Environmental Research 146 (2016) 245–251

Contents lists available at ScienceDirect

Environmental Research

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

# Urinary concentrations of parabens and their association with demographic factors: A population-based cross-sectional study

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ARTICLE INFO

*Article history:*
Received 1 September 2015
Received in revised form
27 December 2015
Accepted 28 December 2015
Available online 15 January 2016

*Keywords:*
Paraben
Urine
Biomonitoring
Demographic characteristic
Korean population

ABSTRACT

Parabens are broad-spectrum antimicrobial agents used in a range of consumer products, including personal care products, cosmetics, and food. Recently, the widespread use of parabens has raised concerns about the potential health risks associated with their endocrine-disrupting effect. In the present study, 2541 urine samples were collected and analyzed by liquid chromatography-mass spectrometry for the determination of the concentrations of methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP) and butyl paraben (BuP). The detection rate and geometric mean concentrations of parabens in the general population followed the order MeP (97.7%; 116 ng/mL) > EtP (97.2%; 24.7 ng/mL) > PrP (96.7%; 11.0 ng/mL) > BuP (83.5%; 1.13 ng/mL). The composition profiles showed that MeP and EtP accounted for > 90% of the urinary paraben concentration. We performed statistical analysis in order to evaluate differences between demographic variables and urinary concentrations. Our results showed that adjusted proportional change of MeP, PrP, and BuP in adults were 2.67–6.13 times higher in females than in males. The urinary concentrations of PrP in adults increased significantly with age. The adjusted proportional changes of MeP and PrP in adults were associated with increased body mass index (BMI). The adjusted proportional changes of BuP and PrP in children and adolescents were 1.44 and 1.69 times higher in females than in males. However, there was no clear association between paraben concentrations and demographic variables in the children and adolescents groups. The estimated daily intake ($EDI_{urine}$) of MeP and EtP in adults were 301 μg/kg bw/day, which is lower than the acceptable daily intake (ADI; 10 mg/kg bw/day). In summary, our results revealed that the general population in Korea was exposed to parabens during 2009–2010, and most Koreans are exposed to parabens. The urinary levels of parabens varied by age group with demographic factors in the Korean population. The results of study may be used to establish a nationally representative baseline of exposure to parabens in risk assessment.



## 1. Introduction

Parabens (*p*-hydroxybenzoic acid esters) are used extensively in cosmetics, foods, and pharmaceutical products since they have low toxicity, broad inertness, low cost, and worldwide regulatory acceptance (Soni et al., 2005). However, parabens have recently been recognized as chemicals of concern for their potential risks to human health are still unknown. Although parabens have a long history of safe use as antimicrobial preservatives, recently, several *in vitro* studies have shown that parabens exhibit specific activity toward the estrogen receptor, and may lead to a prolonged estrogenic effect in the skin (Gomez et al., 2005; Prusakiewicz et al., 2007). *In vivo* studies have indicated that, parabens, at a dose of approximately 10 mg/kg bw/day, induce oxidative stress via lipid peroxidation (Shah and Verma, 2011) and reduce testosterone secretion in male rodents (Oishi 2004). Human studies have revealed a weak but significant relationship between urinary paraben concentration and sperm DNA damage, oxidative stress biomarker levels, and serum thyroid hormone levels (Meeker et al., 2011; Kang et al., 2013; Koeppe et al., 2013).

The acceptable daily intake (ADI) of parabens for the sum of methyl paraben (MeP), ethyl paraben (EtP), and propyl paraben (PrP) is < 10 mg/kg/day, as set by the Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives (JECFA/WHO) in 1974 (JECFA, 1974). However,

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http://dx.doi.org/10.1016/j.envres.2015.12.032
0013-9351/

growing evidence of the reproductive toxicity of PrP resulted in its removal from the group ADI in 2007 (JECFA, 2007). In the past decade, parabens have received noticeable attention from the Scientific Committee on Consumer Safety (SCCS) because of their widespread exposure, estrogenic activity, and reproductive toxicity. PrP and butyl paraben (BuP) have been banned from use in cosmetics for children less than 3 years of age in Denmark (SCCS, 2011). The Ministry of Food and Drug Safety of Korea allows the use of parabens at levels up to 0.4% for single ester and 0.8% for mixtures of all parabens in cosmetic ingredients (MFDS, 2015).

Parabens and their metabolites do not accumulate in the body, and are eliminated within a few hours of exposure (Aubert et al., 2012; Wang and Kannan, 2013). Serum paraben concentrations, even after intravenous injection, decline quickly and remain low in the blood (Abbas et al., 2010). The parent compounds and their metabolites are conjugated and excreted in urine. Therefore, urinary measurements in humans can be used to estimate paraben uptake (Boberg et al., 2010). Urinary levels of parent parabens can be used as biomarkers of recent human monitoring (Ye et al., 2006; Calafat et al., 2010; Ma et al., 2013; Wang et al., 2013).

Most human exposure to parabens occurs with the consumption of food or pharmaceutical products or use of personal care products containing parabens (Guo and Kannan, 2013; Liao et al., 2013; Guo et al., 2014; Moreta et al., 2015). Biomonitoring can be used to estimate the aggregate exposure from all routes and sources among individuals in a population (Cowan-Ellsberry and Robison, 2009). Furthermore, population-based exposure surveillance is being conducted worldwide to protect public health, and it is essential to determine the mean population exposure level and understand demographic differences (Kim et al., 2011; Lee et al., 2014). Large sample-size studies involving humans are necessary to assess the potential adverse effects of parabens. Nevertheless, nation-wide biomonitoring data and evaluations of human paraben exposure remain limited in Korea. In this study, we measured the urinary concentrations of various parabens in 2541 human urine samples obtained via a national representative survey. Here, we report the level of paraben exposure in relation to demographic factors among the Korean population.

## 2. Materials and methods

### 2.1. Survey population

The Korean National Human Biomonitoring Survey was conducted by the Ministry of Food and Drug Safety. Participants, aged 3–69 years ($n=2541$), were recruited from the general population residing in the Republic of Korea between July 2009 to September 2010. The survey populations were collected by a stratified two-stage cluster random sampling design based on the National Census Registry. One-hundred census blocks were determined randomly based on the population of each city. All individuals completed interviews without missing data and provided urine samples to a public health center; the overall response rate was 93.7%. This study was approved by the Asan Medical Center (Seoul, Korea) Human Research Ethics Committee. All participants provided written informed consent, as defined by the Helsinki Declaration, prior to data collection.

### 2.2. Data collection

Urine samples were collected in polypropylene cup at different times throughout the day, and creatinine adjustments were used to correct for urine dilution. All samples were stored at −80 °C prior to analysis. Demographic information regarding sex, age, education, income, smoking status, and current residence was collected through face-to-face interviews. Body mass index (BMI) was calculated from self reported height ($m^2$) and weight (kg). We categorized education level for adults group as less than a high school diploma, high school diploma, and college or higher. Monthly household income was categorized as $<991$; 991–2973; 2,974–4956; and $>4956$ US dollars. Smoking history was divided into never, former, or current. Current residences were classified into rural or urban. BMI for adults was divided into underweight ($BMI<18.5$), normal weight ($18.5\leq BMI<23.0$), overweight ($23.0\leq BMI<25.0$), and obesity ($25.0\leq BMI$) as defined by the WHO for Asian populations.

### 2.3. Chemicals and reagents

Analytical standards of MeP, EtP, PrP, and BuP were purchased from Sigma-Aldrich (St. Louis, MO). As internal standards, methyl 4-hydroxybenzoate-2,3,5,6-d4 (MeP d4, 99.2%); ethyl 4-hydroxybenzoate-2,3,5,6-d4 (EtP d4, 98.8%); *n*-propyl 4-hydroxybenzoate-2,3,5,6-d4 (n-PrP d4, 98.8%); and *n*-butyl 4-hydroxybenzoate-2,3,5,6-d4 (n-BuP d4, 98.8%) were purchased from CDN ISOTOPES (Point-Claire, Quebec). The target analytes and internal standards are presented in Table S1. $\beta$-Glucuronidase (3015000 unit/g solid) and sulfatase (16020 unit/g solid) from *Helix pomatia* were purchased from Sigma-Aldrich. Analytical-grade acetonitrile, methanol, and water were purchased from J. T. Baker (Center Valley, PA). The stock solutions of target analytes and internal standards were stored at −80 °C.

### 2.4. Analysis of parabens

The urine samples were analyzed by combined solid-phase extraction and high-performance liquid chromatography (HPLC) coupled with the triple quadrupole tandem mass spectrometry (MS/MS) method (Lee et al., 2013). In brief, 1 mL of an aliquot of urine was mixed with 10 μL of the internal standard solution (MeP d4, EtP d4, n-PrP d4, and n-BuP d4) and 50 μL of enzyme solution (sulfatase and $\beta$-glucuronidase) in a 2.0 mL conical tube. Samples were incubated for 4 h at 37 °C and then added to 730 μL of acetic acid. The mixture was applied to Strata-X (30 mg, 1 ml; Phenomenex, Torrance, CA, USA), which had been conditioned by sequential elution with 3 mL of acetonitrile and 3 mL deionized water. Adsorbed parabens were rinsed with 5% methanol, dried for 2 h *in vacuo* at room temperature, and eluted with 1 mL of acetonitrile. Finally, parabens were quantified by LC-MS/MS with an Agilent 1200 series HPLC system (Palo Alto, CA, USA) connected to an API 4000 (triple quadrupole mass spectrometer; Applied Biosystems, Foster, CA, USA) used in the negative ion electrospray ionization mode. The chromatographic separation was carried out on a reverse-phase Synergi 4 μ Fusion-RP column (75 × 2.0 mm, 80 Å; Phenomenex). The mobile phase for elution was 0.1% acetic acid in water and 0.1% acetic acid in acetonitrile. The flow rate was 300 μL/min, and the injection volume was 5 μL, with a column oven temperature of 35 °C. Multiple reaction monitoring transitions were created for the analytes and internal standards (Table S2). Data acquisition was performed using the Analyst™ 1.42 software (Applied Biosystems).

### 2.5. Quality assurance and quality control

Procedural blanks (acetonitrile) were analyzed for each batch of 24 samples. Method blanks spiked with all target chemicals below the limit of quantification (LOQ) were analyzed to check for drift in instrumental sensitivity and carryover of target compounds between samples. Calibration check standards were injected regularly during the instrumental analysis sequence. Linearity in these analytes was checked from 0.1–500 ng/mL with a correlation