

# Human urine contamination with environmental pollutants: simultaneous determination using UPLC-MS/MS

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## ABSTRACT

Paraben derivatives are widely used as an antifungal, antimicrobial preservative in cosmetic products, pharmaceuticals, and food. These molecules are called endocrine disruptors (EDCs). The exposure of the human body to paraben derivatives needs further study and for this purpose 200 urine samples were collected from Tunisian men and women aged between 5 and 90 years to determine three paraben derivatives: methylparaben (MP), ethylparaben (EP) and propylparaben (PP) using ultra performance liquid chromatography–tandem mass spectrometer (UPLC-MS/MS). The three major parabens were found in 95 urine samples. The obtained results indicate that MP, EP, and PP were detected in 57%, 46%, and 40% of all samples, respectively. Urinary concentration for the three parabens was in the range of 0.88–84.46 ng/mL, 0.52–29.2 ng/mL, and 0.51–28.17 ng/mL of PP, MP, and EP, respectively. In addition, the concentrations of the paraben derivatives in women were higher than those of men. These findings indicate that the exposure occurs from common products (foods, cosmetics, and pharmaceuticals). The Tunisian authorities should control the composition of packaging of these common products in order to protect humans against EDCs.

**Key words** | biomonitoring, endocrine disruptors, environmental contamination, paraben derivatives, UPLC-MS/MS

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## INTRODUCTION

Parabens are esters of para-hydroxybenzoic acid. The most commonly used parabens are methylparaben (MP), ethylparaben (EP), propylparaben (PP), and butylparaben (BP) in combination or alone (Soni *et al.* 2005). Their widespread use has led to their prevalent distribution in the

environment. In fact, they have been detected in wastewater, sewage sludge, and other environmental samples (Tohidi & Cai 2015; Camino-Sánchez *et al.* 2016). Otherwise, they are known to be used as anti-microbial preservatives in a range of consumer products, especially in cosmetics,

pharmaceuticals, and food products (Elder 1984; Soni et al. 2005; Tillett 2012). Consequently, humans are exposed to these derivatives by dermal (cosmetic products), oral (food, medicines), and inhalation routes. Then, they are absorbed in the intestine, metabolized in the liver, and excreted through urine as parabens in free form or in the form of two biomarkers: p-hydroxybenzoic acid (PHBA) and p-hydroxyhippuric acid (PHHA) (Kiwada et al. 1980; Soni et al. 2005; Ye et al. 2006a, 2006b; Boberg et al. 2010). Urine is the most suitable matrix used for the diagnosis and the assessment of paraben exposure (Boberg et al. 2010). Thus, urinary levels of parent parabens can be considered as biomarkers of recent human monitoring (Ye et al. 2006a, 2006b; Calafat et al. 2010; Ma et al. 2013; Wang et al. 2013). Indeed, the total consumption of parabens from foods, cosmetics, and pharmaceuticals has been estimated to be 0.017, 0.833, and 0.417 mg/kg BW/day, respectively (Soni et al. 2005).

Several *in vitro* studies (Routledge et al. 1998; Nishihara et al. 2000) and *in vivo* studies (Oishi 2001; Lemini et al. 2003) have indicated the estrogenic activity of parabens and demonstrated many adverse effects of endocrine disruption (Ji et al. 2013), reproductive toxicity (Laing et al. 2016), and genotoxicity (Lee et al. 2013a, 2013b), leading to obesity, type 2 diabetes (Kuo et al. 2013), breast cancer (Deb et al. 2016), and other diseases (Zhou et al. 2017). Meeker et al. (2011) also found an association between urinary levels of parabens and sperm DNA damage. In that regard, the joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives (JECFA/WHO) in 1974 showed that the acceptable daily intake (ADI) of parabens for the sum of MP, EP, and PP is lower than 10 mg/kg/day (JECFA 1974).

Despite the health concerns about the possible toxic effects of parabens, particularly as endocrine disruptors (EDCs) in recent years, no data devoted to human exposure, distribution, and excretion of parabens in the Tunisian population are available.

The objective of this study is to evaluate the extent of the Tunisian population exposure to different parabens (ME, EP, PP). Urine samples were collected from subjects living in three separate regions in Tunisia (Mahdia, Sfax, and Gafsa) in order to elucidate geographic trends in exposure to parabens. Measurements were performed with a sensitive

and specific method using liquid chromatography–tandem mass spectrometry (LC-MS/MS).

## EXPERIMENTAL WORKS

### Chemicals

All parabens, methyl, ethyl, n-butyl, and benzylparabens and their internal standards were purchased from Sigma-Aldrich (Bornem, Belgium).  $\beta$ -Glucuronidase-sulfatase (*Helix pomatia*) was obtained from Sigma-Aldrich (Bornem, Belgium). Acetonitrile, methanol, and ammonia (HPLC-grade) were purchased from Merck (Darmstadt, Germany). Ultra-pure water was obtained using MilliQ quality (Millipore Corp., Bedford MA, USA). The stock solution of parabens (1 mg/mL) was prepared in MeOH and stored at  $-20^{\circ}\text{C}$ . Working solutions were prepared by diluting the appropriate amount of the stock solution in MeOH. The whole solutions were brought to an ambient temperature just before use.

### Study participants, sample collection, and storage

The study was conducted on a group of women and men aged between 5 and 90 years, randomly selected from three regions of Tunisia, who participated at University Hospital Mahdia Taher Sfar, Regional Hospital Gafsa Houcine Bouzeine, and University Hospital Sfax. In total, 200 samples of human urine were collected for this study. The urine collection of men was done in the following steps: (i) wash the hands thoroughly, (ii) retract completely the foreskin and clean the tip of the penis, (iii) remove the pot, the bottle and then the bottle lid, (iv) begin to urinate to fill half of the pot, then remove the bottle from the pot, and close the lid on the bottle when the bottle is full. Finally, write the name, date of birth, and date/time of collection on the urine label. The samples were collected in amber glass bottles and stored at  $-80^{\circ}\text{C}$ . All participants completed the questionnaire about age, weight, education, medical status, diet, and smoking habits. The research protocol was accepted by the ethics committee of Sfax, Mahdia, and Gafsa hospitals.

## Sample preparation

Urine samples (500  $\mu\text{L}$  each) were added into 2 mL glass vials, fortified with 100  $\mu\text{L}$  ammonium acetate (1 M) and vortex mixed for 10 s. Ten  $\mu\text{L}$  of enzyme solution (sulfatase and  $\beta$ -glucuronidase) was added, then the glass vials were incubated overnight at 37 °C. Thereafter, 25  $\mu\text{L}$  of the internal standard of parabens and 365  $\mu\text{L}$  of mobile phase were placed in the already prepared solution. All solutions were vortex agitated and centrifuged at 10,000 rpm for 5 min (10 °C). A 10  $\mu\text{L}$  aliquot was injected into the UPLC-MS/MS (Lee et al. 2013a, 2013b).

## Instrumental method

Paraben was analyzed by UPLC-MS/MS. The UPLC analysis was performed using an Acquity<sup>®</sup> sample and solvent manager from Waters (Milford, MA, USA). Chromatographic separation was achieved using an Acquity UPLC<sup>®</sup> BEH C18 column (2.1 mm i.d., 1.7  $\mu\text{m}$ ) from Waters. The autosampler and the column were kept at 8 and 40 °C, respectively. Mobile phases consisted of 0.1% ammonia in water and in acetonitrile. A gradient program was run to analyze the samples within 10 min. The column effluent was analyzed by a XEVO TQ MS<sup>®</sup> triple quad mass spectrometer (Waters) operating in the negative electrospray ionization (ESI-) MS/MS mode. The limit of detection (LOD) of MP, EP, and PP in urine was 0.5 ng/mL, which was calculated as 3  $S_0$ , where  $S_0$  was the standard deviation as the concentration approaches zero (Taylor 1987).

## Statistical analysis and estimated daily intake

The paraben concentrations were expressed as minimum or maximum and selected percentile values were calculated to describe the distribution of paraben levels. Statistical analysis of the results was performed using SPSS standard version 13.0 software. Urinary metabolite concentration was calculated in ng/mL. The estimated daily intake (EDI) of parabens was calculated using the equation described by Ma et al. (2013), as shown in Equation (1):

$$EDI = 50 \times Ci \times V / BW \quad (1)$$

where EDI ( $\mu\text{g}/\text{kg BW}/\text{day}$ ) is the estimated daily intake of individual parabens,  $Ci$  ( $\mu\text{g}/\text{L}$ ) is the measured urinary concentration of individual parent parabens,  $V$  (L/day) is the daily urine excretion rate (a value of 1.7 L was used in this study) (Perucca et al. 2007), and  $BW$  (kg) is body weight (62.7 kg for males and 54.8 kg for females were applied in this study) (Yang et al. 2005). Factor 50 was applied to account for metabolites (p-HB) whose proportions were 50 times higher than that of the parent paraben.

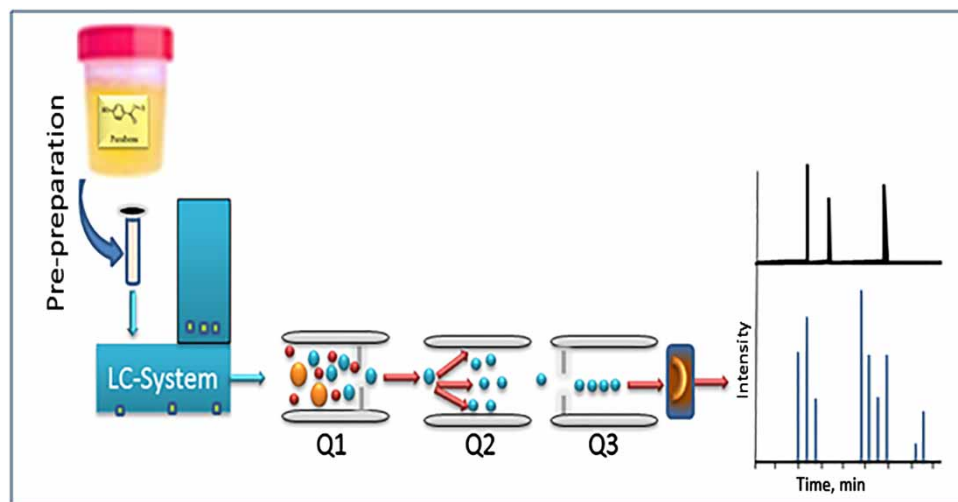
## RESULTS

Urine samples of both young and old Tunisian population were analyzed by using UPLC-MS/MS, as shown in Figure 1, in order to determine paraben rate.

The geographic characteristics of the Tunisian population are detailed in Table 1. Participants were aged between 5 years and 90 years. Focusing on the urinary paraben levels according to sex, age, and other geographic factors (evenly distributed), participant rate did not significantly differ.

MP, EP, and PP were detected in all samples ( $n = 95$ ). MP, EP, and PP were detected in 57%, 46%, and 40% of adults examined, respectively (Table 2). The geometric means and medians of urinary concentration in all samples were detected at concentrations 6.38 ng/mL with median 5.35 ng/mL, 2.78 ng/mL with median 2.54 ng/mL, and 1.88 ng/mL with median 1.59 ng/mL for PP, MP, and EP, respectively. The three parabens were detected in urine at concentrations ranging from 0.88 to 84.46 ng/mL for PP, from 0.52 to 29.2 ng/mL for MP, and from 0.51 to 28.17 ng/mL for EP.

In addition, MP, EP, and PP were detected in 40%, 35%, and 28% of women, respectively. The geometric mean levels and selected percentile (95%) concentrations of PP, MP, and EP found in the women's group ( $n = 67$ ) were respectively 5.82 ng/mL (47.87 ng/mL), 2.91 ng/mL (18.07 ng/mL), and 1.91 ng/mL (8.05 ng/mL). However, MP, EP, and PP were positively detected in 16%, 12%, and 12% of the men's group ( $n = 28$ ), respectively. In fact, the geometric mean levels and selected percentile (95%) concentrations of parabens in men were 7.89 ng/mL (65.08 ng/mL), 2.74 ng/mL (12.13 ng/mL), and 1.56 ng/mL (5.86 ng/mL).



**Figure 1** | The preparation step and analysis of parabens using UPLC-MS/MS.

**Table 1** | Geographic characteristics of the Tunisian population

Variable	Subgroup	N
Sex	All	95
	Female	67
	Male	28
Age	Children	1
	Adolescents	2
	Adults	
	20–30	18
	30–40	19
	40–50	21
	50–60	13
	60–70	14
	Old	
70–80	6	
Education level	<High school	10
	High school	40
	>High school	45
Smoking status	Precedent	19
	Never	76
Region residence	Mahdia	24
	Gafsa	25
	Sfax	46

of PP, MP, and EP, respectively, as shown in Table 3. In the same way, the level of parabens in the women's urinary concentrations was significantly higher than in men (Figure 2).

The average concentration, geometric means, and 95th percentile of urinary parabens based on the regions of Tunisia are illustrated in Table 4. EP, MP, and PP levels were

lower in the Mahdia women's group compared to the Sfax and Gafsa women's groups. The highest geometric means of parabens were found in women from Gafsa region with 6.50 ng/mL, 4.05 ng/mL, and 1.98 ng/mL for PP, MP, and EP, respectively. The 95th percentile in this study showed that concentrations of PP in women from the Gafsa region was 68.40 ng/mL, 23.61 ng/mL of MP, and 8.85 ng/mL of EP.

We noticed a significant difference in MP between the Sfax women's group and that of Mahdia (Figure 2(b)). The human urinary concentrations of PP, MP, and EP are presented in Figure 3(a)–3(c), respectively. EDCs were revealed at high concentrations in the urine collected from all study regions (Sfax, Mahdia, and Gafsa cities).

## DISCUSSION

In our study, PP, EP, and MP were the important parabens detected in the urine samples from the Tunisian population in comparison with other studies carried out in Korea and the USA (Kang *et al.* 2016). PP was the highest paraben then MP and EP. The reason for this high paraben concentration in human urine is due to the greater use of products containing personal care products (Soni *et al.* 2005). According to the results, they found that personal care products are the major source of parabens. The detection limits of MP,

**Table 2** | Concentration (ng/mL) of parabens in urine samples ( $n = 95$ ) from Tunisian adults

Compounds	GM	5th	25th	Median	75th	95th	Average	Range	DR (%)
All ( $n = 95$ )									
PP	6.38	1.36	2.72	5.35	13.62	54.44	13.85	0.88–84.46	40
EP	1.88	0.64	0.93	1.59	3.85	8.31	3.04	0.51–28.17	46
MP	2.78	0.58	1.46	2.54	4.68	18.03	4.75	0.52–29.2	57

PP, propylparaben; EP, ethylparaben; MP, methylparaben; GM, geometric mean; DR, detection rate (%); percentile (5th, 25th, 75th and 95th); range (min–max).

**Table 3** | Urinary concentration (ng/mL) of parabens (geometric means and 95th percentile) in the Tunisian population according to sex

Compounds	GM	5th	25th	Median	75th	95th	Average	Range	DR (%)
Men ( $n = 28$ )									
PP	7.89	1.64	2.02	9.61	17.39	65.08	18.35	1.62–84.1	12
EP	1.56	0.84	0.97	1.17	2.05	5.86	2.15	0.84–8.67	12
MP	2.74	1.07	1.43	2.02	5.09	12.13	4.32	0.58–18.09	16
Women ( $n = 67$ )									
PP	5.82	1.36	2.98	4.97	8.71	47.87	11.92	0.88–84.46	28
EP	1.91	0.52	0.87	1.82	3.94	8.05	3.27	0.35–28.17	35
MP	2.91	0.67	1.68	2.60	4.49	18.07	5.02	0.52–29.2	40

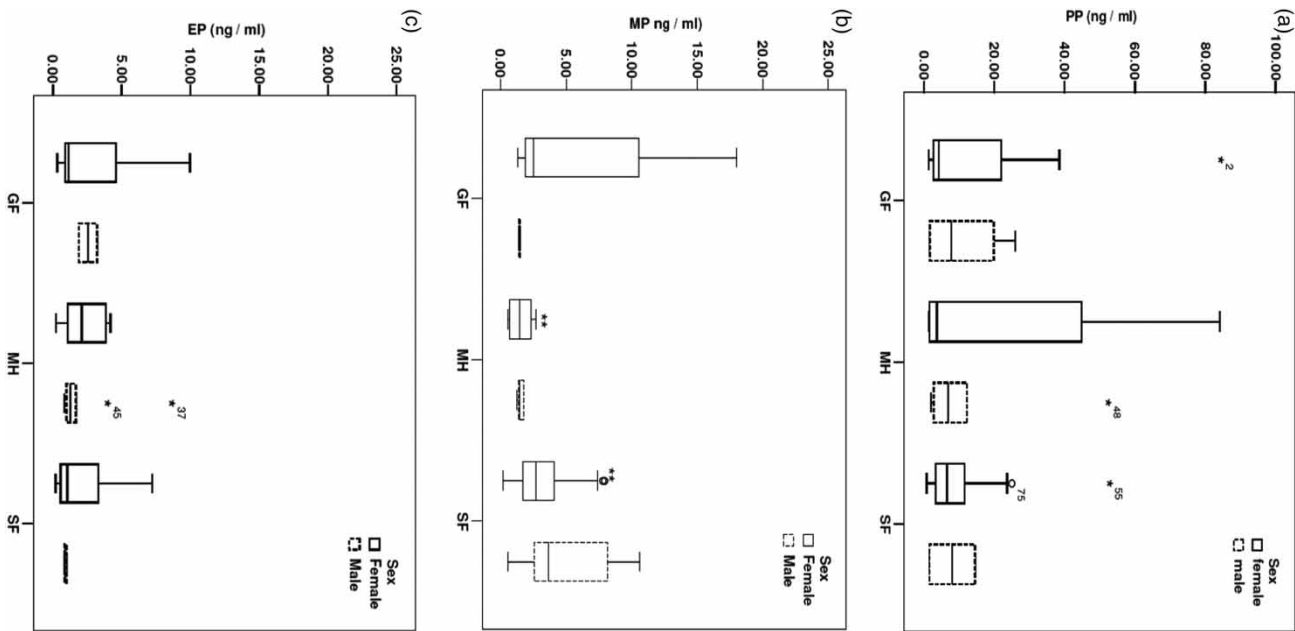
PP, propylparaben; EP, ethylparaben; MP, methylparaben; GM, geometric mean; DR, detection rate (%); percentile (5th, 25th, 75th, and 95th); range (min–max).

EP, and PP found in urine samples ranged from 0.5 ng/mL and the limit of quantification was 0.5  $\mu$ g/mL.

The high concentrations of PP and MP found in these results were indicated as being present in human urine samples from China, Denmark, Spain, and the USA (Ye et al. 2006a, 2006b; Casas et al. 2011; Frederiksen et al. 2011; Ma et al. 2013). However, the median concentration of parabens in urine samples from Tunisia vacillated compared with other countries, for example, the USA with 191 ng/mL for MP (Calafat et al. 2010) and China (Ma et al. 2013) with 6.50 ng/mL. High PP, MP, and EP concentrations were measured in all samples from the different regions of Tunisia for female and male subjects. Table 5 compares urinary paraben concentrations of this study with those reported in other studies (Ye et al. 2006a, 2006b; Shirai et al. 2013); we found that the geometric means and average urinary concentrations were lower than those in the previous studies. According to the USA, the urinary paraben concentrations detected in all 100 sample subjects (women and men) ranged between 43.9 ng/mL for MP and 9.1 ng/mL for PP (Ye et al. 2006a,

2006b) which are higher than those found in our study. The remarkable difference in urinary concentrations of parabens between Tunisia and other developed countries might hinge on the lower consumption rate of cosmetics and personal care products in Tunisia.

Indeed, the results of increased concentrations of MP and EP found in the women's groups were in agreement with a previous study (Moos et al. 2014). The United States National Health and Nutrition Examination Survey (NHANES) reported comparable gender-specific differences as in this study. In the NHANES study, women had three-fold higher adjusted geometric mean levels of MP and seven-times higher adjusted geometric mean levels of PP compared to men (Calafat et al. 2010). A significant difference in urinary concentration of the three parabens between males and females was also observed in several other studies (Smith et al. 2012; Engel et al. 2014; Kang et al. 2016). MeP and n-PrP are frequently used parabens and are often used in combination in personal care products (Soni et al. 2005; Guo & Kannan 2013). The major reason for the detection of these compound parabens MP, E, P and PP is that they



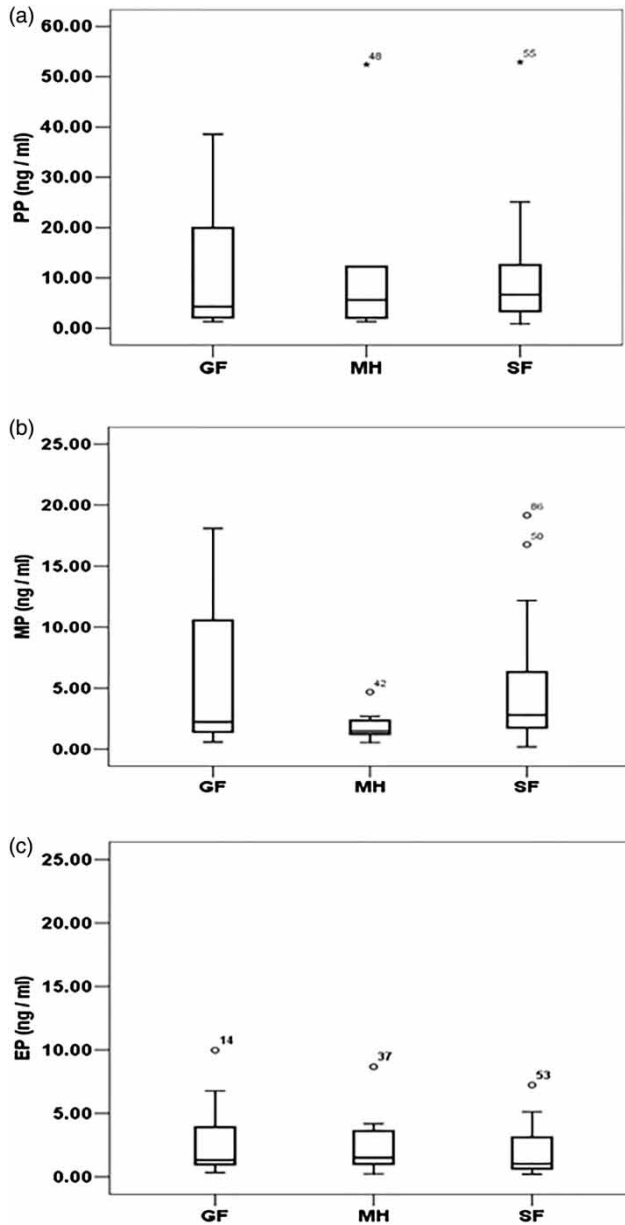
**Figure 2** | Boxplots of the urinary PP (a), MP (b), and EP (c) concentrations of the human monitoring study classified by sex.

are used as personal care products, especially cosmetic products (Andersen 2008). The second major reason is that they are used in food packaging. Both oral and dermal administrations are most likely to lead to hydrolysis of parabens (Soni *et al.* 2005; Ye *et al.* 2006a, 2006b). Thus, the levels of parabens we measured in human urine presumably reflect

**Table 4** | Concentration (ng/mL) of parabens in urine samples ( $n = 95$ ) of three regions of Tunisia

Compounds	Mahdia region						Sfax region						Gafsa region					
	Women ( $n = 11$ )			Men ( $n = 13$ )			Women ( $n = 38$ )			Men ( $n = 8$ )			Women ( $n = 18$ )			Men ( $n = 7$ )		
	PP	EP	MP	PP	EP	MP	PP	EP	MP	PP	EP	MP	PP	EP	MP	PP	EP	MP
GM	2.39	2.22	1.28	11.40	1.62	1.82	6.47	1.77	2.97	4.92	0.92	3.67	6.50	1.98	4.05	5.76	2.46	1.99
95th	5.23	4.16	2.63	76.17	6.79	4.09	30.61	8.27	16.07	13.86	0.99	10.43	68.40	8.85	23.61	24.21	3.15	14.77
Average	2.92	2.61	1.55	26.77	2.30	2.10	10.65	3.50	4.62	8.09	0.92	5.17	17.98	3.28	7.48	10.85	2.55	4.60

PP, propylparaben; EP, ethylparaben; MP, methylparaben; GM, geometric mean; percentile (95th).



**Figure 3** | Boxplots of the urinary PP (a), MP (b), and EP (c) concentrations of the human monitoring study of three regions from Tunisia (Gafsa, Mahdia, and Sfax).

only a fraction of human paraben exposure. The fraction of the parent compound of paraben excretion in urine may depend on the route of exposure. Uptake of parabens through the skin may result in more parent compound avoiding degradation in the liver before urinary excretion compared with oral uptake. Considering that parabens are rapidly metabolized into non-specific metabolites, the higher urinary excretion of parent parabens seen in some

**Table 5** | EDI urine ( $\mu\text{g}/\text{kg}$  bw/day) calculated from urinary concentration of parabens for Tunisian adults

Sex	Compounds	MP	PP	EP
Male Tunisian adults	GM	3.71	10.69	2.11
	5th percentile	1.45	2.22	1.13
	Median	0.02	13.02	1.58
	95th percentile	16.67	88.22	7.94
Female Tunisian adults	GM	4.51	9.02	2.96
	5th percentile	1.03	2.10	0.80
	Median	4.03	7.70	2.82
	95th Percentile	28.02	74.25	12.48
Chinese male adults	GM	6.69	2.50	3.63
	5th percentile	0.73	0.35	0.35
	Median	5.20	1.83	2.96
	95th percentile	154	84.2	125
Chinese female adults	GM	15.9	3.06	8.94
	5th percentile	2.60	0.26	0.65
	Median	15.5	2.50	10.7
	95th percentile	242	74.9	125

PP, propylparaben; EP, ethylparaben; MP, methylparaben; GM, geometric mean; percentile (95th).

urine specimens could also be explained by a less continuous exposure to parabens.

In this study, the total concentration of parabens is higher in adults than in children. CDC (2015) showed the opposite relationship in the USA, where adults had a higher concentration of parabens than children, suggesting that the observed age trend in the US population is most likely due to child-specific exposure sources such as baby products (wipes, lotions, etc.). This may be the result of behavioral or physiological differences between children and adults, such as chewing behaviors (Tulve et al. 2002; Xue et al. 2007) or a larger surface area-to-body weight ratio that may increase the potential for exposure via dermal absorption (WHO 2011).

High urinary concentrations of parabens in Tunisian women have been found in the city of Gafsa, and weak concentrations were detected in Mahdia city; a significant difference was observed in particular for MP. The reason for this high concentration in women from Gafsa was greater use of cosmetic products, especially solar creams and lotions.

The EDI values of parabens measured from the urinary concentrations in Tunisian adults are shown in Table 5. The EDI for MP, PP, and EP in males was 3.71, 10.69, and 2.11  $\mu\text{g}/\text{kg}$  BW/day, respectively. Compared to Tunisian women, for MP, PP, and EP, identical EDI values were

found in male Tunisians (4.51, 9.02, 2.96  $\mu\text{g}/\text{kg}$  BW/day and 3.71, 10.69, 2.11  $\mu\text{g}/\text{kg}$  BW/day, respectively). The EDI values of parabens for young Tunisian adults were lower than the ADI (10 mg/kg/day).

## CONCLUSION

In this study, we have determined the levels of parabens in the urine of young and elderly Tunisians. Studies on human exposure to parabens in Third World countries are still rare. The present study revealed that most Tunisians had detectable levels of parabens in their urine, thus indicating ubiquitous exposure to these compounds in Tunisia. Furthermore, 200 urine samples were collected from various geographic regions. The results showed that the urinary concentration of parabens MP, PP, and EP differed according to age, sex, and demographic factors in the Tunisian population. Dietary and dermal exposure data for parabens in the Tunisian population are still limited. The presence of parent compounds in the urine indicates that paraben uptake results in the exposure of human tissues to the potentially harmful parent compounds. In this way, further research is necessary to assess whether the paraben concentrations to which the Tunisian population is exposed are high enough to cause a threat to human health.

## REFERENCES

- Andersen, A. 2008 Final amended report on the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzylparaben as used in cosmetic products. *Int. J. Toxicol.* **27**, 1–82.
- Boberg, J., Taxvig, C., Christiansen, S. & Hass, U. 2010 Possible endocrine disrupting effects of parabens and their metabolites. *Reprod. Toxicol.* **30**, 301–312.
- Calafat, A. M., Ye, X., Wong, L. Y., Bishop, A. M. & Needham, L. L. 2010 Urinary concentrations of four parabens in the U.S. population: NHANES 2005–2006. *Environ. Health Perspect.* **118**, 679–685.
- Camino-Sánchez, F., Zafra-Gómez, A., Dorival-García, N., Juárez-Jiménez, B. & Vílchez, J. L. 2016 Determination of selected parabens, benzophenones, triclosan and triclocarban in agricultural soils after and before treatment with compost from sewage sludge. A lixiviation study. *Talanta* **150**, 415–424.
- Casas, L., Fernández, M. F., Llop, S., Guxens, M., Ballester, F., Olea, N., Irurzun, M. B., Rodríguez, L. S., Riaño, I., Tardón, A., Vrijheid, M., Calafat, A. M. & Sunyer, J. 2011 Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. *Environ. Int.* **37**, 858–866.
- CDC (United States Centers for Disease Control) 2015 *Fourth National Report on Human Exposure to Environmental Chemicals*. U.S. Department of Health and Human Services, Atlanta, GA, USA.
- Deb, P., Bhan, A., Hussain, I., Ansari, K. I., Bobzean, S. A., Pandita, T. K., Perrotti, L. I. & Mandal, S. S. 2016 Endocrine disrupting chemical, bisphenol-A, induces breast cancer associated gene HOXB9 expression in vitro and in vivo. *Gene* **590**, 234–243.
- Elder, R. L. 1984 Final report on the safety assessment of methylparaben, ethyl paraben, propyl paraben, and butylparaben. *J. Am. Coll. Toxicol.* **3**, 147–209.
- Engel, L. S., Buckley, J. P., Yang, G., Liao, L. M., Satagopan, J., Calafat, A. M., Matthews, C. E., Cai, Q., Ji, B. T., Cai, H., Engel, S. M., Wolff, M. S., Rothman, N., Zheng, W., Xiang, Y. B., Shu, X. O., Gao, Y. T. & Chow, W. H. 2014 Predictors and variability of repeat measurements of urinary phenols and parabens in a cohort of shanghai women and men. *Environ. Health Perspect.* **122**, 733–740.
- Frederiksen, H., Jørgensen, N. & Andersson, A. M. 2011 Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). *J. Expo. Sci. Environ. Epidemiol.* **21**, 262–271.
- Guo, Y. & Kannan, K. 2013 A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure. *Environ. Sci. Technol.* **47**, 14442–14449.
- JECFA (Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives) 1974 *17th Report of the Joint FAO/WHO Expert Committee on Food Additives*. World Health Organization Technical Report Series 539.
- Ji, K., Hong, S., Kho, Y. & Choi, K. 2013 Effects of bisphenols exposure on endocrine functions and reproduction of zebrafish. *Environ. Sci. Technol.* **47**, 8793–8800.
- Kang, H. S., Kyung, M. S., Ko, A., Park, J. H., Hwang, M. S., Kwon, J. E., Suh, J. H., Lee, H. S., Moon, G. I. & Hong, J. H. 2016 Urinary concentrations of parabens and their association with demographic factors: a population-based cross-sectional study. *Environ. Res.* **146**, 245–251.
- Kiwada, H., Awazu, S. & Hanano, M. 1980 The study on the biological fate of paraben at the dose of practical usage in rat. II. The pharmacokinetic study on the blood concentration after the administration of ethyl paraben or p-hydroxybenzoic acid. *J. Pharmacobio-Dynam.* **3**, 353–363.
- Kuo, C. C., Moon, K., Thayer, K. A. & Navas-Acien, A. 2013 Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. *Curr. Diab. Rep.* **13**, 831–849.



- Laing, L. V., Viana, J., Dempster, E. L., Trznadel, M., Trunkfield, L. A., Uren Webster, T. M., Van Aerle, R., Paull, G. C., Wilson, R. J., Mill, J. & Santos, E. M. 2016 Bisphenol A causes reproductive toxicity, decreases dnmt1 transcription, and reduces global DNA methylation in breeding zebrafish (*Danio rerio*). *Epigenetics* **11**, 526–538.
- Lee, S. Y., Liu, X., Takeda, S. & Choi, K. 2013a Genotoxic potentials and related mechanisms of bisphenol A and other bisphenol compounds: a comparison study employing chicken DT40 cells. *Chemosphere* **93**, 434–440.
- Lee, S. Y., Son, E. J., Kang, J. Y., Lee, H. S., Shin, M. K., Nam, H. S., Kim, S. Y., Jang, Y. M. & Rhee, G. S. 2013b Development of a quantitative analytical method for determining the concentration of human urinary parabens by LC-MS/MS. *Bull. Korean Chem. Soc.* **4**, 1131.
- Lemini, C., Jaimez, R., Avila, M. E., Franco, Y., Larrea, F. & Lemus, A. E. 2003 In vivo and in vitro estrogen bioactivities of alkyl parabens. *Toxicol. Ind. Health* **19**, 69–79.
- Ma, W. L., Wang, L., Guo, Y., Liu, L. Y., Qi, H., Zhu, N. Z., Gao, C. J., Li, Y. F. & Kannan, K. 2013 Urinary concentrations of parabens in Chinese young adults: implications for human exposure. *Arch. Environ. Contam. Toxicol.* **65**, 611–618.
- Meeker, J. D., Yang, T., Ye, X., Calafat, A. M. & Hauser, R. 2011 Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. *Environ. Health Perspect.* **119**, 252–257.
- Moos, R. K., Angerer, J., Wittsiepe, J., Wilhelm, M., Brüning, T. & Koch, H. M. 2014 Rapid determination of nine parabens and seven other environmental phenols in urine samples of German children and adults. *Int. J. Hyg. Environ. Health* **217**, 845–853.
- Nishihara, T., Nishikawa, J., Kanayama, T., Dakeyama, F., Saito, K., Imagawa, M., Takatori, S., Kitagawa, Y., Hori, S. & Utsumi, H. 2000 Estrogenic activities of 517 chemicals by yeast two-hybrid assay. *J. Health Sci.* **46**, 282–298.
- Oishi, S. 2001 Effects of butylparaben on the male reproductive system in rats. *Toxicol. Ind. Health* **17**, 31–39.
- Perucca, J., Bouby, N., Valeix, P. & Bankir, L. 2007 Sex difference in urine concentration across differing ages, sodium intake, and level of kidney disease. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R700–R705.
- Routledge, E. J., Parker, J., Odum, J., Ashby, J. & Sumpter, J. P. 1998 Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol. Appl. Pharmacol.* **153**, 12–19.
- Shirai, S., Suzuki, Y., Yoshinaga, J., Shiraiishi, H. & Mizumoto, Y. 2013 Urinary excretion of parabens in pregnant Japanese women. *Reprod. Toxicol.* **35**, 96–101.
- Smith, K. W., Braun, J. M., Williams, P. L., Ehrlich, S., Correia, K. F., Calafat, A. M., Ye, X., Ford, J., Keller, M., Meeker, J. D. & Hauser, R. 2012 Predictors and variability of urinary paraben concentrations in men and women, including before and during pregnancy. *Environ. Health Perspect.* **120**, 1538–1543.
- Soni, M., Carabin, I. & Burdock, G. 2005 Safety assessment of esters of phydroxybenzoic acid (parabens). *Food Chem. Toxicol.* **43**, 985–1015.
- Taylor, J. K. 1987 *Quality Assurance of Chemical Measurements*. Lewis Publishers, Chelsea, MI, USA.
- Tillett, T. 2012 Here today, here tomorrow? Urinary concentrations of parabens over time. *Environ. Health Perspect.* **120**, a437.
- Tohidi, F. & Cai, Z. W. 2015 GC/MS analysis of triclosan and its degradation by-products in wastewater and sludge samples from different treatments. *Environ. Sci. Pollut. Res.* **22**, 11387–11400.
- Tulve, N. S., Suggs, J. C., McCurdy, T. R., Cohen Hubal, E. A. & Moya, J. 2002 Frequency of mouthing behaviour in young children. *J. Expo. Anal. Environ. Epidemiol.* **12**, 259–264.
- Wang, L., Wu, Y., Zhang, W. & Kannan, K. 2013 Characteristic profiles of urinary p-hydroxybenzoic acid and its esters (parabens) in children and adults from the U.S. and China. *Environ. Sci. Technol.* **47**, 2069–2076.
- WHO (World Health Organization) 2011 *Summary of Principles for Evaluating Health Risks in Children Associated with Exposure to Chemicals in Children's Environmental Health*. World Health Organization, Geneva, Switzerland, pp. 1–58.
- Xue, J., Zartarian, V. G., Moya, J., Freeman, N., Beamer, P. & Black, K. 2007 A meta-analysis of children's hand-to-mouth frequency data for estimating nondietary ingestion exposure. *Risk Anal.* **27**, 411–420.
- Yang, X., Li, Y., Ma, G., Hu, X., Wang, J. & Cui, Z. 2005 Study on weight and height of the Chinese people and the differences between 1992 and 2002. *Chin. J. Epidemiol.* **26**, 489–493.
- Ye, X., Bishop, A. M., Reidy, J. A., Needham, L. L. & Calafat, A. M. 2006a Parabens as urinary biomarkers of exposure in humans. *Environ. Health Perspect.* **114**, 1843–1846.
- Ye, X., Kuklennyik, Z., Bishop, A. M., Needham, L. & Calafat, A. M. 2006b Quantification of the urinary concentrations of parabens in humans by on-line solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry. *J. Chromatogr. B* **844**, 53–59.
- Zhou, A., Chang, H., Huo, W., Zhang, B., Hu, J., Xia, W., Chen, Z., Xiong, C., Zhang, Y., Wang, Y., Xu, S. & Li, Y. 2017 Prenatal exposure to bisphenol A and risk of allergic diseases in early life. *Int. Pediatr. Res. Found.* **81**, 851–856.

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