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Urinary phthalate metabolites and environmental phenols in university students in South China

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ABSTRACT

In China, university students have unique lifestyles compared with the rest of the youth population, as they are almost entirely isolated in campuses. The number of university students is large, and since students represent the future of human reproduction, exposure to environmental endocrine disruptors (EEDs) may have a large impact on society. In this study, levels of several EEDs, including phthalate metabolites, parabens, bisphenol A (BPA) and its analogues, triclosan (TCS), and benzophenone-3, were determined in 169 urine samples collected from university students in Guangzhou, South China. In addition, to further understand the potential sources of EEDs in their daily lives, a survey of students' lifestyles was conducted. Based on the urinary concentrations of EEDs and the survey results, daily exposure doses of target EEDs and their potential sources were investigated. Our results indicated that nine phthalate metabolites, three parabens, and BPA were ubiquitous (detection frequency > 60%) in the urine of university students. The concentrations of total phthalates (median: 99.4 $\mu g\,L^{-1}$) were orders of magnitude higher than those of total parabens (7.30 $\mu g\,L^{-1}$) and of other environmental phenols (0.40 $\mu g\,L^{-1}$). Significantly higher concentrations of phthalates, parabens, and TCS were found in female versus male students, partly due to the higher usage of personal care products (PCPs) by female students ($p < 0.05$). The estimated daily intakes (EDIs) of phthalates, parabens, BPA, and TCS were 0.46–1.35, 3.29–10.3, 0.007, and 0.67 μg/kg-bw/day, respectively. The EDIs of phthalates and BPA were much lower than those suggested by the European Food Safety guidelines (10, 50, and 50 μg/kg-bw/day for dibutyl phthalate, diethylhexyl phthalate, and BPA, respectively). Our results indicated that university students were widely exposed to EEDs, but at relatively low doses. PCP usage was the main reason for differences in levels of phthalates (especially diethyl phthalate) and parabens between male and female students in South Chinese universities.

1. Introduction

Phthalates and environmental phenols are widely used as solvents, plasticizers, preservatives, or as intermediates in numerous consumer products, such as polyvinyl chloride materials, personal care products (PCPs), pharmaceuticals, foodstuffs, and other products (Barusic et al., 2015; Guo et al., 2014, 2012; Jia et al., 2016). They have been detected in indoor dust, air, PCPs, food and drinking water, as documented in various papers (Guo and Kannan, 2011, 2013; Guo et al., 2012; Liao and Kannan, 2014). These chemicals, known as environmental endocrine disruptors (EEDs), may have potentially adverse effects on human health, and humans are exposed to them on a daily basis through inhalation, oral ingestion, and dermal absorption (Wittassek et al., 2011).

Several epidemiological studies have reported that exposure to phthalates or their metabolites may lead to reproductive and developmental toxicities (Hauser et al., 2016; Scinicariello et al., 2016), and that these substances may have positive relationships with obesity, headaches, coughing, and itching (Buckley et al., 2016; Kim et al., 2016). Like phthalates, parabens also act as a group of weak estrogenic and anti-androgenic chemicals (Soni et al., 2005). Inverse associations in concentrations between parabens and thyroid hormones were found in adults, and parabens were reported to be negatively associated with menstrual cycle length in women (Koeppe et al., 2013). Concurrently, 3,4-dihydroxybenzoic acid, a paraben metabolite, has also been associated with obesity and higher levels of this chemical were found in obese children than in non-obese children (Xue et al., 2015). In addition, recent studies have indicated that type 2 diabetes, hypertension, early microvascular diseases among middle-aged and elderly Chinese

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people, and wheezing in early life were associated with BPA exposure (Kim and Park, 2013; Spanier et al., 2012; Wang et al., 2015b). However, the potential biological mechanisms by which EEDs affect human health are not clear and those proposed by different studies are inconsistent. For instance, Reddy et al. reported that exposure to phthalates was associated with endometriosis, while another study concluded that no relationship existed between the two (Itoh et al., 2009; Reddy et al., 2006a, 2006b). In addition, one study reported a negative relationship between levels of urinary monoethyl phthalate and sperm motility in men, while no associations were found in another similar study (Joensen et al., 2012; Jonsson et al., 2005).

There is a diversity of biomonitoring data on human exposure to phthalates, parabens, and environmental phenols, including background exposure investigations for general or vulnerable populations, exploration of potential sources, and environmental epidemiologic studies of disease (Guo and Kannan, 2011; Guo et al., 2011; Lind et al., 2012; Xue et al., 2015). For example, studies have assessed phthalate exposure in children, babies, pregnant women, and in the general populations of many countries (Asimakopoulos et al., 2014; Axelsson et al., 2015; Bamai et al., 2015; Guo et al., 2011; Han et al., 2014; Kim et al., 2016). In addition to such studies, it is also essential to assess exposure levels of EEDs in the young population, such as in university students who will be important for societal development and human reproduction in the very near future. In China, the number of full-time undergraduate and graduate students in universities exceeded 33.8 million in 2014 (National Bureau of Statistics of China, 2014). Chinese university campuses are usually built in big cities, but are relatively isolated from the surrounding environment. Students have a unique lifestyle compared with the rest of the young population, e.g., staying in simply furnished dormitories or classrooms, eating in the school canteen together, having daily dermal contact with books and laptops, and using several varieties of PCPs. Therefore, university students may have a distinct fingerprint of EED exposure.

In the present study, 11 phthalate metabolites, six parabens, and 11 other environmental phenols (including nine bisphenol analogues, triclosan (TCS), and benzophenone-3 (BP-3), all containing two phenyl rings) were analyzed in 169 urine samples collected from students (who had already been living on campus for at least three months) at eight universities (all ranked in the Top 10 in Guangdong province) in Guangzhou, South China. Following this analysis, a questionnaire survey was carried out in order to further explore the potential sources of EED exposure, either in hard copy in classrooms or via a dedicated mobile phone application (APP) in the same universities to study the lifestyles of the students. The objectives of this study were as follows: 1) measure concentrations and profiles of phthalate metabolites, parabens, bisphenols, and other environmental phenols in university students; 2) investigate the potential sources of these EEDs; and 3) assess the daily exposure doses of these EEDs in university students in South China.

2. Materials and methods

2.1. Study population and sample collection

The target EEDs in the present study are metabolized in a short time, e.g., several hours, after entering the human body (Koch et al., 2005; Ma et al., 2013). In order to avoid the influence of chemical exposures from students' hometowns, urine samples were collected from volunteers in January 2016, immediately prior to the winter holidays, when the students had already lived on the university campus for at least three months. The first morning, urine was collected in a polypropylene tube, quickly transferred to a lab, and maintained at − 20 °C until analysis. A total of 169 urine samples, including those from 107 male and 62 female students (Table S1, "SI" designates figures and tables in the Supporting information thereafter) were collected from ten campuses of eight universities distributed in different districts of Guangzhou, South China (Fig. S1). Approvals for urine analysis were obtained from the Jinan University Review Board, Jinan University, China (2015-LSPK-029). After urine collection, a questionnaire survey of daily life of the students was carried out in October 2016 in the same universities. A similar questionnaire was answered in hard copy by students in classrooms or via a mobile phone APP, and a total of 702 completed and valid questionnaires were collected (169 by APP and 533 in hard copy) for final analysis.

2.2. Sample preparation and instrumental analysis

Three groups of EEDs were analyzed, including 11 phthalate metabolites (monomethyl phthalate (mMP), mono (3-carboxypropyl) phthalate (mCPP), monoethyl phthalate (mEP), mono (2-ethyl-5carboxypentyl) phthalate (mECPP), monoisobutyl phthalate (miBP), mononbutyl phthalate (mBP), mono (2-ethyl-5-oxohexyl) phthalate (mEOHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), monocyclohexyl phthalate (mCHP), monobenzyl phthalate (mBzP), and mono(2-ethylhexyl) phthalate (mEHP)), six parabens (methyl-paraben (MeP), ethyl-paraben (EtP), propyl-paraben (PrP), butyl-paraben (BuP), benzyl-paraben (BzP), and heptyl-paraben (HepP)), and nine bisphenol anologues ((bisphenol A (BPA), bisphenol S (BPS), bisphenol AF (BPAF), bisphenol AP (BPAP), bisphenol Z (BPZ), bisphenol G (BPG), bisphenol PH (BPPH), bisphenol BP (BPBP), and bisphenol F (BPF)), TCS, and BP-3.

Concentrations of the target EEDs in urine were determined with an isotope-diluted method (Iyer et al., 2018; Wang and Kannan, 2013). Briefly, urine samples were allowed to thaw to room temperature in the dark, and 0.5 mL of urine was transferred into a glass tube. Upon addition of 250 μL of ammonium acetate buffer (pH = 4.5), 50 μL of labeled internal standards (IS: phthalate metabolites (250 μg L^{-1}); parabens (200 μg L^{-1}), bisphenols (200 μg L^{-1}), TCS (200 μg L^{-1}), and BP3 (200 μg L^{-1})), 50.0 μL of β-glucuronidase (Helix pomatia, β-glucuronidase/sulfatase) and 0.5 mL of HPLC-grade water, the sample was incubated at 37.0 °C overnight for deconjugation. All analytes were liquid-liquid extracted with ethyl acetate (3.0 mL) three times by shaking for 30 min. The combined supernatant was concentrated under a gentle stream of nitrogen to near-dryness and 0.5 mL of solvent (90% HPLC water/10% acetonitrile) was added for instrumental analysis. Urine creatinine levels and specific gravity were also determined. For creatinine estimation, 40 μL of thawed urine was diluted to 14 mL with water, and 50 μL of diluent and 750 μL of labeled internal standards were added. The instrumental analysis was conducted over two days. Specific gravity was determined using a digital handheld refractometer. Fifteen isotope-labeled chemicals were used as internal standards to quantify the concentrations of target EEDs and creatinine, including ^{13}C-MeP, ^{13}C-TCS, ^{13}C-BPS, ^{13}C-BPA, ^{13}C-mMP, ^{13}C-mEP, ^{13}C-mBP, ^{13}C-mBZP, ^{13}C-mCHP, ^{13}C-mCPP, ^{13}C-mECPP, ^{13}C-mEOHP, ^{13}C-mEHHP, D_4-miBP, and D_5-creatinine.

All analytes including urine creatinine were analyzed using an AB-Sciex 5500 triple quadrupole mass spectrometer (ESI-MS-MS; Applied Biosystems, Foster City, CA) equipped with a Shimadzu Nexera-XZ LC system (Shimadzu Corporation Inc. Kyoto). The details of the reagents and instrumental analysis protocols are shown in Table S2-S3.

2.3. Quality assurance and quality control (QA/QC), and data analysis

For each batch of 25 urine samples, two blanks (with HPLC water instead of urine), two spiked blanks (with a known amount of each target analyte added into HPLC water instead of urine) and three matrix-spiked samples (with a known amount of each target analyte added into urine samples) were analyzed. The results of the QA/QC system are shown in Table S4. As shown, trace levels of mMP, miBP, mBP, mEHP, BPF, and BPA (respective average concentrations: 0.24, 0.86, 0.62, 6.76, 2.09, and 1.03 μg L^{-1}) were found in the blanks, and were subtracted from the final reported data. The detection limits of all analytes ranged from 0.2 to 0.5 μg L^{-1}, and samples with analyte concentrations