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Urinary parabens and triclosan concentrations and associated exposure characteristics in a Korean population—A comparison between night-time and first-morning urine

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ABSTRACT

Parabens and triclosan have been widely used in many personal care products and cosmetics. The endocrine disrupting potential of these compounds is of increasing public health concern. The aim of this study is to understand the current exposure profile of these chemicals in last void before bedtime (night-time) and first-morning void (first-morning) urines among a Korean population and to characterize their exposure sources and pathways.

A total of 261 people, including infants (0–2 years), toddlers (3–6 years), children (7–12 years), adolescents (13–18 years), and adults (≥19 years), were recruited, and sampled for night-time urine and first-morning urine of the following day. Methyl (MeP), ethyl (EtP), propyl (PrP) and butyl paraben (BuP), and triclosan were measured in urine. The demographic characteristics, use of personal care products, and food consumption were obtained through a questionnaire.

Among the target compounds, EtP and MeP were most frequently detected at the highest concentrations. The median concentration of EtP in night-time urine was 32.4 μg/L (interquartile range: 8.37–82.8 μg/L), which is higher than previously reported worldwide. Unlike other test compounds, compared to those measured from first-morning urine, the EtP concentrations were significantly higher in night-time urine, suggesting the presence of different exposure sources. Among adults, the MeP and PrP concentrations in night-time urine were associated with frequent use of skin care products, colored cosmetics, bath products, toothpaste, vinyl food packaging, or consumption of canned food. The MeP and PrP concentrations were higher in females than in males, especially in night-time urine. The results of this study also show that multiple urine samples are necessary to capture the diurnal variation of non-occupational exposure to environmental chemicals, such as parabens.

1. Introduction

Parabens (alkyl esters of *p*-hydroxybenzoic acid) and triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) have been widely used as antimicrobial agents in many consumer and personal care products. Among parabens, methyl paraben (MeP), ethyl paraben (EtP), and propyl paraben (PrP) are commonly added to pharmaceuticals, cosmetics, lotions, and hair products. Additionally, some parabens are used as anti-spoiling agents in foods, beverages, and food packaging materials (Soni et al., 2005). Triclosan has been used in soaps, body wash, shampoo, toothpastes, mouthwash, and other household products (Dann and Hontela, 2011).

Reflecting the widespread use of these compounds in consumer products, parabens and triclosan have been frequently reported in humans worldwide. MeP and PrP have been detected in the urine of > 94% of the population in the United States (U.S.) who participated

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in the National Health and Nutrition Examination Survey (NHANES) 2011–2012 (n = 2489; calculated from datasets provided by CDC, 2015). Of 660 urine samples collected in Germany between 1995–2012, MeP, EtP and PrP were detected in 79–99% of samples, followed by butyl paraben (BuP) in only 40% of samples (Moos et al., 2017; Moos et al., 2015). Among 143 Danish children, 79% of urine samples contained triclosan (Frederiksen et al., 2013), which was also detected in the urine of 93% of Korean adults (Kim et al., 2011).

Several *in vivo* and *in vitro* studies have reported endocrine and reproduction related effects of parabens (Oishi, 2002; Routledge et al., 1998; Taxvig et al., 2008) and triclosan (Lan et al., 2015; Zorrilla et al., 2009). Epidemiological studies also support their associations with decreased sperm quality (Meeker et al., 2011) and serum thyroid hormones (Koeppe et al., 2013). Because of increasing concerns regarding the potentially adverse health consequences of exposure to parabens and triclosan, these chemicals have been regulated in many products worldwide. According to the European Commission Regulation (EU) No 358/2014, the maximum level of parabens was restricted to 0.4% for one ester, 0.8% for a mixture, and 0.3% for triclosan in cosmetics (EC, 2009). In 2014, the maximum permitted concentration of the total amount of PrP and BuP in cosmetics was set at 0.14%, and other parabens (*e.g.*, *iso*-PrP and *iso*-BuP) were banned (EC, 2014). In the European Union, only MeP and EtP are permitted as food additives (EFSA, 2004). Triclosan was not included in the list of food additives, and its use in food contact materials is banned according to a March 2010 Commission Decision. In Korea, the Ministry of Food and Drug Safety has restricted the use of certain parabens and triclosan in mouthwashes since 2016 (Korean MFDS Notification No. 2016-113).

This study was conducted to determine the current occurrence of major parabens and triclosan in the urine of a Korean population and to identify their major exposure sources and pathways. Since these chemicals have relatively short biological half-lives in humans (Janjua et al., 2008; Moos et al., 2016; Sandborgh-Englund et al., 2006) and may have various exposure sources and pathways, the urinary concentrations of these chemicals could vary significantly by time (Koch et al., 2014). Therefore, by collecting both last void before bedtime (night-time) and first-morning void (first-morning) urines and by comparing the differences in the paraben and triclosan concentrations, more representative exposure profiles of these chemicals were obtained. The results of this study will help understand the characteristics of exposure to parabens and triclosan and develop exposure mitigation measures for major parabens and triclosan among the Korean population.

2. Materials and methods

2.1. Study population, sample collection, and questionnaire

A total of 261 subjects were recruited mostly during autumn between the end of July and mid-November, 2015 in Seoul, Korea and its vicinity. Participating subjects were recruited through convenience sampling, *e.g.*, advertisements in online communities and personal networks. The subjects included different age groups, *i.e.*, 'infant' (up to 2 years old, n = 31), 'toddler' (between 3 and 6 years of age, n = 45), 'child' (between 7 and 12 years of age, n = 48), 'adolescent' (between 13 and 18 years of age, n = 46), and 'adult' (≥19 years of age, n = 91), with 108 males and 153 females. When the mother of a participating infant, toddler, or child wished to participate, she was also included in our study population as an adult woman. As a result, a total of 78 mother-offspring pairs were recruited. All participants were instructed to collect two urine samples at home in sterilized polypropylene tubes, one at night before bedtime (hereafter 'night-time urine') and the other, first in the next morning ('first-morning urine'). A detailed guide booklet explaining the sampling, storing, and delivery procedures with photos was provided. For infants, organic cotton cloth diapers were used for urine collection following a protocol employed previously for the measurement of phthalate metabolites, with some modifications (Kim et al., 2017). Briefly, cloth diapers were purchased and pre-washed/dried at least four times without detergents in the laboratory. Mothers were instructed to wash their hands before squeezing the wet diapers to prevent potential contamination, and to collect urine in a sterilized collection cup. A detailed instructional video with a demonstration was also prepared and provided. For toddlers, the urine were collected using sterilized collection cups, as all the participating toddlers were toilet-trained. For this sampling, the same protocol as the sampling for children, adolescents, and adults was used. Immediately after the collection, urine samples were moved into the tubes, and stored in a home freezer (−20 °C). Later, the samples were shipped in a cooler with ice packs to keep them cold (< −4 °C) during the delivery to the laboratory. Most of the samples were delivered within 24 h (all within three working days). Upon delivery to the laboratory, urine samples were stored at −40 °C until analysis. Since stability of the urinary phenolics under −20 °C has not been guaranteed and potential degradation could be an issue, the storage duration in the home freezer was limited as short as possible (average 4.6 d, SD 7.4 d). A self-administered questionnaire was filled out and provided information on the demographic parameters, average frequency of personal care product use within the previous 1–2 months, and average frequency of food consumption within a year. For infants, toddlers, and children, their parents or guardians were instructed to answer the questionnaire on their behalf. The Institutional Review Board of Seoul National University approved the study (Approval No. 1506-002-004), and informed consent was obtained from each of the participants or their parents/guardians.

2.2. Chemical analysis

Four major parabens, including MeP, EtP, PrP, and BuP, and triclosan were measured in both night-time and first-morning urine. Target chemicals, internal standards, *i.e.*, $^{13}C_6$-MeP, $^{13}C_6$-EtP, $^{13}C_6$-PrP, $^{13}C_6$-BuP, and $^{13}C_{12}$-triclosan, and β-glucuronidase (Helix pomatia, H1) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). The urine samples were treated and analyzed following the methods of Ye et al. (2005, 2006) with some modifications. Briefly, 500 μL of urine sample was mixed with 10 μL of 2 μg/mL internal standard solution, 20 μL of a β-glucuronidase/sulfatase solution (10,000 units/mL), and 100 μL of 1 M ammonium acetate buffer (pH 6.5). After a 4 h incubation at 37 °C and a 10 min sonication, 100 μL of 0.1 M formic acid was added. Samples were then centrifuged at 10,000 rpm for 10 min, and the supernatant was analyzed by an online solid-phase extraction-liquid chromatography–tandem mass spectrometry (SPE–HPLC–MS/MS) system. HPLC analysis was performed on a Nexera system (Shimadzu, Kyoto, Japan) equipped with an autosampler, dual pump, column oven, vacuum degasser and switching valve. An SPE column in gradient mode was employed. The target compounds were detected by an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, U.S.A.). The detailed HPLC and mass spectrometer conditions are described in Tables S1 and S2 of the Supplementary material, respectively.

For quality assurance, procedure blanks were included for each instrumental run. Five field blanks, including four for polypropylene tubes and one for cloth diapers, and two trip blanks were analyzed, and no target chemicals were detected above quantifiable concentrations. Accuracy was considered to be acceptable when deviations from the nominal spiked value were within 20%. Precision was considered to be acceptable when the intra- and inter-day coefficients of variation were within 20%. Both the accuracy and precision of analysis for each compound in this study were within the acceptable range (Table S3). The limits of detection (LODs) were 0.2 μg/L for MeP, 0.5 μg/L for EtP, 0.1 μg/L for PrP, 0.2 μg/L for BuP, and 0.3 μg/L for triclosan.

Additionally, for external quality assurance, we performed a cross-validation of the analytical procedure with a subset of the samples