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Metabolites of organophosphate ester flame retardants in urine from Shanghai, China

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ABSTRACT

The metabolites of nine organophosphate ester (OPE) flame retardants were measured in 180 urine samples collected from a population (including adults and children) in western Shanghai, China, using liquid chromatography-tandem spectrometry (LC-MS/MS). The total urinary concentrations of nine OPE metabolites ranged 100–23800 pg/mL, with a geometric mean (GM) value of 1450 pg/mL. The concentrations of alkyl-OPE metabolites (879 pg/mL) were approximately an order of magnitude higher than those of aryl-OPE (53.7 pg/mL) and chlorinated-OPE metabolites (52.7 pg/mL). Diphenyl phosphate (DPHP), diethyl phosphate (DEP), di-n-butyl phosphate (DNBP), bis(2-ethylhexyl) phosphate (BEHP), and bis(2-butoxyethyl) phosphate (BBOEP) were the dominant OPE metabolites found in urine. The results showed that an increase in age was associated with a significant decrease in urinary DPHP ($r = -0.278$, $p < 0.01$) and DNBP ($r = -0.314$, $p < 0.01$) concentrations. The highest concentrations of DPHP (GM = 80.7 pg/mL) and DNBP (GM = 16.9 pg/mL) were found in urine from people living in homes that were less than 10 years old. The urinary DNBP concentration was significantly associated with self-reported symptoms of allergy. Our result establishes baseline value for OPE exposure in a population in China for comparison in future studies.

1. Introduction

Organophosphate esters (OPEs) are used as flame retardants, plasticizers, and antifoaming agents in a broad spectrum of industrial and commercial applications, such as electronic equipment, textiles, furniture, lacquers, floor polishes, building materials, and hydraulic fluids (Marklund et al., 2005; Stapleton et al., 2009; Van den Eede et al., 2011). OPEs are esters of phosphoric acid and are either halogenated (mostly, chlorinated alkyl phosphates) or non-halogenated (aryl & alkyl substituted) compounds. A major application of OPEs is in flame retardation and approximately 209,000 t (Möller et al., 2012), accounting for 70% of the market demand, were used in 2004 (Mäkinen et al., 2009). Following the phase-out of polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA), and hexabromocyclododecanes (HBCDs) (Stockholm Convention, 2009; Covaci et al., 2011; Stapleton et al., 2012; Van der Veen and de Boer, 2012), the use of OPEs as flame retardants increased. For instance, tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCiPP), and tris(1,3-dichloro-2-propyl) phosphate (TDCiPP) are used as substitutes for penta-BDE mixture (Covaci et al., 2011; Stapleton et al., 2012; Van der Veen and de Boer, 2012). In addition, non-halogenated OPEs are widely used as plasticizers and for other applications with unknown consumption volume (Möller et al., 2012). The production and usage of OPEs have increased sharply worldwide in recent years (from 296,000 t in 2004 to 500,000 t in 2011), and these chemicals have been listed as high production volume chemicals (Mihajlovic et al., 2011; Chen et al., 2018).

Because OPEs are used as additives and are not chemically bonded to the polymer backbone, they can easily leach out into the surrounding environment during manufacture and usage. Recent investigations point to their occurrence in air (Salamova et al., 2014), water (Luo et al., 2014; Gao et al., 2015; Lee et al., 2016; Kim et al., 2017), soil (Mihajlovic et al., 2011), sludge (Cristale and Lacorte, 2013; Celano et al., 2014), sediments (Cristale and Lacorte, 2013), fish (Malarvannan

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et al., 2015; Giulivo et al., 2017), house dust (Cristale and Lacorte, 2013; Kim et al., 2013), human hair (Liu et al., 2015), nails (Liu et al., 2015), serum (Li et al., 2015), placenta (Ding et al., 2016), urine (He et al., 2018), and adipose tissue (Hammel et al., 2016). Human exposure to OPEs is a matter of concern because studies have shown that certain OPEs are neurotoxic, carcinogenic, and endocrine disruptors (Du et al., 2015; Xu et al., 2017a). TCEP and TCiPP were associated with locomotor deficits and dopaminergic degeneration in *Caenorhabditis elegans* (Xu et al., 2017b). Tris(2-butoxyethyl) phosphate (TBOEP) disrupted reproductive performance and the development of progeny in adult zebrafish (Xu et al., 2017a). TCEP exposure resulted in tumor growth in the kidney and thyroid, and TDCiPP was a cancer promoter in the brain, liver, and testes (W.H.O, 1998; Lu et al., 2017). Aryl-OPEs were reported as developmental toxicants (Du et al., 2015). Epidemiological studies have shown an association between exposure to TDCiPP or triphenyl phosphate (TPHP) and hormonal changes and poor semen quality in men (Meeker and Stapleton, 2010; Meeker et al., 2011, 2013). TPHP exposure was associated with increased total thyroxine levels, especially in women (Preston et al., 2017). Paternal preconception exposure to TDCiPP has been reported to affect successful oocyte fertilization, whereas maternal preconception exposure to OPEs was related to adverse pregnancy outcomes (Carignan et al., 2018). Environmental agencies have been assessing the risks of some OPEs for making policy decisions (Rodil et al., 2005).

Human exposure to OPEs can arise from ingestion, inhalation, and dermal contact with household dust, among other sources (Hartmann et al., 2004; Meeker et al., 2013; Lee et al., 2016). Human dietary intakes of TPHP, triethyl phosphate (TEP), tri-n-butyl phosphate (TNBP), tricresyl phosphate (TMPP), tris(2-ethylhexyl) phosphate (TEHP), and TBOEP through fish consumption were 0.19, 2.0, 1.0, 0.07, 1.5, and 0.08 ng/kg/day, respectively (Kim et al., 2011). Additionally, studies have ranked human exposure pathways to OPEs as: dust ingestion > indoor air inhalation > food and drinking water ingestion (Gunderson, 1988; Yang et al., 2014). However, limited data are available on the levels of internal doses of OPEs in the general population, especially in China.

OPEs are readily metabolized to their respective diesters in the human body (Liu et al., 2016), and produce various phase II conjugate metabolites (Van den Eede et al., 2013a, 2016; Butt et al., 2014, 2016). OPE diester metabolites were used as markers of OPEs exposure because these compounds have been observed nearly ubiquitously in adult urine (Cooper et al., 2011; Van den Eede et al., 2013b; Hoffman et al., 2014). Urinary OPE diester metabolites such as bis(1,3-dichloro-2-propyl) phosphate (BDCiPP) and diphenyl phosphate (DPHP) were used as markers of human internal doses of exposure (Meeker et al., 2013; Butt et al., 2016; Kosarac et al., 2016; Lu et al., 2017). OPE diester metabolites were the main OPE metabolites found in laboratory animal studies (Dirtu et al., 2012). Recent studies have reported urinary OPE metabolites for the populations in Australia, Canada, Norway, the U.S., and China, with the data indicating widespread human exposure to OPEs (Cooper et al., 2011; Cequier et al., 2015; Van den Eede et al., 2015; Kosarac et al., 2016; Lu et al., 2017). Despite the large production and consumption of OPEs, little is known about the exposure of the general population in China. To the best of our knowledge, there are only three studies that report urinary levels of OPE metabolites in children, e-waste recycling workers, and guests in hotel rooms from the central and southern China (Lu et al., 2017; Chen et al., 2018; Tao et al., 2018). The urinary levels of OPE metabolites in populations in eastern China are not known.

In the present study, 180 urine samples collected from a population in western Shanghai, including children and adults, were analyzed for nine OPE metabolites. The objectives of this study were: a) to validate a method for the analysis of nine OPE metabolites by LC-MS/MS, b) to determine the concentrations and profiles of OPE metabolites in urine samples from a population in Shanghai, and c) to investigate associations between the urinary OPE metabolites levels and demographic characteristics of a population in Shanghai.

2. Materials and methods

2.1. Sample collection and preparation

Urine samples were collected in western Shanghai during July 2016 to February 2017. One hundred and thirty Shanghai residents (males, $n = 74$; females, $n = 56$; and age range, 25–90 yrs) were recruited through Shanghai No. 3 Rehabilitation Hospital when they were undergoing routine physical examination. Twenty-three children (boys, $n = 19$; girls, $n = 4$; and age range, 4–17 yrs) were recruited from a local Taekwondo training institution. Twenty-seven undergraduate and postgraduate students (males, $n = 10$; females, $n = 17$; and age range, 18–24 yrs) were recruited from Shanghai University. The Shanghai No. 3 Rehabilitation Hospital Institutional Review Board reviewed and approved the study protocol. All the participants and parents/guardians of the children gave informed consent. Before sample collection, each participant completed a questionnaire, which covered demographic information such as sex, age, occupation, smoking habit, symptoms of allergy, and house characteristics (including age of homes, daily dwelling time in homes, residence area, and number of household electrical appliances) (Table S1). All participants were selected randomly but required to have resided in Shanghai for more than two years in good health with no documented occupational exposure to OPEs. No female participant was on their menstrual period during urine sample collection. A total of 180 convenience early morning void urine samples were obtained. Each urine sample was collected into a 20 mL glass vial that was muffled at 450 °C prior to use. After collection of urine, the vials were kept in a cooler with ice packs during transportation to the laboratory. The urine samples were stored at − 29 °C until further analysis.

The method for the extraction of OPE metabolites in urine was similar to that described previously (Cooper et al., 2011). Briefly, 5 mL of urine was transferred into a glass tube and spiked with 10 ng of each of internal standards (d_{10}-diphenyl phosphate (d_{10}-DPHP), d_{10}-bis(1,3-dichloro-2-propyl) phosphate (d_{10}-BDCiPP), and d_8-bis(2-butoxyethyl) phosphate (d_8-BBOEP)). The sample was then diluted 1:1 (*v/v*) with Milli-Q water and acidified to pH 6.5 with 0.1 M acetic acid. Solid phase extraction (SPE) cartridges (Phenomenex, Strata-X-AW, 60 mg/3 mL) were conditioned with 3 mL of methanol and 3 mL of Milli-Q water. After loading the sample at a rate of 0.5 mL/min, the cartridges were rinsed with 3 mL of Milli-Q water and dried under nitrogen for 5 min. Target compounds were eluted with 2 mL of acetonitrile containing 5% pyrrolidine twice. The combined eluate was concentrated under a gentle stream of nitrogen to near dryness, reconstituted with 0.5 mL of Milli-Q water/MeOH (4:1, *v/v*) and filtered through a 0.22 μm hydrophilic polytetrafluoroethylene filter (ANPEL, Shanghai, China).

2.2. Target compounds and instrumental analysis

Nine OPE metabolites were analyzed: diphenyl phosphate (DPHP), di(methylphenyl) phosphate (DMPP), diethyl phosphate (DEP), di-n-butyl phosphate (DNBP), bis(2-ethylhexyl) phosphate (BEHP), bis(2-butoxyethyl) phosphate (BBOEP), bis(2-chloroethyl) phosphate (BCEP), bis(1-chloro-2-propyl) phosphate (BCiPP), and bis(1,3-dichloro-2-propyl) phosphate (BDCiPP). DNBP and DEP were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany) and AccuStandard (New Haven, CT, USA) respectively, and others including three deuterated internal standards were purchased from TRC (Toronto, Ontario, Canada).

The analysis of nine OPE metabolites was performed using an Agilent 1260 liquid chromatography coupled to an Agilent 6460 triple quadrupole mass spectrometry (LC-MS/MS; Palo Alto, CA, USA). Five microliters of the extract were injected onto a Poroshell 120 EC-C18 reverse-phase column (3 × 100 mm, 2.7 μm; Agilent) that was