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CASE STUDY

Assessment of urinary paraben levels and associated health risks in adults: a preliminary study on methyl and propyl paraben exposure

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ABSTRACT
Parabens, often used as preservatives in consumer products, have raised concerns due to their endocrine-disrupting properties. The aim of this study was to quantify the levels of methyl and propyl paraben in adult urine samples and to assess potential health risks. Using high-performance liquid chromatography (HPLC), methyl and propyl parabens were detected in 20 participants at different concentrations. Methylparaben was more prevalent than propylparaben. Risk assessment was performed by calculating the estimated daily intake (EDI) and the hazard quotient (HQ), with HQ values indicating no significant health risk for the participants. Although current exposure levels appear to be safe, the long-term effects of chronic exposure remain uncertain, highlighting the need for further research. This preliminary study provides insight into paraben exposure in adults and contributes to the growing literature on the safety and prevalence of parabens.

KEY WORDS: paraben exposure; endocrine disruptors; urinary biomarkers; health risk assessment

Introduction

Parabens, a group of aliphatic esters derived from p-hydroxybenzoic acid, are widely used as preservatives in various consumer products such as cosmetics, pharmaceuticals and foods. Since the 1920s, parabens have been known for their antimicrobial properties, which enable them to prevent the growth of bacteria, yeast and mold, thus extending the shelf life of products. The most commonly used parabens in commercial applications are methyl-, ethyl-, propyl- and butylparaben, while others, such as isobutyl- and benzylparaben, are only used to a limited extent ("Final Amended Report on the Safety Assessment of Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben as Used in Cosmetic Products," 2008; Petric *et al.*, 2020).

Despite their widespread use, concern has grown about the safety of parabens due to their potential estrogenic and anti-androgenic effects. As endocrine disruptors (EDs), parabens can mimic or disrupt the body's hormone balance, which can lead to health problems such as breast cancer, reproductive disorders and developmental abnormalities (Oishi, 2002; Charles & Darbre, 2013; Sanchis *et al.*, 2020; Hager *et al.*, 2022). Their ability to interact with estrogen receptors, albeit in a much weaker form than endogenous estrogen, has made them a focus of toxicological studies (Darbre & Harvey, 2008).

Human exposure to parabens occurs primarily through the application of cosmetics to the skin, ingestion of food and pharmaceuticals, and environmental contamination from sources such as sewage (Liao *et al.*, 2013; Guo & Kannan, 2013). Parabens are frequently detected in human biological samples such as blood, urine and even breast tissue (Ye *et al.*, 2006; Barr *et al.*, 2012). In particular, paraben levels in urine have been shown to be a reliable biomarker for the assessment of human exposure. Studies have shown that parabens are rapidly absorbed, metabolized and excreted, predominantly in conjugated form in urine, making it possible to

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assess exposure and associated risks through biomonitoring (Hager *et al.,* 2022).

The metabolism of parabens involves enzymatic hydrolysis by esterases found in the skin, intestine and liver. These processes convert parabens into p-hydroxybenzoic acid derivatives, which undergo further conjugation reactions such as glucuronidation and sulfation before excretion. However, factors such as the rate of absorption through the skin, formulation characteristics and individual enzyme activity can influence the extent of systemic exposure and potential bioaccumulation. For example, methylparaben has been detected in unmetabolized forms in human body fluids due to the lower activity of skin esterases compared to esterases in the gut or liver, indicating possible differences in the exposure and risk profiles of individuals (Petric *et al.*, 2020; Hager *et al.*, 2022).

The regulation and safety assessment of parabens has evolved over time. For example, European Union regulations allow the use of certain parabens in cosmetics in controlled concentrations, with stricter guidelines for longer-chain parabens such as propyl and butyl parabens due to potential reproductive toxicity concerns (*Uredba - 1223/2009 - EN - EUR-Lex*, n.d.). The Scientific Committee on Consumer Safety (SCCS) has provided assessments on the safety of methylparaben and its permitted concentration limits in cosmetic formulations, further emphasizing the ongoing review of its safety profile (*SCCS - Final Opinion on Methylparaben - European Commission*, n.d.).

Given the complex interplay of exposure pathways and health concerns, research into the toxicological effects of parabens remains essential. This study focuses on the quantification of methyl and propyl parabens in urine samples to assess the level of human exposure and associated health risks. By using high performance liquid chromatography (HPLC) for the detection and quantification of these compounds, this research aims to provide a comprehensive understanding of the extent of human exposure to parabens and their potential toxicological effects.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of the Faculty of Pharmacy, University of Sarajevo (Approval number: 0101-1214/24). Written informed consent was obtained from each participant prior to study enrollment.

Chemicals and reagents

The creatinine standard was purchased from Thermo Scientific, China. Hydrochloric acid (35–38%) was sourced from Avantor Performance Materials, Poland, and sodium hydroxide from Merck KGaA, Darmstadt, Germany. Picric acid and methyl 4-hydroxybenzoate (analytical grade) were obtained from Sigma-Aldrich, USA. Propyl 4-hydroxybenzoate (analytical grade) was provided by Fagron d.o.o., Zagreb. Methanol was purchased from Merck KGaA, Germany, while ethyl acetate (analytical grade) was sourced from Sigma-Aldrich, USA.

Participant selection

The study included a total of 20 adult participants who were selected on the basis of specific inclusion and exclusion criteria. The primary inclusion criteria were age over 18 years, generally good health with no chronic medical conditions and no long-term use of medications that could potentially affect urinary biomarker levels. All participants were informed about the aims, procedures and potential risks of the study and gave written informed consent prior to participation. Exclusion criteria included chronic conditions such as diabetes, hypertension, kidney disease or other conditions known to affect urinary biomarkers. Participants were also excluded if they had a recent infection, were pregnant or breastfeeding, had recently undergone surgery, or were taking prescription medications, supplements or recreational drugs that could affect urine biomarker levels. In addition, subjects who were unable or unwilling to comply with study procedures were not eligible to participate. Participants were asked to provide a urine sample on the first morning to minimise variability in biomarker concentrations due to diurnal changes in urine excretion. Attention was paid to a balanced representation of male and female participants to investigate possible gender differences in paraben concentrations and exposure. Demographic and anthropometric data, including age, weight, height and body mass index (BMI), were collected to allow detailed analyses and comparisons.

Sample collection

Urine samples (approximately 50 ml from the first morning void) were collected from the study participants, stored in polypropylene (PP) containers and transported to the laboratory, where they were frozen at –20 °C until further analysis.

Creatinine analysis

The concentration of creatinine in urine samples was determined using the Jaffe reaction (Toora & Rajagopal 2002), which involves a colorimetric reaction between creatinine and picric acid in an alkaline medium. Urine samples were diluted, reacted with picric acid and sodium hydroxide, and the absorbance of the resultant complex was measured at 530 nm using a UV mini-1240 spectrophotometer (Shimadzu Corporation, Japan). A calibration curve prepared from serial dilutions of a creatinine standard was used for quantification.

Paraben analysis

Methyl and propyl parabens were extracted from urine samples by liquid-liquid extraction after acid hydrolysis of their conjugated forms (Lu *et al.*, 2015). The urine samples were first thawed, brought to room temperature and mixed thoroughly. A 0.5 mL portion of each sample was mixed with 125 µL of 12 mol/L HCl, then mixed

and hydrolyzed at 90 °C for one hour. After hydrolysis, the samples were extracted three times with 3 mL ethyl acetate and the combined organic layers were dried under a stream of nitrogen before reconstitution in 0.5 mL methanol (Honda *et al.,* 2018). Quantification was performed by HPLC using a diode array detector (1260 Infinity II, Agilent Technologies, USA) and a C18 column according to a previously developed method (Imamović *et al.*, 2012) which was slightly modified and revalidated for the purposes of this study. Key parameters such as linearity, limit of detection (LoD), and limit of quantification (LoQ) were evaluated. The coefficients of determination (R^2) for the calibration curves were 0.9997 for methylparaben and 0.9993 for propylparaben indicating good linearity. The mobile phase consisted of methanol and water (60:40) under isocratic conditions, with a flow rate of 0.5 mL/min at 42°C. Detection was performed at 254 nm, and the injection volume of the sample was 10 µL. All solutions were filtered through a 0.2 µm membrane filter (Regenerated Cellulose 47 mm, pore size 0.2 µm, Agilent Technologies, Inc., Santa Clara, USA). The LoD and LoQ of the applied method were 0.36 and 1.21 µg/L for methylparaben and 0.021 and 0.07 µg/L for propylparaben, respectively.

Risk assessment

The toxicological risk assessment of paraben exposure was conducted by estimating the daily intake of parabens from their concentrations in urine using established mathematical models.

The estimated daily intake (EDI) of parabens was calculated using the formula:

$$EDI = (C \times R)/(BW \times F) \quad (1)$$

where, EDI is the estimated daily intake (µg/kg body weight/day), C is the concentration of parabens in urine (µg/L), R is the daily urine output volume, assumed to be 1.7 L/day (as per Honda *et al.*, 2018), BW is the body weight of the individual in kilograms and F is the urinary excretion factor (17.4 for methyl paraben and 10.2 for propylparaben) (Moos *et al.,* 2017).

The hazard quotient (HQ) was calculated to compare the EDI against the tolerable daily intake (TDI) values. An HQ greater than 1 indicates a potential health risk, necessitating further evaluation and potential management measures.The hazard quotient is determined by dividing the EDI by the TDI:

$$HQ = EDI/TDI \quad (2)$$

A HQ value less than 1 indicates that the exposure level is below the threshold of concern for potential health risks, while an HQ value greater than 1 suggests a potential health risk.

On the other hand, the Margin of Exposure (MOE) is a concept also used in toxicological risk assessment to determine the relative safety of exposure to a given substance or toxic factor. The basic idea of MOE is to compare the amount of a substance to which humans are exposed with a dose that is considered harmful. A higher MOE indicates a greater difference between exposure and harmful dose, which is considered safer. A lower MOE suggests that exposure is closer to or even exceeds the harmful dose, which may increase the risk of adverse effects (Sanchis *et al.*, 2020).

The MOE is calculated as follows:

$$MOE = NOAEL/EDI \quad (3)$$

No observed adverse effect level (NOAEL) is the maximum dose at which no adverse effects are observed. For methylparaben, this value is 1000 mg/kg/day, and for propylparaben, it is 2 mg/kg/day (Petric *et al.,* 2020). These reference values provide a basis for assessing the potential health risk associated with paraben exposure, using urinary concentrations as biomarkers of internal exposure.

Results

Participant characteristics

A total of 20 participants were included in the study, including both males and females. The demographic and anthropometric characteristics of the participants are summarized below (Table 1).

This balanced representation of the genders made it possible to investigate possible gender-specific differences in paraben concentrations.

Concentration of creatinine in urine samples

The creatinine concentration in the first-morning urine samples ranged from 291.10 mg/L to 2,880.65 mg/L across the 20 participants, with an average of 1,109.56 mg/L. This normalization of paraben concentrations to creatinine was used to correct for variations in urine dilution.

Paraben concentration in urine samples

Methyl and propyl parabens were detected in varying concentrations, with methylparaben being more prevalent (Table 2).

The presence of the two parabens analyzed was detected in all urine samples (n=20, 100%). Methylparaben could not be quantified (<LoQ) in four urine samples (samples 6, 8, 10 and 17), while propylparaben could only be quantified in four samples (samples 9, 11, 16 and 19) using the analytical method applied. The concentrations of methylparaben in the urine samples in which it was quantified were higher than the concentrations of propylparaben and ranged from 1.21 to 25.71 µg/L urine and 1.33 to 12.84 µg/g creatinine, respectively. The concentrations of propylparaben were lower and ranged from 0.07 to 1.44 µg/L urine and 0.07 to 1.29 µg/g creatinine, respectively.

Table 1. Participant demographic and anthropometric data.

Characteristic	Value
Age Range (years)	22–27
Gender Distribution	55% Female, 45% Male
Average Weight (kg)	57.5±6.04 (Female), 97.3±19.89 (Male)
Average Height (cm)	163.2±5.02 (Female), 186.4±6.60 (Male)

Table 2. Concentration of methyl and propyl parabens in urine samples.

Participant ID	Methylparaben (µg/L)	Methylparaben (µg/g creatinine)	Propylparaben (µg/L)	Propylparaben (µg/g creatinine)
1	25.71 ± 0.15	12.84	<LoQ	< LoQ
2	5.13 ± 2.27	6.15	<LoQ	< LoQ
3	1.91 ± 0.44	3.59	<LoQ	< LoQ
4	6.12 ± 0.05	2.77	<LoQ	< LoQ
5	1.21 ± 0.03	1.33	<LoQ	< LoQ
6	<LoQ*	<LoQ	<LoQ	< LoQ
7	3.13 ± 0.15	2.04	<LoQ	< LoQ
8	<LoQ	<LoQ	<LoQ	< LoQ
9	2.81 ± 0.57	2.92	0.07 ± 0.08	0.07
10	<LoQ	<LoQ	<LoQ	< LoQ
11	5.55 ± 0.02	3.40	0.43 ± 0.19	0.26
12	7.56 ± 3.76	2.62	<LoQ	< LoQ
13	4.18 ± 1.34	1.86	<LoQ	< LoQ
14	5.52 ± 0.39	3.78	<LoQ	< LoQ
15	4.89 ± 0.97	12.07	<LoQ	< LoQ
16	2.73 ± 0.04	5.25	0.42 ± 0.15	0.81
17	<LoQ	<LoQ	<LoQ	< LoQ
18	6.62 ± 0.11	5.27	<LoQ	< LoQ
19	6.73 ± 2.69	6.06	1.44 ± 0.65	1.29
20	5.70 ± 3.37	3.12	<LoQ	< LoQ

*< LoQ indicates values below the limit of quantification.

Table 3. Risk assessment results for paraben exposure.

Participant ID	EDI Methylparaben (µg/kg/day)	EDI Propylparaben (µg/kg/day)	MOE Methylparaben	MOE Propylparaben	HQ Methylparaben	HQ Propylparaben
1	0.04186	N/A	23,889.17	N/A**	0.004186	N/A
2	0.00836	N/A	119,618.1	N/A	0.000836	N/A
3	0.00415	N/A	240,837.5	N/A	0.000415	N/A
4	0.00980	N/A	102,018.1	N/A	0.000980	N/A
5	0.00157	N/A	636,175.6	N/A	0.000157	N/A
6	<LoQ*	<LoQ	N/A	N/A	N/A	N/A
7	0.00546	N/A	183,015.3	N/A	0.000546	N/A
8	<LoQ	<LoQ	N/A	N/A	N/A	N/A
9	0.00451	0.0002	221,892.9	9,356.07	0.000472	0.000020
10	<LoQ	<LoQ	N/A	N/A	N/A	N/A
11	0.00542	0.0007	184,414.1	2,998.16	0.000609	0.000070
12	0.00605	N/A	165,208.4	N/A	0.000605	N/A
13	0.00378	N/A	264,529.6	N/A	0.000378	N/A
14	0.00568	N/A	176,161.2	N/A	0.000568	N/A
15	0.00405	<LoQ	246,941.8	N/A	0.000405	N/A
16	0.00444	0.00059	225,112.3	3,363.08	0.000504	0.000059
17	<LoQ	<LoQ	N/A	N/A	N/A	N/A
18	0.01198	<LoQ	83,473.83	N/A	0.001198	N/A
19	0.01133	0.0041	88,241.86	484.60	0.001546	0.000410
20	0.00625	<LoQ	159,886.3	N/A	0.000625	N/A

*< LoQ indicates values below the limit of quantification; **N/A - not applicable; EDI - Estimated Daily Intake; MOE - Margin of Exposure; HQ - Hazard Quotient.

The mean concentration of methylparaben was generally higher than that of propylparaben in all samples, with methylparaben being detected more frequently. Propylparaben was only quantified in a limited subset of the samples, which limits the significance of comparative analyzes. The gender-specific differences in paraben concentrations were determined using a two-tailed t-test for independent samples. While female participants had slightly higher normalized concentrations of methylparaben (6.15±6.70 µg/L or 10.92±7.37 µg/g creatinine) than male participants (4.96±1.77 µg/L or 7.05±5.60 µg/g creatinine), the difference was not statistically significant (p=0.603). Propylparaben levels were analyzed in a similar manner, but did not show sufficient frequency for meaningful statistical comparisons.

To examine associations between participant characteristics (age, BMI and creatinine levels) and normalised paraben concentrations, correlation analyses were performed using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-normally distributed data. No statistically significant correlations were found between paraben concentrations and age (p=0.367) or BMI (p=0.457 for both methyl and propyl parabens).

Risk assessment

To evaluate the toxicological risk of paraben exposure, the EDI and the HQ were calculated based on the measured concentrations of methyl and propyl parabens (Table 3). All HQ values were below 1, indicating that the exposure levels for the study population were within acceptable risk thresholds. The MOE values also supported the safety margin for paraben exposure in this cohort.

Discussion

This study investigated the presence and levels of methyl and propyl parabens in the morning urine samples of a cohort of adult participants to assess their exposure and potential health risks. Parabens are commonly used as preservatives in various consumer products and have been associated with endocrine-disrupting properties, raising public health concerns (Monneret, 2017; Petric *et al.,* 2020; Hager *et al.*, 2022). Our findings provide insight into the extent of exposure within this sample population and contribute to the growing body of research on parabens.

The study showed that methyl parabens were detected more frequently and at higher concentrations than propyl parabens in the urine samples. Several studies have investigated the prevalence and concentrations of methyl and propyl parabens in human urine samples, highlighting the more frequent detection and higher concentrations of methyl parabens compared to propyl parabens. For example, a study analyzing urine concentrations of parabens in the US population found that methylparaben was detected in 99.9% of samples, while propylparaben was present in 96.3% of samples, with methylparaben concentrations higher than propylparaben (Feizabadi *et al.*, 2020). Another study examining the paraben concentrations in the urine of Iranian men found that methylparaben was detected in 95% of the samples, while propylparaben was detected in 94% of the samples, with the methylparaben concentrations being significantly higher (Hajizadeh *et al.*, 2021). These findings are in line with previous research findings indicating an increased use and prevalence of methylparabens in consumer products such as cosmetics and personal care items. The higher detection rates and concentrations of methylparaben in human urine samples suggest greater exposure to this compound.

Propyl parabens are detected less frequently and at lower concentrations, but still deserve attention due to their potential health effects, especially in sensitive populations or during sensitive life stages (Vandenberg & Bugos, 2021).

The variability of paraben concentrations can be influenced by individual factors such as the use of personal products, differences in metabolism and lifestyle habits. For example, studies have shown that women in service and sales occupations may have higher levels of parabens and phthalates in their urine compared to other women, possibly due to increased use of personal care products (Kim *et al.,* 2023). In addition, the differences in urinary paraben concentrations before and during pregnancy and the increased variability in methylparaben and propylparaben concentrations could be due to changes in the use of personal care products, medications or food consumption during pregnancy (Smith *et al.,* 2012).

In this study, the female participants had slightly higher average concentrations of methylparabens, which could be due to the greater use of cosmetics and personal care products by women. In terms of gender differences, some studies have found that women tend to have higher concentrations of certain parabens in their urine, which could be related to their more frequent use of cosmetics and personal care products. For example, one study examining occupational differences in personal care product use found that women in service and sales occupations may have higher urinary levels of parabens and phthalates than other women (Kim *et al.,* 2023). However, the extent of these differences may vary and not all studies have found statistically significant gender differences (Kiani Feizabadi *et al.,* 2020). These findings suggest that while there are trends indicating higher exposure in certain groups, broader population studies are needed to better characterize and understand gender differences in paraben exposure.

The EDI and HQ calculations showed that paraben exposure was below the established thresholds for all participants, with HQ values consistently below 1. This result indicates that exposure to methyl and propyl paraben did not pose a significant health risk to the participants in this study. The calculated MOE values support this conclusion as they are above the safety margins normally required for risk assessment.

Although these findings suggest a relatively low risk from paraben exposure, it is important to consider the effects of cumulative and long-term exposure, particularly in vulnerable populations such as pregnant women, young children and people who use many cosmetic products. In addition, parabens are often found in a variety of consumer products, which can lead to cumulative exposure from multiple sources. This aspect emphasises the need for continued monitoring and more comprehensive studies to evaluate the potential health effects over longer periods of time and across different demographic groups.

The findings of this study are consistent with previous research indicating widespread exposure to parabens in the general population. Large-scale biomonitoring studies in the United States and Europe have documented comparable concentrations of methyl and propyl parabens, underscoring their ubiquity and consistent patterns

of human exposure (Calafat *et al.*, 2010). Of note, data from the US population show higher detection frequencies and concentrations of methylparaben compared to propylparaben (Ward *et al.*, 2020).), which supports our observed trend.

However, some studies have reported associations between higher paraben concentrations and adverse health effects, such as reproductive problems and endocrine disruption (Mitra *et al.*, 2021).

Although our study did not find evidence of exposure levels that pose immediate health risks, the possibility that chronic exposure at low levels may contribute to long-term health effects remains a concern. The scientific community continues to research the effects of such exposure, including possible interactions with other endocrine disrupting chemicals. For example, a study examining the effects of parabens on breast cancer cells in black women found that parabens increased the growth of a black breast cancer cell line (Tapia *et al.*, 2023). These findings emphasise the importance of ongoing research to fully understand the health effects of paraben exposure.

Limitations and future directions

Limitations of the study include the relatively small sample size, which could limit the generalizability of the results. The low frequency of detectable propyl parabens further limited the statistical analyses, emphasising the need for studies with larger and more diverse populations. In addition, factors such as diet, occupation, and geographic location that may influence paraben exposure were not fully controlled or examined. An important limitation is that a default value of 1.7 L/day for urine excretion was assumed for all participants, as no 24-hour urine collection was performed in this study. Although this value derived by Honda *et al.* (2018) was applied uniformly for all participants, the lack of 24-hour urine collection means that individual variations in urine excretion could not be accounted for. To our knowledge, this is the first study to monitor paraben exposure in the population of Bosnia and Herzegovina and provides an important foundation for future research in this region.

Future studies should aim to include a larger number of participants and consider longitudinal studies to better understand the dynamics of paraben exposure over time. Investigating the sources and routes of paraben exposure, including specific product use, could provide valuable insights into strategies to reduce exposure, particularly among at-risk groups. Further research on the combined effects of multiple parabens and other endocrine disruptors in the environment is also warranted to fully assess their potential health effects.

Conclusion

To summarise, this study provides a snapshot of paraben exposure in a specific population. It shows measurable concentrations of methyl and propyl parabens in urine samples and indicates that current exposure does not pose significant health risks. However, the potential for cumulative and long-term effects and the need for broader and more detailed investigations remain a priority for future research. Addressing these challenges will help to ensure effective public health policies and risk management strategies regarding the use of parabens in consumer products.

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