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Urinary metabolites of organophosphate esters in children in South China: Concentrations, profiles and estimated daily intake☆

Yi Chen [a, b], Jianzhang Fang [a], Lu Ren [a, b], Ruifang Fan [c], Jianqing Zhang [b], Guihua Liu [b], Li Zhou [b], Dingyan Chen [b], Yingxin Yu [d], Shaoyou Lu [b, *]

[a] School of Chemistry and Environment, South China Normal University, Guangzhou, 510006, China
[b] Shenzhen Center for Disease Control and Prevention, Shenzhen, 518055, China
[c] School of Life Science, South China Normal University, Guangzhou, 510631, China
[d] School of Environmental and Chemical Engineering, Shanghai University, Shanghai, 200444, China

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ABSTRACT

Organophosphate esters (OPEs) are widely used in household products as flame retardants or plasticizers and have become ubiquitous pollutants in environmental media. However, little is known about OPE metabolites in humans, especially in children. In this study, eight OPE metabolites were measured in 411 urine samples collected from 6 to 14-year-old children in South China. Bis(2-chloroethyl) phosphate (BCEP), bis(1-chloro-2-propyl) phosphate (BCIPP) and diphenyl phosphate (DPHP) were the dominant OPE metabolites, and their median concentrations were 1.04, 0.15 and 0.28 μg/L, respectively. The levels of urinary OPE metabolites in the present study were much lower than those in participants from other countries, with the exception of BCEP, suggesting widespread exposure to tris(2-chlorethyl) phosphate (TCEP, the parent chemical of BCEP) in South China. No significant difference in the concentrations of any of the OPE metabolites was observed between males and females ($p > .05$). Significant negative correlations were observed between age and BCEP, BCIPP, bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), di-o-cresyl phosphate (DoCP) and di-p-cresyl phosphate (DpCP) (DCP), or DPHP ($p < .05$). Pearson correlation coefficients between urinary OPE metabolites indicated multiple sources and OPE exposure pathways in children. The estimated daily intake suggested that children in South China have a relatively high exposure level to TCEP. To the best of our knowledge, this is the first study to report the urinary levels of OPE metabolites in Chinese children.

1. Introduction

Flame retardant chemicals are frequently added to various household products such as plastics, textiles and electronic equipment to provide fire protection and meet flammability standards (Hoffman et al., 2014). Among these flame retardants, polybrominated diphenyl ethers (PBDEs) historically played an important role in polyurethane foam and electronic applications. However, concern about the use of PBDEs has been raised due to their persistence, bioaccumulation and toxicity, leading to the phase-out or discontinuation of PBDE mixtures since the mid-2000s (Butt et al., 2014; Hoffman et al., 2015; Veen and Boer, 2012). As one of the main alternatives to PBDEs, organophosphate esters (OPEs) are extensively used in residential furniture to maintain compliance with fire safety standards (Veen and Boer, 2012; Mäkinen et al., 2009). For example, triphenyl phosphate (TPHP) is considered a substitute for deca-BDE, while tris(2-chlorethyl) phosphate (TCEP) and tris(1-chloro-2-propyl) phosphate (TCIPP) are potential alternatives to penta-BDE (Zheng et al., 2015; Covaci et al., 2011). Moreover, some OPEs such as triortho-cresyl phosphate (TCP) and TPHP are often added as plasticizers in polyvinylchloride (PVC), cellulosic polymers, unsaturated polyester resins, and synthetic rubber (Veen and Boer, 2012). Due to the sharp increase in demand and production, the global consumption of OPEs reached 500,000 tons in 2011 (Ou, 2011). In China, the production of OPEs was estimated to be 100,000 tons in 2011 and the demand for OPEs is expected to increase by 15% per year (Wang et al., 2015).

OPEs are additives rather than chemically bonded to the

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* Corresponding author.
E-mail address: lushaoyou2005@163.com (S. Lu).

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products, allowing their release into the environment via volatilization, abrasion and dissolution (Veen and Boer, 2012; Wang et al., 2015). In recent years, OPEs have been ubiquitously detected in various environmental media such as drinking water, indoor air and dust, implying potential human exposure through ingestion, inhalation, and dermal absorption (Bacaloni et al., 2007; Hoffman et al., 2015). Toxicological experiments have suggested that TCEP and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) may be carcinogenic (Dodson et al., 2014; OEHHA, 2014). TPHP and TDCIPP were found to be associated with increased prolactin levels and reduced sperm concentrations in males, indicating endocrine disruption (Liu et al., 2012). Moreover, tri-*n*-butyl phosphate (TNBP) and TCP exhibited neurotoxic effects following chronic exposure (WHO, 1991; WHO, 1990). Thus, it is necessary to pay attention to the health effects and exposure level of OPEs in humans.

Recent studies have suggested that OPEs, such as TPHP and TDCIPP, are readily hydrolyzed in the body and are finally excreted via urine in the form of dialkyl or diaryl phosphate metabolites (Cequier et al., 2015; Dodson et al., 2014). Therefore, urine was found to be a valid biomarker to assess human exposure to OPEs in epidemiological investigations and biomonitoring studies (Dodson et al., 2014; Hoffman et al., 2014). Compared to environmental monitoring, the measurement of urinary OPE metabolites could directly reflect integrated exposure covering different sources and pathways (Cequier et al., 2015).

The Pearl River Delta, located in Guangdong Province, is one of the most economically developed and urbanized regions in China. Guangzhou and Shenzhen are both major cities in Pearl River Delta. Of these two cities, Guangzhou is the capital of Guangdong Province and the most populated city in this region. Shenzhen is the first special economic zone of China which was established in the 1980s (special economic zone: areas established by the Chinese government for foreign investment and industrial development). In the past three decades dynamic economic development in Guangzhou and Shenzhen has led to serious environmental pollution (Lu et al., 2015; Li et al., 2013; He et al., 2015). The high degree of commercialization and industrialization is accompanied by flame retardant pollution. Flame retardant chemicals, such as PBDEs, hexabromocyclododecanes and tetrabromobisphenol A, have been frequently detected in environmental and biological samples in this region (Zhang and Sun, 2012; Huang et al., 2010; Meng et al., 2012; Ni and Zeng, 2013; Feng et al., 2012). However, data on other frequently used flame retardants, such as OPEs, in this region are very limited. Only a few studies on the occurrence of OPEs in environmental media have been conducted (He et al., 2015; Tan et al., 2016; Li et al., 2014), and there is a lack of human exposure studies in South China. Children are more susceptible to chemical hazards than adults due to their higher exposure dose per body weight and more vulnerable immune systems (Van den Eede et al., 2015; Meeker, 2012; Dourson et al., 2002). Therefore, the potential exposure of children from this region to OPEs merits special concern.

The aims of this study were to: (1) investigate the concentrations and profiles of eight urinary OPE metabolites in 6 to 14-year-old children from South China; (2) determine the correlations between individual metabolites in order to further understand the sources of these chemicals; and, (3) estimate the daily intake of OPEs in children from South China. This is the first study to report the urinary levels of OPE metabolites in Chinese children.

2. Materials and methods

2.1. Study subjects and sample collection

Urine samples were collected in September 2015. A total of 411 children were recruited from primary schools in Guangzhou and Shenzhen. Of these, 212 children, 8–12 years old, were from different primary schools covering all six administrative districts in Shenzhen (male: n = 108, female: n = 104). Another 199 children, 6–14 years old, were from primary schools in Guangzhou (male: n = 120, female: n = 79). All participants were required to complete a questionnaire under the direction of their parents or teachers. The questionnaire covered personal information including name, age, gender, weight and height. Detailed information on the subjects included in the present study is summarized in Table S1, and the sampling locations are shown in Fig. S1.

First-voided morning urine samples were collected in 50-mL glass bottles that had been cleaned with 0.1 M HCl followed by pure water. Specific gravity was measured in each urine sample by a digital handheld refractometer (Atago, Bellevue, WA, USA) to quantify urine dilution. Creatinine adjustment was not adopted for its considerable variation by age and gender (James et al., 1988). Then the urine samples were immediately frozen at −20 °C and sent to the laboratory in dry ice. The urine samples were kept frozen in glass bottles at −20 °C until extraction and chemical analysis.

2.2. Chemicals and reagents

Eight OPE metabolites including bis(2-butoxyethyl) phosphate (BBOEP, 99% purity), bis(2-chloroethyl) phosphate (BCEP, 99% purity), bis(1-chloro-2-propyl) phosphate (BCIPP, 99% purity), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP, 97% purity), dibutyl phosphate (DBP, 99% purity), di-o-cresyl phosphate (DoCP, 97% purity), di-p-cresyl phosphate (DpCP, 97% purity), diphenyl phosphate (DPHP, 99% purity), and their corresponding deuterated internal standards, d_8-BBOEP, d_8-BCEP, d_{12}-BCIPP, d_{10}-BDCIPP, d_{18}-DBP, d_{14}-DoCP, d_{14}-DpCP, and d_{10}-DPHP, were all purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Methanol (LC-MS grade, purity> 99.9%) was purchased from Fluka (St. Louis, MO, USA). Formic acid, ammonium acetate and ammonia were high performance liquid chromatography (HPLC) grade and obtained from Fisher Scientific (Houston, TX, USA). All other reagents were of analytical grade and used without further purification. Solid phase extraction (SPE) cartridges (CNW P-WAX, 60 mg/3 mL) were obtained from Anpel (Shanghai, China).

2.3. Sample preparation and instrumental analysis

The pretreatment procedure for urinary OPE metabolites was as follows: First, 2 mL of urine sample was spiked with 20 ng internal standards, and then the pH value of the urine sample was acidified to 3 by adding 12 μL of formic acid. The urine sample was then loaded onto a P-WAX SPE cartridge, which had been conditioned with 2 mL of 5% ammonia in methanol followed by 3 mL of 0.6% formic acid in water. After sample loading, 2 mL of 30% methanol in water was used to elute matrix interference from the cartridge. After the cartridge was completely dried under vacuum, target analytes were eluted with 2 mL of 5% ammonia in methanol. The eluent was evaporated to near dryness under nitrogen gas and redissolved in 200 μL of methanol. Finally, the solution was filtered through a 0.22 μm membrane filter (Anpel, Shanghai, China) and stored at −20 °C for instrumental analysis.

Urine samples 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) with electrospray ionization. Ten microliters of the final solution was injected into the instrument and analyzed in negative ion mode. The ion source temperature was 450 °C and the ionization voltage was −4500 V. The MS/MS was operated in multiple reaction