where substantial contamination by BPs has recently been noted (Song et al., 2012; Yang et al., 2014). The developed methods may be of great usefulness for future biomonitoring efforts, which are expected to involve screenings at a larger scale.

2. Materials and methods

2.1. Chemicals and reagents

BPS (purity >98.0%), BPF (purity >99.0%), BPA (purity 98.5%), BPA-d_4 (purity >97.8%), BPB (purity >98.0%) and BPAF (purity 98.0%) were all purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). TCBPA (purity >99.0%), TCBPA-$^{13}C_{12}$ (purity >99.0%), TBBPA (purity >99.0%) and TBBPA-$^{13}C_{12}$ (purity >99.0%) were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). BPF-d_{10} and BPS-$^{13}C_{12}$ (purity >99.9%) were purchased from Toronto Research Chemicals Inc. (Ontario, Canada). LC–MS-grade water, methanol and acetonitrile were supplied by Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade ethyl acetate was supplied by Dickma (Lake Forest, CA, USA). Acetic acid (purity >99%) and sodium acetate anhydrous (purity >99%) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). Glucuronidase/arylsulfatase from *Helix pomatia* was purchased from Roche Diagnostics GmbH (Mannhein, Germany). Stock standard solutions (1000 mg L^{-1}) were individually prepared by dissolving standard compounds in methanol and stored at −20 °C. Working solutions were prepared by the serial dilution of the stock solutions with methanol/water (50:50, v/v).

2.2. Sample collection

This study was conducted according to the principles of the Declaration of Helsinki. The urine samples were collected from individuals residing near a BPAF manufacturing plant in south China in June 2013. Spot urine samples (approximately 20 mL) were collected from 94 participants: 50 female (aged 26–79 years) and 44 male (aged 26–84 years), at an arbitrary time of day. For sample collection, the urine collection tubes were screened to confirm the absence of BPs prior to use by placing them in contact with methanol for 12 h at room temperature and then analysing the methanol for BPs. After collection, urine samples were immediately transported on dry ice to the lab and stored at −80 °C until analysis.

2.3. Analysis of BPs in urine

Frozen urine samples were thawed, vortex-mixed and centrifuged at 2500*g* for 10 min. A portion of the clear supernatant (2.0 mL) was transferred to a glass centrifuge tube. Internal standards (0.25 ng for BPS-$^{13}C_{12}$ and 2.5 ng for BPF-d_{10}, BPA-d_4, TCBPA-$^{13}C_{12}$ and TBBPA-$^{13}C_{12}$), 20 μL of β-glucuronidase/sulfatase (2000 Units) and 1 mL of 0.2 M sodium acetate buffer (pH 5.4) were added to the sample tube. The mixtures were vortex-mixed and incubated at 37 °C for 12 h in the dark. After incubation, 2.0 mL acetonitrile was added to facilitate protein precipitation, and 3.0 mL ethyl acetate was then added to the sample tube for liquid–liquid extraction (LLE). The mixtures were sonicated at 30 °C for 10 min and centrifuged at 2500*g* for 10 min. The supernatants (approximately 4.5 mL) were collected and evaporated to dryness in a gentle stream of nitrogen; the residual was reconstituted to 500 μL with methanol/water (50:50, v/v) for LC–MS/MS analysis. The total concentration of BPs (conjugated plus free forms) was measured using this procedure. The same procedure was performed in parallel without β-glucuronidase/sulfatase treatment to measure the free BP (unconjugated BP) forms. In light of the variance observed in the matrix effects between different urine samples, isotopic internal standards were added for calibration. The calibration curves with seven points were created using a series of standard solutions (BPF, BPB and TBBPA: 0.125, 0.25, 0.5, 1.25, 2.5, 5.0 and 12.5 ng mL^{-1}; BPA and TCBPA: 0.0625, 0.125, 0.25, 0.625, 1.25, 2.5 and 6.25 ng mL^{-1}; BPS and BPAF: 0.0125, 0.025, 0.05, 0.125, 0.25, 0.5 and 1.25 ng mL^{-1}) spiked with the fixed, labelled internal standards. The BPA-d_4 was selected as internal standard for BPAF and BPB because no commercially labelled-standards are available.

The LC–MS/MS analysis was performed using an Acquity ultra-high-performance liquid chromatography system (UPLC) coupled