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Urinary paraben concentrations among pregnant women and their matching fetuses, and the association with health damages

산모와 태아의 소변 중 파라벤 노출 수준과 건강영향 지표와의 상관관계

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<Abstract>

Urinary paraben concentrations among pregnant women and their matching fetuses, and the association with health damages

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Parabens have been used in multiple products including personal care products, pharmaceuticals, and foods for more than 50 years but several studies have raised concerns on their safety. The present study was designed to determine urinary paraben levels among pregnant women and their matching fetuses, and the association between paraben levels and stress markers. Pregnant women (n=46) and their matching children were recruited from four university hospitals located in Seoul, Ansan and Jeju of Korea, 2011. Parabens including methyl paraben (MP), ethyl paraben (EP), n-propyl paraben (PP), and n-butyl paraben (BP) were measured in urine using an automatic, high throughput online SPE-LC-MS/MS method. Urinary concentrations were normalized with specific gravity. Free cortisol, malondealdehyde (MDA) and 8 -hydroxydeoxyguanosine (8-OHdG) were

measured in urine as stress marker and the transcription of *NOX1* gene in placenta was quantified using quantitative PCR. Urinary MP was detected as the highest, and BP was detected as the lowest paraben in urine samples of both pregnant women and their fetuses. The levels of urinary parabens among Korean pregnant women are comparable to those reported elsewhere, except for EP which was 4-9 folds higher than pregnant women of other countries. The ratios of fetal to maternal paraben concentrations varied between 0.5 and 0.6 for MP and PP, but approximately 10 fold lower for EP. Urinary MP or EP levels were associated with several oxidative stress related biomarkers such as urinary 8-OHdG and MDA, even after the adjustment of relevant covariates such as maternal age, mode of delivery, pre-pregnancy BMI, gestational age and parity. This is the first study that reported the levels of major parabens in the first urines of newborn infants. Further studies are warranted to understand the implications of paraben exposure among biologically susceptible human populations.

Keywords: urine, methyl paraben, ethyl paraben, placenta, oxidative stress

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<Contents>

< List of tables>

< List of figures>

1. Introduction

Parabens are esters of *p*-hydroxybenzoic acid, having antifungal and antibacterial properties. For this reason, these compounds have been used in multiple products including personal care products, pharmaceuticals, and foods (Andersen, 2008). Antimicrobial activity of parabens is thought to increase with the length of alkyl group from methyl to n-butyl (Han and Washington, 2005). The octanol/water partition coefficients of parabens increase as the carbon number of the alkyl chain of parabens increases (logKow value for methyparaben, MP=1.66, ethylparaben, EP=2.19, propylparaben, PP=2.71, and butylparaben=3.24).

Recently parabens have been detected in high levels in humans (Calafat et al., 2010; Meeker et al., 2011). Since parabens and their metabolites can be measured in urine, urinary levels of parent parabens may be used as biomarkers of recent human exposure (Ye et al., 2006a). Exposure may occur through ingestion, inhalation, or dermal absorption. Upon absorption, parabens are rapidly metabolized mainly into *p*-hydroxybenzoic acid and its respective glucuronic and sulfuric acid conjugates. Parent parabens in their free or conjugated form can be excreted in urine following the skin application. Urinary paraben concentrations were reported to correlate to those levels in both serum and seminal plasma in 60 healthy Danish men (Frederiksen et al., 2011).

Parabens have been frequently detected in pregnant women worldwide, with MP

being the greatest concentrations, followed by PP, EP, and BP. Among 120 Spanish pregnant women, the average level of MP, PP, EP, and BP was 191, 29.8, 8.8, and 2.4 μg/L, respectively. In their children's urine, the average level of MP, PP, EP, and BP was 150, 21.5, 8.1, and 1.2 μg /L, respectively, suggesting similar sources of exposure (Casas et al., 2011). Similar patterns of paraben concentrations were reported among pregnant women in Japan and USA (Shirai et al., 2012; Smith et al., 2012). While urinary paraben concentrations are quite variable in women during pregnancy, a single urine measurement during pregnancy is thought to represent gestational exposure (Smith et al., 2012). Paraben exposure during pregnancy is important because prenatal exposure to parabens may affect the health of fetus. To our knowledge, the levels of parabens in fetal urine have never been reported.

Although parabens have been used for more than 50 years and are generally considered as safe, several studies have raised concerns on the safety of parabens (Prusakiewicz et al., 2007; Tavares et al., 2009). Exposure to parabens may modulate or disrupt the endocrine system and cause oxidative stress, which may cause harmful consequences in animals and humans (Darbre and Harvey, 2008; McGrath, 2003). In pregnant rats, exposure to BP caused adverse effects on the reproductive organs of the male offspring (Kang et al., 2002). BP can cause oxidative stress by inhibiting anti-oxidants, and was reported to increase malondialdehyde (MDA) in mouse liver (Shah and Verma, 2011). MP and EP may induce oxidative stress also by mediating erythrocyte glutathione (GSH) conjugates of hydroquinone by reacting with $^1O_2$ and GSH (Nishizawa et al., 2006). PP led to

increase of DNA damage in Vero cell (Martin et al., 2010). Oxidative stresses caused by parabens were also reported in other studies (Arikan et al. 2001; Nishizawa et al. 2006), but such links in humans have seldom been reported.

The present study was designed to determine the urinary levels of parabens in pregnant women and their matching fetuses in Korea. The association between urinary paraben levels and the biomarkers of oxidative stresses was also evaluated. The results of this study will help understand the levels of paraben exposure among sensitive human populations, and develop management decisions on these compounds.

2. Materials and Methods

2.1. Study population and sample collection

Pregnant women (n=46) and their matching fetuses (n=46) were recruited from four university hospitals located in Seoul, Ansan and Jeju of Korea between February and December, 2011 (Table 1). Urine samples were collected from pregnant women within a day before delivery and from newborn infants within 48 h after birth. Urine samples were stored at -80^{o}C immediately after sampling, until analysis. In addition, placenta (~1x1x1 cm) was sampled immediately following birth, and was stored in -80^{o}C freezer. Placenta sample was taken approximately 1 – 1.5 cm below the fetal membrane to avoid membrane contamination. A paraben-containing body-wash product was used in one participating hospital (Soonchunhyang University Hospital) for the newborn infants (n=9).

One-on-one interviews with participating pregnant women were conducted, and demographic parameters, physiological data, and pregnancy history at the time of enrollment were asked. Medical records regarding current or previous health status and gestational period were abstracted. Institutional Review Boards of School of Public Health, Seoul National University, and all participating university hospitals approved the study. Informed consents were obtained from the participating women. All samples and data were processed blind.

2.2. Chemicals and analysis

Four parabens including MP, EP, PP, and BP were measured in the urine. Target chemicals and internal standards, i.e., MP-d_4, EP-d_4, PP-d_4, BP-d_4 were purchased from Sigma-Aldrich (Yongin, Korea), and CDN ISOTOPE (Quebec, Canada), respectively. Distilled water and solution were bought from Burdick & Jackson (Morristown, NJ, USA) and ammonium formate, ammonium acetate and formic acid were obtained from Fluka (Buchs, Switzerland).

Urine sample was prepared by enzyme hydrolysis and analyzed using online SPE-LC-MS/MS method, following Ye et al. (Ye et al., 2006a, 2006b) with minor modifications. A 100 μL aliquot of the urine was mixed with 830 μL of 0.1M acetic acid, 10 μL enzyme solution (β-glucuronidase/sulfatase), and 10 μL of internal standard solution (200 ng/mL). After incubating at 37 ^{o}C for 4 h, and subsequent centrifuging at 2500 rpm for 10 min, the supernatant was transferred to glass vial and analyzed by online SPE-LC-MS/MS system. Calibration curve was derived from artificial urines spiked with standard (0.1. 0.5, 1, 2, 5, 10, 50, 100, 250, or 500 ng/mL).

Nanopace II (Shiseido, Tokyo, Japan), equipped with autosampler, dual pump, column oven, vacuum degaser and switching valve, was used for urine analysis in online SPE system. For HPLC condition, refer to supplementary Table S1. SPE column of Synergi 4U Fusion-RP (80A, 2.0×75mm, Rhenomenes, CA, USA) with

gradient mode was employed for analysis. The detection of compounds was performed by API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) with multiple reaction mode (MRM). Operational parameters for detection were shown in supplementary Table S2. The parameters of MS for high sensitivity detection were in electrospary ionization (ESI) negative mode (curtain gas 25Mpsi, GSI 40Mpsi, detector temperature 400 ^{o}C, Entrance potential -10.0V, ion source energy -4500V, Collisionally activated dissociation 6eV). The method limits of detection (LODs) were 0.7 μg/L for MP, 0.2 μg/L for EP, 0.3 μg/L for PP, and 0.5 μg/L for BP.

2.3. Quantification of MDA, 8-OHdG, and free cortisol

Thiobarbituric acid reactive substances (TBARS) assay was conducted to quantify MDA using a commercial kit (OxiSelct TM TBARS Assay Kit; Cell Biolabs Inc., San Diego, CA, USA). A manufacturer provided method was followed. After removing insoluble particles, the supernatant was assayed. The absorbances were read at 532 and 412 nm using a spectrophotometric plate reader (TECAN infinite RM 200, TECAN, Mannedorf, Switzerland) for MDA and 8-hydroxy-2-deoxyguanosine (8-OHdG), respectively. For urinary 8-OHdG, a competitive enzyme-linked immunosorbent assay (ELISA) was conducted using a commercial kit (8-hydroxy-2-deoxy guanosine EIA Kit; Cayman Chemical Company, USA) following Hung et al. (2011).

Free cortisol concentrations in urine were analyzed using a commercially available chemiluminescence immunoassay at Samkwang Medical Laboratories (Seoul, Korea). The assay was conducted with Unicel DxI 800 Immunoassay System (Beckman Coulter, Brea, CA, USA). The detection limit was 0.4 μg/dL and the assay range was 0.4~60 μg/dL.

2.4. Adjustment of urine analytical results

Urinary parabens and protein levels were adjusted by specific gravity (SG) of urine to avoid the influence of urine volume fluctuation. For this purpose, Meeker et al. (2011) was followed, e.g., SG-corrected level = measured level [(mean of SG - 1)/SG - 1], where the mean SG is 1.015 for the pregnant women's, and 1.009 for the fetal urines. Creatinine (CR) adjustment was also performed for comparability with other studies that reported creatinine adjusted urinary paraben levels.

2.5. Quantitative PCR

Approximately 30 mg of placenta tissue was randomly taken to extract the total RNA using RNeasy mini kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol. The integrity and concentration of RNA was determined by Nanodrop (ND-1000 Spectrophotometer, City, DE, USA). RNA extracts were stored at -80 ^{o}C until further use. Real-time RT-PCR was carried out for the selected genes using gene-specific primers (For *NADPH oxidase isoform 1* (*Nox1*), NM_007052.4, 5'-GCC AGT GAG GAT GTT TTC CAG TAT G-3' (forward), 5'-CCC AAA GGA GGT TTT CTG TTT CAG-3' (reverse)) and Power SYBR Green RNA-to-CT 1-Step (Applied Biosystems, City, CA, USA). Quantitative PCR was performed using LightCycler 480 (Roche Applied Science, Indianapolis, IN, USA). Relative changes of target gene expression were calculated by fold upregulation/downregulation compared to that of an internal control, housekeeping gene (*Tyrosine 3-monooxygenase/tryptophan* (*YWHAZ*), NM_003406.3, 5'-AGC TGG TTC AGA AGG CCA AA-3' (forward), 5'-GGC TGC CAT GTC ATC ATA TCG-3' (reverse) (Adibi et al., 2009)).

2.6. Statistical analysis

For parabens of which detection rate is 80% or greater, nondetects were imputed with LOD/sqrt2, and were subject to statistical analysis. For parabens of which detection rates were < 80%, statistical analysis was not conducted. Data analysis was performed using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA). The *p* value less than 0.05 was considered statistically significant. All parabens and stress biomarkers were transformed by the natural logarithm (ln) for statistical analyses. In multiple regression model, maternal age, maternal BMI, mode of delivery, gestational age and parity were used as covariates.

3. Results

3.1. Concentrations of urinary parabens in pregnant women and their fetuses

Urinary MP was detected at the greatest concentration with the detection frequencies of 100% in fetal urines and 98% in pregnant women. For EP, and PP, the detection frequencies range also between 98 and 100%. BP was the least detected paraben with a detection frequency of 28% in maternal urine and 41% in fetal urine. MP constituted the major proportion of the total amount of parabens in both maternal and fetal urines. The concentrations of MP and EP comprised 71-76%, and 18-19% of the sum of all four paraben concentrations in urine, respectively (Fig. 1).

The paraben concentrations were higher among the maternal urine samples compared to those of fetal urine samples. In maternal urines, the median level of MP, EP, and PP was 134.0, 38.0, and 6.6 μg/L, respectively. In fetal urines, median levels of MP, EP, and PP was 79.6, 2.4, and 3.4 μg/L, respectively. Urinary paraben concentrations were associated with characteristics of the subjects. Both maternal and fetal urinary PP concentrations were higher in the group with longer gestational age. Fetal urinary PP concentrations were detected higher in

primipara group. Maternal and fetal urinary PP concentrations differed significantly by maternal BMI groups (Table 2). Unadjusted urinary paraben concentrations exhibited similar trends but significance was not present (see supplementary Table S3).

3.2. Associations with stress markers or birth size

MP or EP levels in urine were associated with several stress markers in urine, even after the adjustment of maternal age, maternal BMI, parity, gestational age and mode of delivery (Table 3). In all newborn infant group (Fetus 2 Group, n=46), the SG-adjusted MP levels in fetal urine were significantly associated with urinary MDA levels (β=0.15, p=0.05), however such significance was not observed with EP (β=0.11, p=0.08). When the infants who did not use paraben-containing body-wash products were considered (Fetus 1 Group, n=37), both MP and EP levels in fetal urines showed significant positive associations with urinary MDA levels (β=0.18, p=0.02 for MP and β=0.13, p=0.05 for EP). In maternal urine, the EP levels were significantly associated with urinary MDA levels (β=0.10, p=0.03), and marginally with urinary 8-OHdG levels (β=0.09, p=0.07). The paraben levels were not associated with transcription of placental *NOX1* gene. Urinary cortisol levels were negatively associated with EP levels of pregnant women, but the significance was disappeared after the adjustment of covariates. Similar trends of associations were also found between unadjusted urinary parabens and oxidative stress biomarkers, except for cortisol (see supplementary Table S4).

4. Discussion

4.1. Urinary paraben levels in pregnant women and their matching fetuses

Frequent detection of parabens in urine samples (Table 2) indicates that exposure to parabens are widespread among pregnant women and their matching fetuses in Korea. In maternal and fetal urine samples of Korea, MP was detected in almost all samples and the level of detection was the highest among the measured parabens, followed by EP and PP. The levels and frequency of detection were the lowest for BP ranging between 28 and 41%, little bit lower than those reported elsewhere (Table 4, Fig. 2).

Urinary MP levels detected in the present study were similar to pregnant women in USA, but lower than those in Spain and higher than in Japan and France. Urinary EP levels detected in pregnant women in Korea (median 38.0 μg/L), however, were 4-9 folds higher than those of other countries. EP has been detected at on average 8.8 μg/L among Spanish pregnant women (n=120), and at 4.1 μg/L among French pregnant women (n=191). In contrast, urinary PP levels were relatively lower than those of other countries. This observation indicates presence of strong contributors of EP exposure among Korean pregnant women, which

warrants further investigation.

The parabens in human urine may be originated from the use of the paraben containing products. MP was detected at the highest concentrations in commercial pharmaceutical formulations (Lokhnauth and Snow, 2005). In foam-shampoos and wash-off cosmetic products, MP was also detected at the highest concentration, followed by BP, EP, and PP (Labat et al., 2000). In commercial sunscreen products sold in Korea, MP was detected in highest frequency with an average concentration of 0.232%, followed by PP, EP, and BP (Kim et al., 2011). MP and PP have been detected at the highest concentrations in commercial cosmetic products (Han et al., 2008).

To our knowledge, paraben levels have never been reported among fetal urines elsewhere. Therefore it is impossible to compare the levels detected in the present study with those of other countries. When being compared with those reported among 4 yrs old children of Spain (n=30, Casas et al., 2011), the observed paraben concentrations among the fetuses of Korea are generally lower. The parabens detected in fetal urine can be generally considered to be originated from maternal exposure. In an experimental study using pregnant rats, cutaneous exposure to EP and BP resulted in detection of these compounds in fetal body as well as in amniotic fluid, and placenta (Frederiksen et al., 2008). However, maternal transfer might not be the only sources of paraben exposure among the fetuses that were studied in the present study. For some participating fetuses, body-wash products that might contain parabens were used just after the delivery at the

hospitals. Among the participating hospitals, Soonchunhyang University hospital routinely used a body-wash product that might contain MP, EP, and PP for newborn infants shortly after birth. A total of 9 children who were born in Soonchunhyang University hospital were therefore expected to be potentially influenced by the use of paraben-containing product. In fact, compared to the infants born in other hospitals (n=37), the babies who used paraben-containing body-wash (n=9) showed relatively greater levels of MP (median 63.4 vs 107.0 μg/L), EP (median 2.0 to 6.9 μg/L) and PP (median 2.2 vs 6.8 μg/L). However the differences were not statistically significant maybe due to the limited number of subjects.

When only babies who used paraben-free products were compared (n=37), there were significant positive associations between maternal and fetal urine levels of MP, EP or PP (Fig. 2a-2c), suggesting the importance of maternal exposure on the levels in fetal urines. Among the infant pairs that used the paraben-containing product (n=9), such significant associations between maternal and fetal urinary concentrations disappeared, suggesting the influences of the sources other than that of maternal origin, e.g., use of a body-wash (Fig. 2d-2f).

The levels of parabens in fetal urine samples were generally lower than those detected in maternal urines. The median levels of MP and PP in fetal urines were about 47 and 95% of those for maternal urines, respectively, while EP concentration in fetal urine was about 9% of that in maternal urine. The ratios of fetal to maternal paraben concentrations varied by the use of paraben-containing body-wash

product: For the subjects who were not exposed to a paraben-containing body-wash (n=37), the ratios of fetal to maternal paraben levels were 45 and 6% for MP and EP, but for those who used a paraben-containing body-wash (n=9), the ratios were 108 and 19%, respectively. For PP, however, such differences were not very notable, e.g., 97 vs 83%, respectively.

The reason for relatively low mother-to-fetus ratio of urinary EP is not clear. One of the reasons can be found from different metabolism and elimination. In rats, fetal levels of EP were detected at greater concentrations compared to BP, after maternal percutaneous exposure, suggesting different metabolic characteristics by chemical (Frederiksen et al., 2008). The metabolism and elimination of parabens might differ depending on their dose, exposure route, and species (Soni et al., 2005).

4.2. Urinary parabens and stress markers

In the present study, the urinary levels of parabens and biomarkers were adjusted with SG to correct for diurnal fluctuations of urinary water content. Because pregnancy may influence CR metabolism (Braun et al., 2011), CR was not employed for this purpose. SG has been suggested as superior for adjustment of adjustment of urine samples for cortisol (White et al., 2010) and hormone (Miller et al., 2004). For comparison, unadjusted or CR-adjusted levels of urinary parabens were also shown.

Urinary MP or EP levels were associated with several oxidative stress related biomarkers such as urinary 8-OHdG or MDA (Table 3). In fetal urine, MP levels were positively associated with MDA levels. Several experimental studies support oxidative stress potentials of MP. MP treated rats had significantly higher plasma MDA levels (Gupta et al., 2009) and increased lipid peroxidation (Popa et al., 2011). Under sunlight, MP exposure led to harmful effects on human skin through oxidative stress and lipid peroxidation (Handa et al., 2006). In maternal urine, the positive association between EP and MDA also supports oxidative stress potential of EP. EP level had marginal positive association with 8-OHdG level as well in the maternal urine samples. 8-OHdG is a biomarker of oxidative DNA damage, formed by hydroxylation at the C8 position of guanosine residues in DNA (Wu et al., 2004). Considering that the levels of detection of EP were lower than those of

MP, such positive association between EP and 8-OHdG is interesting. We excluded BP in statistical analysis because of the small sample size. However, BP has been also reported to increase MDA levels and damaged antioxidative defense system including GSH, catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and superoxide dismutase (SOD) in mice liver leading to hepatotoxicity (Shah and Verma, 2011). Exposure to oxidative stressors in early life stages may influence health in adulthood (Luo et al., 2006), therefore consequences of prenatal exposure to parabens and associated evidence of oxidative damages deserve concerns.

Previously, several parabens have been associated with cortisol levels in urine. In animal study, after EP and BP exposure in rats, dose-dependent reduction of cortisol was observed (Taxvig et al., 2008). In humans, negative association between oxidative stress marker (8-OHdG) and cortisol concentration in cord blood was also reported (Chiba et al., 2010). In the present study urinary cortisol levels were negatively correlated with EP in maternal urines (β=-0.09, p=0.04) in the univariate model, but such association was disappeared after the adjustment of relevant covariates. A possible explanation for this observation might be found from the stress related to birth. Prenatal psychological anxiety or stress may affect on the response on fetal HPA axis, in particular, excretion of cortisol (Azak et al., 2012; Saridjan et al., 2010). Thus, the effects of parabens on fetal cortisol levels could be masked or confounded by such stresses associated with birth.

While oxidative stress was often related to birth weight (Kim et al., 2005), we

could not observe any negative association between urinary parabens and birth size. Similar observation of no association has been reported for BP in rats (Borch et al., 2006). Since birth size is a cumulative response that may reflect various conditions ranging from nutrition and health status of mothers to exposure to environmental contaminants, it is often very difficult to determine relationship with specific chemical exposure (Meeker, 2012).

4.3. Limitations and implications

A cross-sectional design of the present study with limited sample size would restrict our ability to make conclusions regarding causal relationship. It should be also noted that oxidative stresses could be influenced by multiple agents including psychological stress, e.g., labor, not to mention other exogenous compounds that were not considered in the present study. However, this study provided, for the first time, the levels of major parabens in fetal urines. Exposure to MP appears to be the most important concern among fetuses, and its consequences should deserve further investigation. In addition, the associations between paraben exposure and oxidative stress biomarkers were demonstrated in both maternal and fetal biospecimen. High exposure levels of EP among Korean pregnant women suggest different source profiles of parabens among women of child-bearing age. The results of this study will help better develop management options for parabens among the sensitive human populations including newborn infants.

5. Conclusion

Our study is the first attempt to determine the levels of paraben exposure among the newborn infants and their mothers. While the levels of urinary parabens in pregnant women were generally comparable to those of other countries, those of EP were about 4 to 9 fold greater, indicating the presence of strong or additional contributors to EP exposure among Korean pregnant women. The paraben concentrations detected in fetal urines were generally lower than those of the mothers, and EP levels in fetal urine samples were much lesser compared to those detected in maternal urines. Differences in toxicokinetics may explain this observation, but further investigations are warranted. Significant positive associations were detected between urinary MP or EP, and oxidative stress markers such as MDA or 8-OHdG in both maternal and fetal urines, suggesting oxidative stress potentials of parabens. Further studies are warranted to understand the implications of paraben exposure among the sensitive human populations like newborn infants.

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Table 1. Characteristics of pregnant women and their fetuses

Variable	n	Range	Mean	SD	Median
Pregnant women (n=46)					
Age (year)	46	22-39	33	3.54	33
BMI (kg/m^2)	34	13.80-27.80	21.08	3.25	20.75
Parity	46	0: 19, ≥1: 27			
Gestational age at delivery (days)	46	256-288	272.02	9.01	274
Infants (n=46)					
Sex		Male: 25, Female: 21			
Birth weight (kg)	46	2.47-4.15	3.28	0.34	3.27
Birth height (cm)	38	33.50-54.00	49.67	3.23	49.80
Birth circumference (cm)	37	31-49	34	2.83	34

Table 2. Specific gravity adjusted urinary paraben concentrations (μg/L) in pregnant women and their fetuses by the characteristics of study populations

	n	SG-adjusted paraben levels, Median (IQR)							
		Pregnant women				Fetuses			
		MP	EP	PP	BP	MP	EP	PP	BP
	46	169.9 (60.6-451.5)	44.6 (16.9-202.8)	8.6 (0.94-65.4)	<LOD (<LOD-0.47)	97.0 (39.9-272.3)	2.9 (1.0-8.0)	4.0 (0.84-15.2)	<LOD (<LOD-1.7)
No. of detection	46	45	46	45	13	46	45	46	19
Age (year)									
22-29	8	209.1 (79.2-1267.5)	21.9 (5.5-152.3)	28.8 (0.68-271.9)	<LOD <LOD	99.6 (54.9-322.8)	2.2 (1.0-4.7)	12.2 (2.2-24.4)	<LOD (<LOD-2.0)
30-39	38	169.9 (60.6-451.5)	65.6 (17.1-202.8)	6.3 (1.1-44.8)	<LOD <LOD	97.0 (39.9-272.3)	3.0 (1.0-10.7)	3.1 (0.84-7.7)	<LOD (<LOD-1.7)
Parity									
0	19	177.7 (131.0-605.2)	45.0 (17.1-229.3)	11.5 (1.5-82.8)	<LOD (<LOD-1.3)	133.9 (45.3-415.2)	2.5 (0.91-4.9)	9.2* (2.4-24.2)	<LOD (<LOD-2.9)
≥1	27	106.4 (43.7-422.3)	44.2 (13.8-202.8)	3.2 (0.80-65.4)	<LOD <LOD	91.4 (19.9-215.3)	3.1 (1.6-11.2)	1.8 (0.67-7.1)	<LOD (<LOD-1.1)
Delivery									
Normal	31	217.3 (66.1-451.5)	45.0 (8.6-352.5)	11.0 (0.94-67.4)	<LOD <LOD	102.6 (38.9-243.0)	2.4 (0.87-12.5)	6.6 (0.84-21.8)	<LOD (<LOD-2.9)
C-section	15	94.6 (49.8-564.5)	37.0 (17.1-193.5)	4.0 (0.56-33.1)	<LOD (<LOD-1.4)	85.5 (39.9-296.4)	3.0 (1.7-4.6)	1.8 (0.84-7.2)	<LOD (<LOD-1.3)

<LOD: Below LOD. LODs were 0.7 μg/L for MP, 0.2 μg/L for EP, 0.3 μg/L for PP, and 0.5 μg/L for BP.

IQR: interquatile range (median with 25-75%tile).

*: significant differences among groups ($p<0.05$) were determined by Kruskal-Wallis test and Wilcoxon's test.

	n	SG-adjusted paraben levels, Median (IQR)							
		Pregnant women				Fetuses			
		MP	EP	PP	BP	MP	EP	PP	BP
BMI before pregnancy (kg/m^2)									
<18.5	7	397.5 (172.5-675.0)	32.7 (5.2-87.9)	44.8* (11.5-112.5)	<LOD (<LOD-3.5)	133.9 (55.8-415.2)	2.1 (0.91-4.9)	24.2* (1.0-80.3)	<LOD (<LOD-3.8)
18.5-24.9	23	125.9 (49.8-407.3)	45.0 (16.9-202.8)	4.0 (0.80-41.6)	<LOD <LOD	85.2 (36.0-272.3)	2.4 (0.87-4.1)	1.8 (0.84-12.0)	<LOD (<LOD-0.85
≥25	5	118.1 (70.9-177.7)	44.2 (33.2-119.5)	6.5 (1.4-11.0)	<LOD <LOD	19.9 (12.2-215.3)	12.5 (<LOD-22.1)	0.8 (<LOD-7.7)	<LOD (<LOD-3.1)
Gestational age (days)									
<274	22	119.4 (49.8-422.3)	21.3 (9.8-193.5)	1.4* (0.56-24.1)	<LOD <LOD	81.1 (19.9-272.3)	2.5 (1.1-14.6)	1.1* (0.84-5.3)	<LOD <LOD
≥274	24	274.0 (66.1-605.2)	76.2 (27.6-296.3)	14.5 (3.2-75.1)	<LOD (<LOD-0.51)	118.3 (48.1-269.7)	3.5 (0.81-11.0)	7.7 (3.3-23.7)	0.7 (<LOD-3.4)
Region									
Large city	18	392.4 (83.7-675.0)	42.9 (20.2-87.9)	13.1 (1.1-82.8)	<LOD <LOD	102.7 (44.8-243.0)	2.5 (0.87-3.5)	4.0 (1.8-12.0)	0.9 (<LOD-2.1)
Small and medium-sized city	8	132.0 (49.1-287.6)	17.0 (7.8-76.1)	7.1 (1.5-62.0)	<LOD (<LOD-0.62)	186.6 (81.1-308.9)	3.8 (1.9-38.3)	2.1 (0.84-13.4)	<LOD <LOD
Industrial city	20	165.3 (56.8-401.4)	129.6 (19.1-455.4)	3.8 (0.56-38.0)	<LOD (<LOD-0.65)	48.1 (19.4-255.9)	3.2 (1.1-9.6)	6.9 (0.75-19.1)	<LOD (<LOD-1.0)

Table 2. continued.

Table 3. Multiple regression coefficients between SG-adjusted urinary parabens and health markers

			Univariate model [a]					Multivariate model [b]		
Variable	**PB**	**Group**	**β**	**95% CI**	**p**	**PB**	**Group**	**β**	**95% CI**	**p**
MDA	**MP**	Fetus 1 [c]	0.11	(-0.01 -0.24)	0.07	**MP**	Fetus 1	0.18	(0.03 -0.32)	0.02
	MP	Fetus 2 [d]	0.11	(-0.02 -0.24)	0.10	**MP**	Fetus 2	0.15	(0.00 -0.29)	0.05
	EP	Pregnant women	0.09	(0.01 -0.18)	0.03	**EP**	Pregnant women	0.10	(0.01 -0.20)	0.03
						EP	Fetus 1	0.13	(0.00 -0.25)	0.05
						EP	Fetus 2	0.11	(-0.01 -0.23)	0.08
8-OHdG						**EP**	Pregnant women	0.09	(-0.01 -0.19)	0.07
Free cortisol	**MP**	Pregnant women	-0.09	(-0.19 -0.01)	0.09					
	EP	Pregnant women	-0.09	(-0.18 -0.00)	0.04					
						EP	Fetus 1	0.12	(0.00 -0.23)	0.05

All parabens and stress biomarkers were transformed by the natural logarithm (ln) for statistical analyses. Only variables that showed significant associations were shown at $p<0.1$. [a] Univariate model: Simple linear regression model between paraben concentrations and health markers. [b] Multivariate model: Multiple regression model between parabens and health markers adjusted for five covariates, i.e., maternal age, maternal BMI, mode of delivery, gestational age and parity. [c] Fetus 1: Infants who did not use paraben-free products (n=37). [d] Fetus 2: All infants (n=46).

Table 4. Urinary paraben concentrations reported in the present study and previous studies

Country	n	Population	Median urinary paraben concentrations (IQR)					
			MP	EP	PP	BP	Unit	Ref.
Korea	46	Pregnant women	134 (31.7-475.0)	38.0 (9.9-235.0)	6.6 (0.37-55.2)	ND (ND-0.67)	μg/L	This study
			169.9 (60.6-451.5)	44.6 (16.9-202.8)	8.6 (0.94-65.4)	ND (ND-0.47)	SG-adjusted μg/L	
			236.8 (92.2-720.0)	53.9 (21.4-327.8)	9.4 (1.5-88.3)	ND (ND-0.89)	CR-adjusted μg/g	
		% detection	98	100	98	28	%	
	46	fetuses	79.6 (31.2-195.0)	2.4 (0.56-6.2)	3.4 (0.44-16.9)	ND (ND-1.4)	μg/L	
			97.0 (39.9-272.3)	2.9 (1.0-8.0)	4.0 (0.84-15.2)	ND (ND-1.7)	SG-adjusted μg/L	
			106.1 (70.9-842.4)	7.2 (2.7-14.8)	7.9 (2.6-37.3)	ND (ND-3.4)	CR-adjusted μg/g	
		% detection	100	98	100	41	%	
Japan	111	Pregnant women	75.8 (27.4–164)	7.53 (1.37–25.8)	20.2 (7.73–84.6)	0.59 (<0.46–3.34)	μg/L	(Shirai et al., 2012)
			108 (33.0-272)	7.26 (1.88-39.3)	33.3 (8.78-159)	0.80 (ND-4.31)	SG -adjusted μg/L	
		% detection	94	81	89	54	%	
Spain	120	Pregnant women	191 (415.5)	8.8 (25.7)	29.8 (61.3)	2.4 (10.3)	μg/L	(Casas et al., 2011)
		detection	100	88	98	90	%	
	30	Children (4 yrs old)	150 (427.8)	8.1 (26.2)	21.5 (56.4)	1.2 (3.7)	μg/L	
		% detection	100	80	100	83	%	
Denmark	60	Young men	17.7 (6.58-64.6)	1.98 (0.49-5.35)	3.60 (0.85-14.0)	0.19 (0.09-1.01)	μg/L	(Frederiksen et al., 2011)
		% detection	98	80	98	83	%	
France	191	Pregnant women[a]	97.8 (9.1-3520)	4.1 (0.7-62.3)	12.5 (0.5-402.0)	1.7 (0.1-53.8)	μg/L	(Philippat et al., 2012)
		% detection	100	68	97	80	%	

[a]: 5-95%tile range.

[b]: the median of within-person geometric mean urinary papaben concentrations.

IQR: interquartile range (median with 25-75%tile).

Country	n	Population	Median urinary paraben concentrations (IQR)					
			MP	EP	PP	BP	Unit	Ref.
USA	1278	≥ 6 yrs old female	137 (35.4–356)	1.3 (ND–10.0)	29.1 (5.30–93.0)	0.50 (ND–3.70)	µg/L	(Calafat et al., 2010)
			147 (43.2-377)	2.09 (ND-12.7)	34.9 (6.25-114)	0.75 (ND-4.44)	CR-adjusted µg/g	
	1270	≥ 6 yrs old male	23.7 (8.30–97.6)	ND (ND–1.90)	2.30 (0.600–13.8)	ND (ND–0.300)	µg/L	
			21.1 (7.48-72.1)	ND (ND–1.82)	1.84 (0.513-9.52)	ND (ND–0.292)	CR-adjusted µg/g	
		% detection	99	42	93	47	%	
USA	194	Male partner of subfertile couple	27.4 (11.4–64.8)	-	3.45 (0.80–17.1)	ND (ND–0.30)	µg/L	(Meeker et al., 2011)
			32.6 (14.4-85.7)	-	4.45 (1.07-20.9)	ND (ND-0.36)	SG-adjusted µg/L	
		% detection	100	-	92	32	%	
USA	129	Pregnant women[b]	135 (51.3–287)	-	22.8 (7.33–-75.2)	0.88 (0.25–2.88)	µg/L	(Smith et al., 2012)
			185 (69.3-348)	-	36.6 (10.3-89.9)	1.23 (0.42-4.03)	SG-adjusted µg/L	

Table 3. continued.

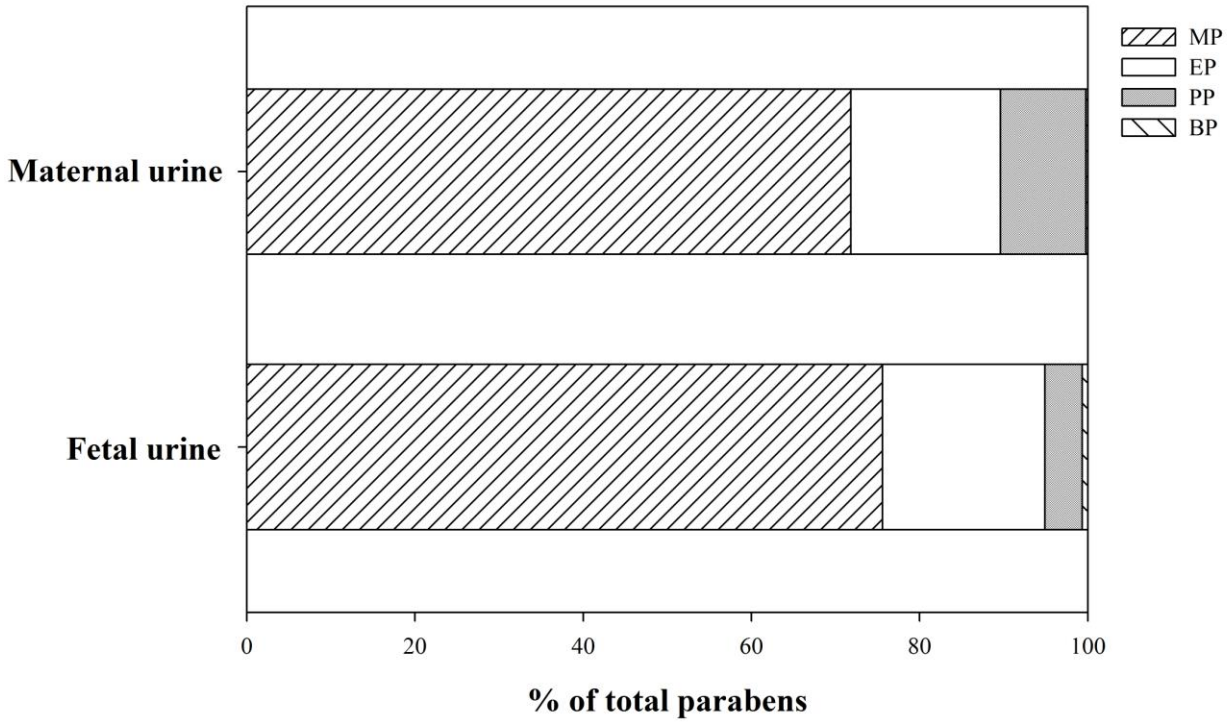
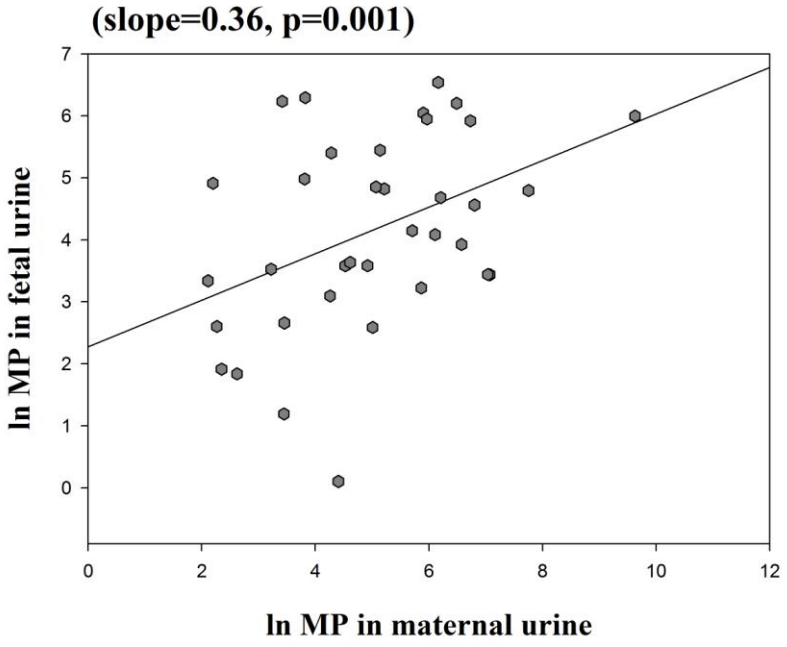
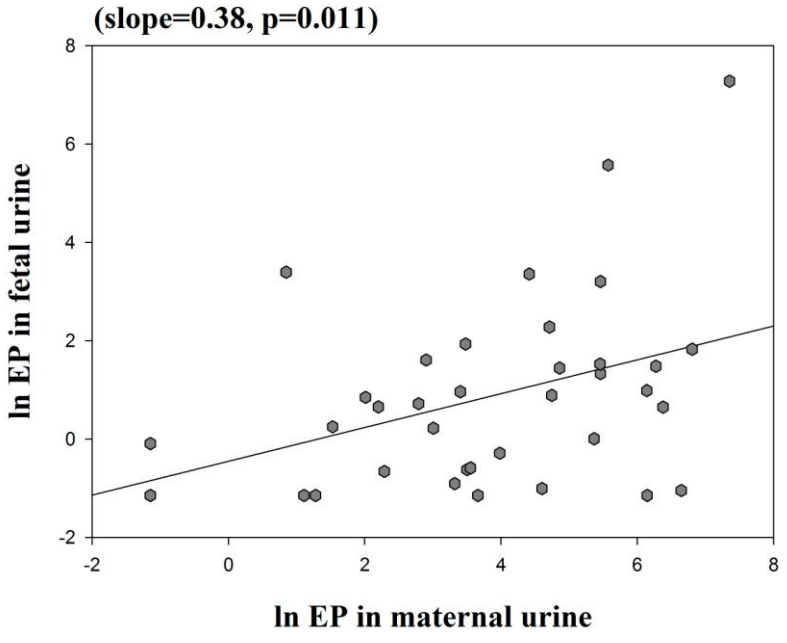


Fig. 1. Relative proportion of individual parabens comprising total parabens in study population (n=46).

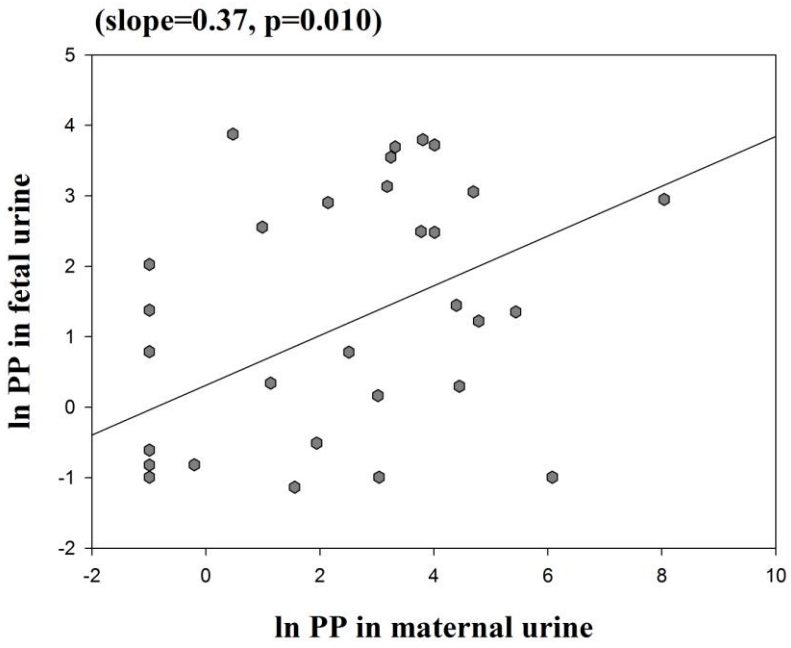
(a) MP in urine



(b) EP in urine

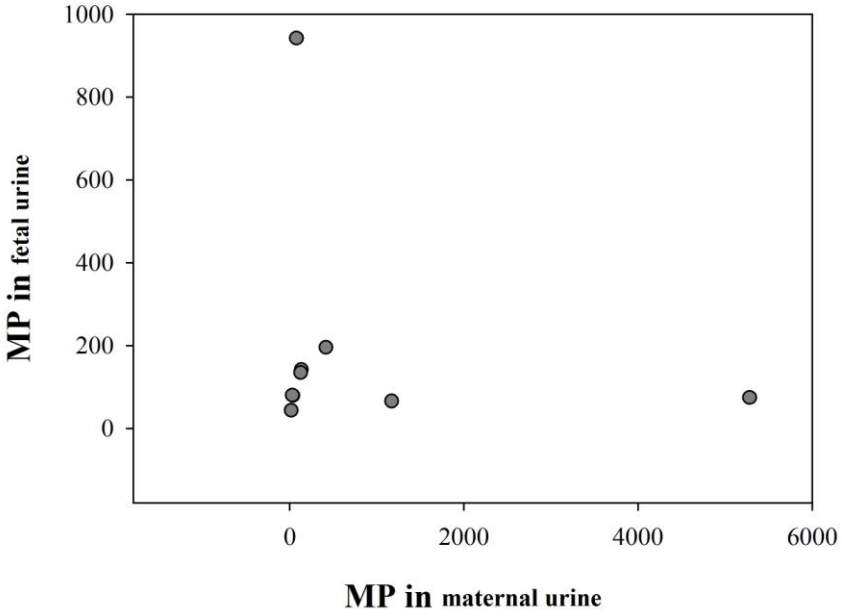


(c) PP in urine

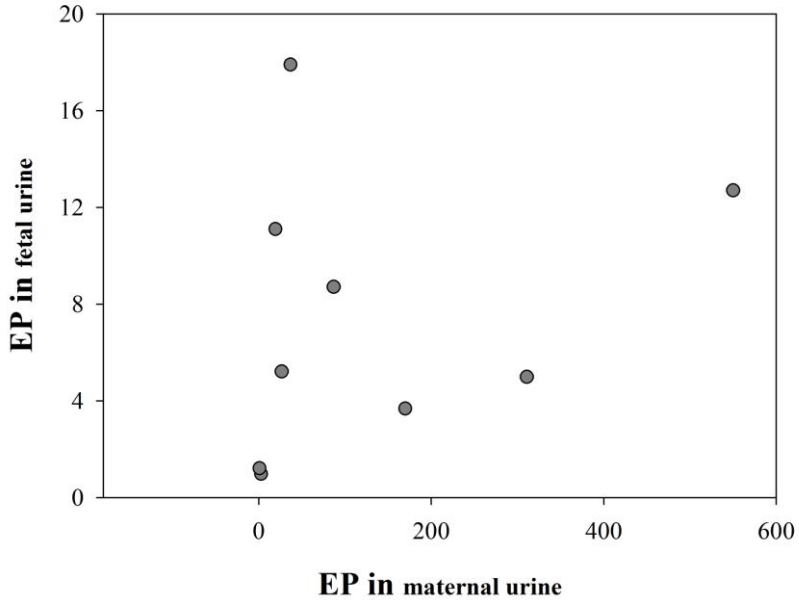


(a-c) Results from mother-fetus pairs used paraben-free products (n=37)

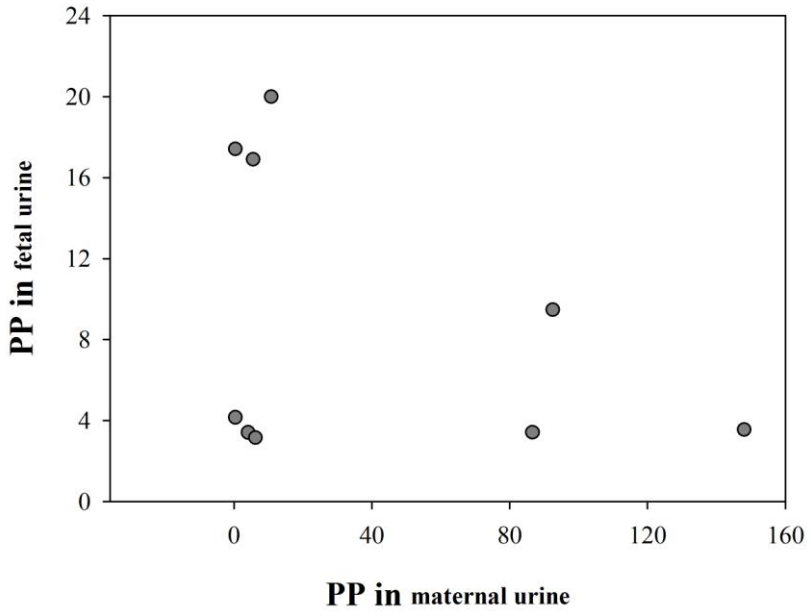
(d) MP in urine



(e) EP in urine



(f) PP in urine



(d-f) Results from mother-fetus pairs used paraben-containing products.

Fig. 2. Scatterplots and simple linear regression line of concentrations of parabens in maternal urine and their urine of fetuses. (a-c) results from mother-fetus pairs used paraben-free products (n=37), (d-f) results from mother-fetus pairs used paraben-containing products.

Supplementary Information

Urinary paraben concentrations among pregnant women and their matching fetuses, and the association with health damages

Table S1. HPLC condition for analysis of parabens

Parameter	Condition
Column	On-line HLB(Waters) Synergi 4U Fusion-RP
Mobile phase	A : 0.1% acetic acid in water B : 0.1% acetic acid in acetonitrile
Gradient	Time(min) 0 10 13 16 16.1 20
(400 μL/min)	B % 30 55 90 90 30 30
Switching valve	Time(min) 0 3 20 Position A B A

Table S2. Operational parameters for mass spectrometer

Compound	Precursor ion Q1 Mass (m/z)	Daughter ion Q3 Mass (m/z)	DP	CE	CXP
Methyl paraben (MP)	151	92	-55	-28	-5
MP-d_4	155	96	-55	-28	-5
Ethyl paraben(EP)	165	92	-60	-32	-15
EP-d_4	169	96	-55	-34	-7
Propyl paraben(PP)	179	92	-65	-30	-5
PP-d_4	183	96	-60	-32	-7
Butyl paraben (BP)	193	92	-70	-35	-14
BP-d_4	197	96	-70	-38	-7

DP : Declustering potential, V.
CE : Collision energy, V.
CXP : Collision cell exit potential, V.

Table S3. Unadjusted urinary paraben concentrations (μg/L) in pregnant women and their fetuses by the characteristics of study populations

		Unadjusted paraben levels, Median (IQR)							
	n	Pregnant women				Fetuses			
		MP	EP	PP	BP	MP	EP	PP	BP
	46	134 (31.7-475.00)	35.95 (9.03-234)	5.86 (<LOD-45.00)	<LOD (<LOD-0.67)	79.6 (31.20-195.00)	2.21 (0.54-6.23)	3.41 (<LOD-12.90)	<LOD (<LOD-1.26)
No of detection	46	45	46	45	13	46	45	46	19
Age (year)									
22-29	8	142 (20.6-807.5)	5.94 (1.16-24.75)	2.78 (<LOD-36.35)	<LOD <LOD	131 (38.65-269)	1.45 (0.46-6.87)	10.39* (<LOD-28.85)	<LOD <LOD
30-39	38	134 (33.8-450)	68.15 (18.2-235)	6.57 (<LOD-55.2)	<LOD (<LOD-1.15)	77 (28.20-195.00)	2.4 (0.54-6.23)	3.28 (<LOD-12.00)	<LOD (<LOD-1.36)
Parity									
0	19	184* (92.50-659.00)	37 (9.03-234.00)	10.8 (<LOD-55.2)	<LOD (<LOD-1.3)	142* (36.00-384.00)	1.21 (<LOD-5.02)	12 (2.19-21.30)	<LOD* (<LOD-2.40)
≥1	27	76.4 (16.1-366.00)	33.2 (7.47-311.00)	4.09 (<LOD-43.60)	<LOD (<LOD-0.67)	63.4 (22.10-128.00)	2.44 (0.75-6.94)	1.35 (<LOD-4.15)	<LOD (<LOD-0.90)
Delivery									
Normal	31	165 (31.50-475.00)	33.65 (3.59-235.00)	6.27 (<LOD-45.00)	<LOD <LOD	72.4 (34.10-171.00)	1.93 (<LOD-9.80)	3.65 (0.55-17.40)	<LOD (<LOD-1.36)
C-section	15	91.5 (45.80-414.00)	45.35 (18.20-214.00)	4.64 (<LOD-55.20)	0.33 (<LOD-2.17)	100.45 (22.10-494.00)	3.16 (1.01-5.02)	2.28 (<LOD-9.46)	<LOD (<LOD-0.77)

<LOD: Below LOD. LODs were 0.7 μg/L for MP, 0.2 μg/L for EP, 0.3 μg/L for PP, and 0.5 μg/L for BP.
IQR: interquatile range (median with 25-75%tile)
*: significant differences among groups ($p<0.05$) were determined by Kruskal-Wallis test and Wilcoxon's test

	n	Unadjusted paraben levels, Median (IQR)							
		Pregnant women				Fetuses			
		MP	EP	PP	BP	MP	EP	PP	BP
BMI before pregnancy (kg/m2)									
<18.5	7	462.5 (171.5-667.50)	18.37 (2.96-102.45)	50.1 (20.00-76.00)	<LOD (<LOD-3.43)	116 (62.50-250.50)	1.42 (0.28-3.74)	12.36 (1.77-40.85)	<LOD (<LOD-1.77)
18.5-24.9	23	82 (30.50-353.00)	53.7 (9.86-235.00)	2.69 (<LOD-20.50)	<LOD <LOD	43.2 (28.20-222.00)	1.29 (0.52-4.25)	1.18 (<LOD-7.60)	<LOD (<LOD-0.57)
≥25	5	70.9 (31.50-391.00)	33.2 (32.40-111.00)	4.75 <LOD-24.10)	<LOD <LOD	22.1 (6.79-95.70)	6.94 (0.54-9.80)	<LOD (<LOD-3.40)	<LOD (<LOD-1.36)
Gestational age (days)									
<274	22	79.2 (30.50-391.00)	33.05 (4.61-129.00)	<LOD (<LOD-20.90)	<LOD (<LOD-0.67)	47 (14.30-146.0)	1.27 (0.75-4.99	<LOD (<LOD-3.55))	<LOD <LOD
≥274	24	177.5 (59.15-578.00)	38 (18.80-235.00)	11.55 (2.15-68.10)	<LOD (<LOD-0.65)	116 (47.75-227.00)	3.74 (<LOD-8.37)	5.93 (2.80-19.15)	<LOD (<LOD-2.49)
Region									
Large city	18	128 (29.10-497.00	28.9* (3.59-87.00)	5.15 (<LOD-55.20)	<LOD (<LOD-0.67))	121 (65.50-195.00)	2.54 (<LOD-2.54)	3.49 (1.18-12.90)	<LOD (<LOD-2.13)
Small a medium-sized city	8	196.75 (27.48-595.50)	559.5 (235.00-835.00)	26.06 (<LOD-95.00)	<LOD <LOD	33.6 (26.70-104.70)	4.11 (0.96-15.46)	4.11 (3.04-15.20)	<LOD (<LOD-2.93)
Iustrial city	20	143.5 (38.75-433.00)	26.75 (6.82-120.00)	5.05 (<LOD-26.70)	<LOD (<LOD-1.46)	73.25 (18.20-308.00)	1.27 (0.55-4.82)	<LOD (<LOD-17.50)	<LOD <LOD

Table S3. Continued.

Table S4. Multiple regression coefficients between unadjusted urinary parabens and health markers

		Univariate model [a]				Multivariate model [b]				
Variable		Group	β	95% CI	p		Group	β	95% CI	p
Fetal weight	MP	Fetus 1 [c]	0.02	(0.00 - 0.05)	0.03					
MDA	MP	Fetus 1	0.23	(0.08 - 0.39)	0.00	MP	Fetus 1	0.26	(0.10 - 0.42)	0.00
		Fetus 2 [d]	0.26	(0.10 - 0.42)	0.00		Fetus 2	0.25	(0.09 - 0.41)	0.00
	EP	Pregnant women	0.21	(0.10 - 0.31)	0.00	EP	Pregnant women	0.19	(0.07 - 0.30)	0.00
		Fetus 1	0.15	(0.02 - 0.28)	0.02		Fetus 1	0.15	(-0.01 - 0.32)	0.07
		Fetus 2	0.20	(0.07 - 0.33)	0.00		Fetus 2	0.16	(0.00 - 0.31)	0.05
	PP	Pregnant women	0.12	(0.02 - 0.22)	0.02	PP	Pregnant women	0.10	(-0.01 - 0.21)	0.06
		Fetus 2	0.15	(0.00 - 0.29)	0.04					
8-OHdG	MP	Fetus 1	0.16	(0.00 - 0.31)	0.05					
		Fetus 2	0.20	(0.05 - 0.35)	0.01					
	EP	Pregnant women	0.13	(0.05 - 0.20)	0.00	EP	Pregnant women	0.16	(0.06 - 0.26)	0.00
		Fetus 2	0.12	(-0.01 - 0.26)	0.07					
	PP	Pregnant women	0.11	(0.05 - 0.17)	0.00	PP	Pregnant women	0.11	(0.02 - 0.20)	0.02
Free cortisol	PP	Pregnant women	0.13	(0.04 - 0.22)	0.01	PP	Fetus 2	0.10	(-0.02 - 0.21)	0.09
	EP	Fetus 1	0.09	(-0.02 - 0.20)	0.09	EP	Fetus 1	0.13	(-0.02 - 0.27)	0.08
		Fetus 2	0.09	(-0.01 - 0.19)	0.09					

All parabens and stress biomarkers were transformed by the natural logarithm (ln) for statistical analyses. Only variables that showed significant associations were shown. [a] Univariate model: Simple linear regression model between paraben concentrations and health markers. [b] Multivariate model: Multiple regression model between parabens and health markers adjusted for five covariates, i.e., maternal age, maternal BMI, mode of delivery, gestational age and parity. [c] Fetus 1: Infants who did not use paraben-free products (n=37). [d] Fetus 2: All infants (n=46).

<국문초록>

산모와 태아의 소변 중 파라벤 노출 수준과 건강영향 지표와의 상관관계

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강 성 은

파라벤은 주로 화장품, 식품, 약품 등에 미생물의 성장을 억제하고 보존기간을 증가시키는데 이용되는 살균성 보존제로 사용되어 왔다. 지난 50여년 간 파라벤은 비교적 그 독성이 낮다고 알려져 널리 이용되어 왔으나, 최근 발표된 연구결과에서 인체 유방암 조직에서 파라벤 농도가 정상인에 비해 높게 검출되었고, 산화적 손상, 내분비계 교란 및 생식에 영향을 미칠 수 있다는 실험 결과들이 제기되면서 파라벤의 안전성이 의심되고 있다. 이에 본 연구는 우리나라 일반인구 중 민감집단인 산모와 태아를 대상으로 파라벤의 노출수준을 파악하고, 파라벤 노출에 의한 스트레스 및 태아성장에 미치는 영향을 알아보기 위해 수행되었다.

2011년에 서울, 안산, 제주 3곳의 산모 46명을 대상으로 소변 및 태반시료를 수집하였다. 분석대상 파라벤은 메틸파라벤 (MP), 에틸파라벤 (EP), 프로필파라벤 (PP), 부틸파라벤 (BP) 4종을 대상으로 하였으며 파라벤의 농도를 소변시료에서 분석하였다. 산화적 손상지표인 MDA 및 8-OHdG 수준은 소변에서, *NOX1* 유전자발현 수준은 태반시료에서 측정하였고, 스트레스 지표인 유리코르티졸은 소변에서 측정하여 각각 파라벤과의 상관관계 분석에 이용하였다. 소변에서 측정한 파라벤 농도와 건강영향지표의 측정결과

는 비중으로 보정하였다. BP는 전체 시료의 80% 미만으로 검출되어 건강영향지표와의 상관성분석에서 제외하였다.

파라벤 중 산모와 신생아 소변 모두에서 MP가 가장 높게 검출되었고, BP가 가장 낮게 검출되었다. 국내 산모 소변에서 검출된 파라벤 농도 수준이 외국 산모의 검출수준보다 4-9배 높게 나타났다. 산모와 신생아 소변 중 검출된 파라벤 농도를 비교하였을 때, 신생아 소변에서의 파라벤 수준이 산모 소변에서의 수준보다 일반적으로 더 낮았다. 다중회귀모델을 이용하여 분석한 결과, 소변에서 MP 또는 EP 농도는 8-OHdG와 MDA 같은 산화적 스트레스 생체지표와 연관성을 나타냈다. 이는 일부 파라벤이 산화적 손상에 영향을 미칠 수 있는 가능성을 제시할 수 있다.

본 연구는 처음으로 신생아 소변을 대상으로 파라벤의 노출정도를 파악한 첫 연구이다. 이와 함께 파라벤이 인체에 미치는 산화적 손상 등 부정적 건강영향에 대한 가능성을 확인하고, 산모 및 태아의 파라벤 노출을 줄일 수 있도록 하여 미래세대 건강을 위한 기초자료로 활용될 수 있을 것으로 기대된다.

주요어: 소변, 메틸파라벤, 에틸파라벤, 태반, 산화적 손상

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