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Urinary concentrations of bisphenol A, parabens and benzophenone-type ultra violet light filters in relation to sperm DNA fragmentation in young men: A chemical mixtures approach

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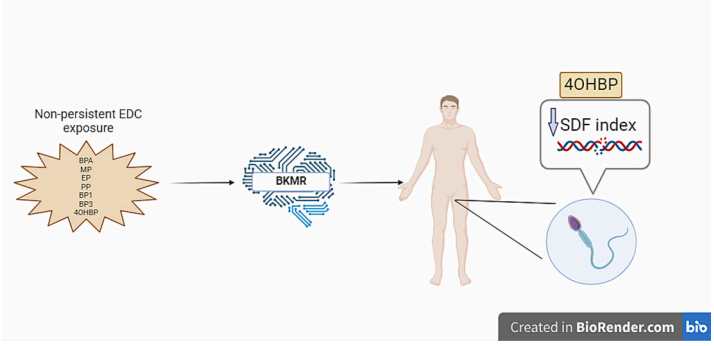
HIGHLIGHTS

- Combined exposure to non-persistent EDCs has been associated with reproductive effects.
- Urinary concentrations of bisphenol A, parabens and UV filters were analysed.
- Sperm DNA fragmentation index is an important factor regarding paternal genetic material.
- Urinary 4OHBP levels were associated with a decrease in Sperm DNA Fragmentation index.

GRAPHICAL ABSTRACT



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ABSTRACT

People are daily exposed to multiple endocrine disruptor compounds (EDCs) that may interfere with different molecular and cellular processes, promoting a potential estrogenic, androgenic, or anti-androgenic state. However, most epidemiological studies attempting to establish relationships between EDCs exposure and health effects are still considering individual compounds. A few studies have shown associations between exposure to individual non-persistent EDCs and sperm DNA fragmentation (SDF) in different male populations. Thus, the aim of this study was to investigate associations between combined exposure to non-persistent EDCs and SDF index in young men. A cross-sectional study was conducted with 158 healthy university students from Southeaster Spain. The participants provided spot urine and semen samples on the same day. The concentrations of urinary bisphenol A (BPA), benzophenones [2,4-dihydroxybenzophenone (BP-1); 2,2′,4,4′-tetrahydroxybenzophenone (BP-2), 2-hydroxy-4-methoxybenzophenone (BP-3), 2,2′-dihydroxy-4-methoxybenzophenone (BP-8), 4-hydroxybenzophenone (4OHBP)], and parabens (methylparaben, ethylparaben, propylparaben, butylparaben) were measured by dispersive liquid-liquid microextraction and ultrahigh-performance liquid chromatography with tandem mass spectrometry detection. SDF was analysed using a Sperm Chromatin Dispersion test. Statistical analyses were carried out using Bayesian Kernel Machine Regression models to evaluate associations between combined exposure to these compounds and SDF index while adjusting by relevant covariates. The increase in urinary concentration of 4OHBP was found to be the most important contributor to the negative association between urinary EDCs concentrations and SDF index, being of −5.5 % [95 % CI: −10.7, −0.3] for those in percentile 50, and − 5.4 % [95 % CI: −10.8, −0.1] for those in percentile 75. No significant associations were observed between other EDCs and SDF index. Our findings show that urinary 4OHBP levels may be associated with a decrease in the SDF index. Nonetheless, the effects we observed were likely to be small and of uncertain clinical significance. Further research is needed to replicate our findings in other male populations.

1. Introduction

The decreasing fertility in humans over the last few decades (Skakkebæk et al., 2022) has potentiated public health investigations (Axelsson et al., 2011; Levine et al., 2023; Rolland et al., 2013). Approximately, a quarter of couples in reproductive age suffer of fertility problems (Agarwal et al., 2015), and around 20 % are explained by male infertility problems (Levine et al., 2023); Zamkowska et al., 2018) being necessary a wider physiological explanation.

Modifiable habits such as having a healthy dietary pattern (Attaman et al., 2012), (Salas-Huetos et al., 2017), maintaining a lean body mass (Sermondade et al., 2013) and being physically active (Gaskins et al., 2012; Priskorn et al., 2016) are widely studied common factors that might contribute to improving or maintaining adequate sperm counts and hormonal levels. However, there are many other modifiable factors to which we are exposed on a daily basis, being the exposure to endocrine disruptor compounds (EDCs) one of the most relevant and latest studied (Adoamnei et al., 2018a, 2018b, 2018c; Castellini et al., 2020).

Regular exposure to these exogenous substances is commonly encountered through our diet (Buckley et al., 2019; Vandenberg et al., 2010), as well as in many other products, such as herbicides, biocides, body lotions and perfumes (Agarwal et al., 2015), being several the routes in which they are introduced into our body (ingestion, inhalation, or skin absorption) (Rehman et al., 2018). These exposures, even when the dosage is relatively low, can disturb hormonal homeostasis (Rubin, 2011; Zoeller et al., 2012). As exogenous substances, a physiological interaction can be established with many metabolic and cellular processes, disturbing and interfering with the production, release, transport, union, and elimination of certain sexual hormones, leading to an estrogenic or androgenic hormonal imbalance (Akingbemi et al., 2004; Castellini et al., 2020; Rehman et al., 2018; Wetherill et al., 2007; Zamkowska et al., 2018).

Several biomonitoring studies around the world have shown that Bisphenol A (BPA), parabens and benzophenone (BP)-type ultra violet (UV) light filters exposure is common among different populations, with detectable concentrations in a very high proportion of study participants (Calafat et al., 2008, 2010; CDC, 2015; Koch et al., 2012; Moos et al., 2015; Philippat et al., 2015; Schlumpf et al., 2010). All these compounds have been frequently evaluated and reported regarding reproductive health outcomes (*e.g.* semen quality, serum hormone levels, endometriosis or couple fecundity) (Adoamnei et al., 2018a, 2018b, 2018c; Buck Louis et al., 2014, 2015; Kunisue et al., 2012).

The relationship between individual exposure to EDCs with sperm and hormonal parameters has been showed in several recent studies (Adoamnei et al., 2018a, 2018b, 2018c), being however scarce for other seminal characteristics such as sperm DNA fragmentation (SDF) (Buck Louis et al., 2015; Goldstone et al., 2015; Kiwitt-Cárdenas et al., 2021; Meeker et al., 2010; Radwan et al., 2018).

As described in the scientific literature, single-pollutant exposure has been assessed for semen quality, however, as human populations are exposed to EDCs mixtures rather than to a single pollutant, it would be more appropriate to establish relationships between exposure to a mixture of chemicals rather than to individual compounds (Sun et al., 2013). Therefore, the objective of this study was to assess the association between urinary concentrations of BPA, parabens and BP-type UV filters, using a mixtures approach, and the SDF index in young men.

2. Material and methods

2.1. Study population

Rationale, study design and methodology have been previously described (Kiwitt-Cárdenas et al., 2021). Briefly, a total of 158 subjects were included in the current study. Participants derived from the Murcia Young Men's Study (MYMS) (Mendiola et al., 2010), were Spanish healthy university students between 18 and 23 years old. Lifestyle questionnaires, seminal analysis, andrological examination, urine and blood analysis were undergone contributing with the data necessary for this study (demographic characteristics, urinary EDCs concentrations and SDF index). Informed consent of all the participating subjects was obtained, as well as the approval of the Commission of Research Ethics of the University of Murcia (no. 495/2010, May 14, 2010).

2.2. Physical examination

The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Testicular examination was carried out by the same investigator and using a Prader orchidometer (Andrology Australia, Clayton, Victoria, Australia), describing the testicular size and the presence or absence of varicocele or other scrotal abnormalities.

2.3. Urinary BPA, BP-type UV filters, and parabens analysis

In order to assess BPA, the five BP-type UV filter and the four paraben concentrations, a single spot sample was obtained for each participant. Polypropylene urine containers of 100 mL were used for collecting urine samples and pretested for ensuring they did not leach or contain the selected EDCs. Later, samples were frozen at −80 °C in 4.5 mL polypropylene vials and sent to the University of Granada. To preserve the samples during the journey, they were put on dry ice and stored at −80 °C until their analysis a few months later.

Detection and quantification of BPA, 2,4-dihydroxybenzophenone (BP-1); 2,2′,4,4′-tetrahydroxybenzophenone (BP-2); 2-hydroxy-4-methoxybenzophenone (BP-3); 2,2′-dihydroxy-4-methoxybenzophenone (BP-8) and 4-hydroxybenzophenone (4OH-BP), and methylparaben (MP), ethylparaben (EP), propylparaben (PP), and butylparaben (BP)] were carried out by dispersive liquid-liquid microextraction (DLLME) and ultra-high performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS) as previously described with minor modifications (Jiménez-Diaz et al., 2016; Vela-Soria et al., 2014). Briefly, urine samples were thawed completely at room temperature, centrifuged at 2600*g* for 10 min to sediment particulate matter and 0.75 mL were taken to carry out the analysis. To determine the total EDCs amount (free plus conjugated) in urine, each sample was spiked with 50 μL of enzyme solution (β-glucuronidase/sulfatase) and incubated at 37 °C for 24 h. The treated urine was placed in a 15 mL screw-cap glass tube and spiked with 30 μL of the surrogate standard solution (1.25 mg/L of BPA-d16, BP-D10 and EP-13C6). Urine was diluted to 10.0 mL with 5 % NaCl aqueous solution (*w/v*) and the pH was adjusted to 2.0. Next, 0.75 mL of acetone and 0.75 mL of trichloromethane were mixed and injected rapidly into the aqueous sample with a syringe. After manual shaking, centrifugation, and evaporation of the extract, the residue was dissolved with 100 μL of a mixture consisting of water (0.1 % ammonia)/acetonitrile (0.1 % ammonia), 70:30 (*v*/v), and finally, 10 μL was injected in the LC system. Urinary creatinine concentration (mg/dL) was determined using an automated colorimetric determination based on the Jaffe assay, in the same urine samples in which environmental chemicals were assessed. Due to the constant excretion rate of creatinine into the urine (that makes urinary creatinine concentration inversely proportional to urine flow rate), creatinine correction is widely used for normalizing analyte concentrations in spot samples for environmental exposure monitoring (Barr et al., 2005).

2.4. Calibration and quality control

Matrix-matched calibration curves were constructed plotting the analyte/surrogate peak area ratio against the analyte concentration, in synthetic urine (Inn et al., 2001). BPA-d16, BP-D10 EP-13C6 were used as surrogate. The limit of detection (LOD) obtained for all the selected ten EDCs was 0.1 ng mL–1, and the limit of quantification (LOQ) 0.3 ng mL–1, except for BP-3 that was 0.2 ng mL–1 and the limit of quantification (LOQ) was 0.6 ng mL–1. Urine samples were extracted in batches of 12, with each batch containing approximately 2 quality-control samples. These quality-control samples included 1 field blank (deionized water which is treated as a sample) and 1 blank urine sample spiked with five benzophenone-UV filters at a final concentration of 2.0 ng/mL. Recoveries in the quality control spiked samples were from 88 % to 104 %, with coefficients of variation (CV) <15 %. Urinary samples that had non-detectable EDCs concentrations were assigned the value of LOD divided by the square root of 2, which is recommended when data are not highly skewed (Hornung and Reed, 1990).

2.5. Semen analysis

The sample was obtained through masturbation in the study centre and analysed within 45 min as described in other studies (Mendiola et al., 2010). Abstinence time (hours) was recorded as the time between current and previous ejaculation as reported by the study subject. WHO criteria (WHO, 2010) were used to analyse seminal parameters (volume, concentration, total sperm count, motility, and sperm morphology). The ejaculated volume was estimated from the weight of the sample, assuming a semen density of 1.0 g/mL. Total sperm count was calculated as the product between volume and sperm concentration. Morphology was assessed using the Papanicolaou stain and strict Kruger criteria (Menkveld et al., 1990). Sperm concentration was evaluated using a haemocytometer (Improved Neubauer; Hausser Scientific Inc., Horsham, PA, USA.). The spermatozoa were classified according to whether they were mobile or immobile to establish the percentage of the first ones (with progressive (P) and non-progressive (NP) movement) (WHO, 2010). All semen analyses were conducted by the same specialized biologist. Moreover, throughout the overall study period, an external quality control on semen samples was conducted in collaboration with the University of Copenhagen's Department of Growth and Reproduction, obtaining satisfactory results.

2.6. Sperm DNA fragmentation analysis

DNA fragmentation analysis has been described and published previously (Sarabia-Cos et al., 2015). Once the semen sample was liquefied, an aliquot was separated and frozen directly without cryoprotectant at −20 °C, and subsequently at −80 °C. SDF analysis was performed using the sperm chromatin dispersion (SCD) test, by using the Dyn-Halosperm® (Halotech S.L., Madrid, Spain) (Fernandez et al., 2005). The samples were thawed by immersion 30s in the water at 37 °C and diluted to an approximate concentration of 15 million/mL. The processed samples were analysed using fluorescence microscopy (SYBR® Green II RNA Gel Stain, Invitrogen™, Life Technologies Corporation, Paisley, United Kingdom) and counted by the same specialized embryologist using the same methodological process earlier described (Sarabia-Cos et al., 2015). The SDF index was defined as the percentage of sperm with fragmented DNA divided by the total sperm analysed, counting 300 sperm for each aliquot. Lastly, the reliability of the SCD test has been shown to be adequate and, for example, exceeded 0.80 using two ejaculates analysed within a three-month interval. Overall evidence supports that a single SCD test for patient classification using predefined SDF thresholds (Esteves et al., 2022).

2.7. Covariates

The covariables included factors previously related to SDF or EDCs exposures regardless of whether they had been previously described as predictors of reproductive health parameters. Age, BMI, smoking, diet patterns, and physical activity were evaluated. Directed acyclic graph (DAG) were used to define potential covariates for sperm DNA fragmentation (Supplementary Fig. 1). Observations were obtained using the administered questionnaires and physical determinations. When the inclusion of a potential covariate triggered a change in the β-coefficient of <10 %, that variable was not retained in final models. Thus, the variables that were finally included in the adjusted models were age and BMI. Urinary concentrations of phenols and parabens were already corrected by creatinine. DAG was created with www.dagitty.net/dags.html tool and modified with the "*ggdag*" package in RStudio v1.4.

2.8. Statistical analysis

Logarithmic transformation of EDCs was carried out to obtain a normal distribution and manage extreme values. Individual BPA, benzophenones and parabens have been described, excluding BP-2 and BP-8 due to high number of individuals under LOD and scarce individual contribution. Descriptive statistics are exposed using crude data, calculating medians and 5th–95th percentiles for population characteristics and mean with standard deviation (SD) for SDF index. Finally, Spearman correlations were carried out for urinary log-transformed compounds to assess correlations between each other.

We employed Bayesian Kernel Machine Regression (BKMR) model, a flexible nonparametric method for analysing chemical mixtures (Bobb et al., 2015; Valeri et al., 2017). This approach models the combined effects of chemicals using a kernel function, considering the correlation between the mixture components. BKMR enables us to assess the relative importance of individual chemicals in the association between the mixture and the outcome. Additionally, it allows us to visualize the relationship between exposure and response for each component of the mixture. To present the results of our BKMR analysis, the percentage difference in the SDF index was calculated within the percentile. An increase in the log-transformed EDC from the 25th to the 75th percentile (p) was associated with a posterior mean of %SDF while holding the other components in the EDCs mixture constant at their median levels (q.fixed = 0.5). We also included reliable intervals for this difference. In our analysis, we treated each measurement of log-transformed EDCs concentration (corrected by creatinine) as a continuous predictor, while keeping the other mixture components at their median values, while adjusting by relevant covariates. During BKMR modelling, 10,000 iteractions (iter) were performed. Finally, multiple linear regression models were also used to evaluate associations between individual urinary EDCs concentrations and SDF index; or semen parameters and SDF index, following similar adjustments previously mentioned. Statistical analyses were performed using JASP v0.16.3 and RStudio v1.4. with the R package "*bkmr*" and "*ggplot2*" for creating graphs.

3. Results

3.1. Population characteristics and relationship between semen parameters and SDF

Study participants were young college students [median (5th–95th) 20.5 (18.5–22.9)] years old, with BMI of 23.6 (19.4–39.8) kg/m^2. More than one-third of the subjects smoked (35 %) and varicocele was detected in 15 % of the participants. Median abstinence time was 70.0 (39.0–123.0) hours, median sperm concentration 41.9 (9.3–128) million/mL, total sperm count 132 (36.3–407.0) millions, motile sperm (PR + NP) 57.1 % (39.3–72.8), morphologically normal sperm 9.0 % (3.0–23.0), semen volume 3.1 (1.3–6.8) mL and mean of sperm DNA fragmentation index of 28.6 % (SD: 14.7) (Table 1). Regarding full-adjusted linear models between semen parameters and SDF index, a negative significant relation for total sperm count (−0.05 [95%CI: −0.09; −0.01]); % motile sperm (PR + NP) (−0.05 [95%CI: −0.08; −0.01]) and morphologically normal sperm (−0.06 [95%CI: −0.11; −0.01]) with SDF was observed (Supplementary Table 1).

3.2. Non-persistent EDC concentrations and their correlations

The distributions of non-persistent EDC logarithmic concentrations are represented in Supplementary Fig. 2, 3 and 4, and crude data is presented in Table 2. EDCs were detected in all the samples with a median urinary concentration of 2.9 ng/mL [IQR 4.1] for BPA; MP of 15.8 ng/mL [IQR 36.6]; EP of 1.7 ng/mL [IQR 3.9]; PP of 0.69 ng/mL [IQR 3.7]; BP-1 of 2.1 ng/mL[IQR 3.0]; BP-3 of 0.9 ng/mL [IQR 5.7]; 4OHBP of 0.2 ng/mL [IQR 0.3] (Table 2). For BP-2 and BP-8, percentage of values above the LOD were fairly small and therefore were not further included in the statistical analysis. EDCs were significantly correlated with Spearman correlation coefficients between 0.1 (logBPA-logPP) and 0.7 (logBP1-logBP3) (Supplementary Table 2).

3.3. BKMR

When BKMR analyses were conducted focusing on SDF index, a first section of interest is the univariate relationship between each covariate and the outcome, being all the other exposures fixed to a particular percentile (fixed at p50) (Fig. 1 and Supplementary Fig. 5). We can conclude from these figures that all selected covariates had weak to moderate associations, and that all dose-responses seem to be linear. In addition, after running individual effects from BKMR in the simulated dataset, 4OHBP was found to be the most important contributor to the negative association between EDCs and SDF index (Table 3 and Fig. 1). We can observe this when an increase in percentile in 4OHBP was associated with a decrease of SDF index: -5.5 (95 % CI: −10.7, −0.3) for p50 and − 5.4 % (95 % CI: −10.8, −0.1) for p75. No other associations were observed between the rest of urinary metabolite concentrations and SDF index, as shown in Table 3. Lastly, concerning linear regression models between single-chemical and SDF index, a negative significant relation between 4OHBP and SDF (−0.11 [95%CI: −0.20; −0.02]) was observed. Nevertheless, this significance became borderline in the full adjusted models (Supplementary Table 1). No other associations were detected.

Table 1
Descriptive characteristics of participants.

Variables	Total (n = 158)
	Median (5th–95th) or n (%)
Age (years)	20.5 (18.5–22.9)
BMI (kg/m^2)	23.6 (19.4–29.8)
Ejaculation abstinence time (h)[a]	70.0 (39.0–123.0)
Mean testicular volume (ml)	21.0 (15.5–26.0)
Current smokers	55.0 (35.0)
Presence of varicocele	24.0 (15.0)
Have had prolonged disease[b]	14.0 (9.0)
Taken any medication[c]	36.0 (22.8)
Semen parameters	
Seminal volume (mL)	3.1 (1.3–6.8)
Sperm concentration (Mill./mL)	41.9 (9.3–128.0)
Total sperm count (Mill.)	132.0 (36.3–407.0)
% Motile sperm (PR + NP)[d]	57.1 (39.3–72.8)
% Morphologically normal sperm	9.0 (3.0–23.0)
Sperm DNA fragmentation (SDF) (n, %)	
SDF Total (mean, SD)	28.6 (14.7)
Lifestyle	
Moderate-vigorous excercise, h/w	5.0 (0.0–14.0)
Prudent pattern score[e]	0.1 (−0.9–1.1)
Western pattern score[e]	0.1 (−1.0–1.1)

[a] Ejaculation abstinence time (hours): reported as the time between current and previous ejaculation as reported by the study subject.
[b] Long-lasting disease (including diabetes/thyroid disease), sexually transmitted diseases (diagnosed with epididymitis, chlamydia, or gonorrhea).
[c] Taken any medication for 3 months before participation in the study (mostly antibiotics or medication against allergy).
[d] Percentage of motile sperm [progressive + non-progressive (PR + NP)].
[e] Dietary patterns were constructed by factorial analysis as described in Gaskins et al. (2012). A higher score indicates a higher adherence to the Prudent or Western dietary pattern.

Table 2
Descriptive characteristics of urinary EDC concentrations.

EDC (ng/mL)	Median	IQR	5th percentile	95th percentile	% above LOD
BPA	2.9	4.1	0.4	10.1	96.2
MP	15.8	36.6	0.1	116.0	88.7
EP	1.7	3.9	0.1	19.7	73.6
PP	0.7	3.7	0.1	29.1	58.5
BP-1	2.1	3.0	0.2	11.9	96.9
BP-3	0.9	5.7	0.1	19.6	62.9
4OHBP	0.2	0.3	0.1	0.8	71.7

EDC: Endocrine disrupting compound.
IQR: Interquartile Range.
% above LOD: % above of limit of detection: BP-2 and BP-8 were excluded with values of 18.3 % and 24.6 %, respectively.

4. Discussion

Our mixture approach results identified that urinary concentrations

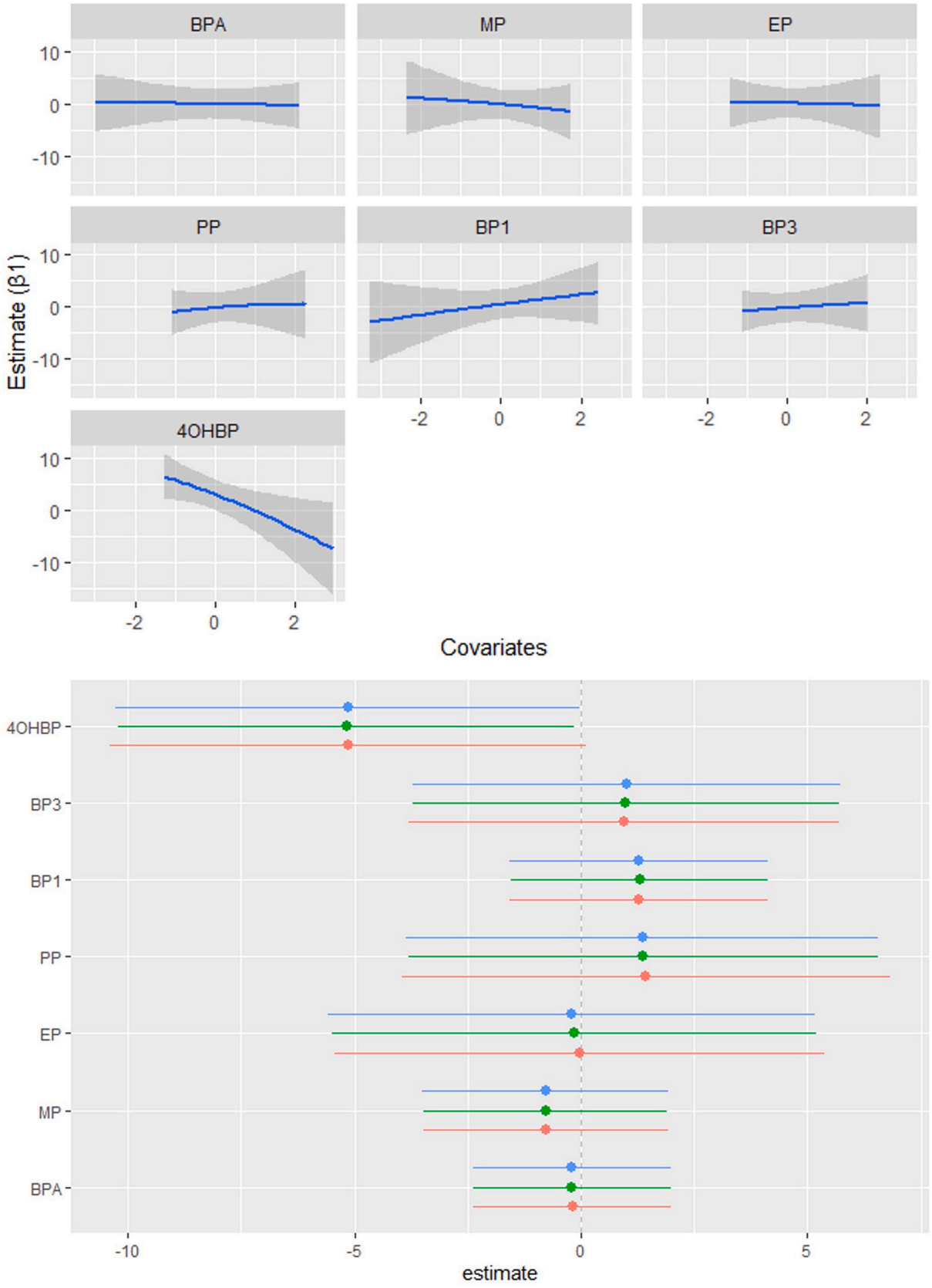

Fig. 1. Univariate dose-response associations from BKMR in the simulated dataset. Single metabolite effect. Differences within individual percentiles (p25 [red], p50 [green] and p75 [blue]) of SDF index in each log-transformed EDC urinary metabolite. We used Bayesian Kernel machine regression (BKMR) models adjusting for age and body mass index. Urinary concentrations of phenols and parabens were already corrected by creatinine.

Table 3
BKMR analysis between urinary EDC concentrations and SDF index.

		p25		p50		p75	
Metabolite	PIP	SDF % change	95 % CI	SDF % change	95 % CI	SDF % change	95 % CI
BPA	0.0114	-0,4	(−3.2, 2.5)	-0,4	(−3.2, 2.4)	-0,5	(−3.3, 2.4)
MP	0.0720	-1,1	(−4.1, 1.8)	-1,1	(−4.0, 1.8)	-1,1	(−4.1, 1.9)
EP	0.0456	0,0	(−5.7, 5.6)	-0,2	(−5.7, 5.4)	-0,3	(−5.9, 5.3)
PP	0.1046	1,6	(−4.0, 7.1)	1,5	(−3.7, 6.8)	1,5	(−3.8, 6.8)
BP1	0.0322	2,3	(−1.8, 6.3)	2,3	(−1.7, 6.3)	2,3	(−1.7, 6.4)
BP3	0.0302	0,4	(−5.2, 6.0)	0,5	(−5.0, 6.1)	0,6	(−5.0, 6.1)
4OHBP	**0.2156**	−5,5	(−11.1, 0.1)	**−5,5**	**(−10.7, −0.3)**	**−5,4**	**(−10.8, −0.1)**

Models adjusted by age and body mass index. Urinary concentrations of phenols and parabens were already corrected by creatinine. PIP: posterior inclusive probability for the exposures. Most important EDC of the mixture in bold. p: percentile.

of 4OHBP were the main contributor to the decrease in the SDF index, while the other EDCs evaluated showed no effect. Moreover, somewhat consistent results very obtained when analysing the data with a single-chemical approach. As far as we know, this is the first study to describe the relationship between SDF in a multipollutant environment in young unselected men.

The SDF index is a relatively new and interesting tool for examining and assessing male infertility (Gosálvez et al., 2009; Aitken and Bakos, 2021; Santi et al., 2018). The importance of this modified genetic information marker relies in the possibility of evaluating the altered transmission of paternal genetic material to the embryo (Aitken and Bakos, 2021), which could potentially hindering embryo implantation and subsequent fetal development (Santi et al., 2018).

Focusing on parabens, our results are not in agreement with those reported by Jurewicz et al. (2017), who found that urinary paraben concentrations in men attending an infertility clinic for diagnostic purposes were significantly associated with an increase in the percentage of sperm with high DNA instability. This mechanism of DNA instability in sperm cells has been known for a long time. Glander et al. (1984) investigated primary microbiological contamination in human ejaculates and secondary contamination after cryopreservation with methylparaben. These authors found that methylparaben reduced microbiological contamination of the cryoprotective medium, but it also decreased human sperm motility. In the same study on the *in vitro* spermicidal activity of methyl-, ethyl-, propyl-, and butylparaben in humans, it was found that these four parabens were effective spermicides (Glander et al., 1984). These authors also stated that the tested doses of 0,2 % of nipagin (methylparaben) was sufficient to decrease seminal parameters and male fertility. Nowadays, normal concentrations between 0.02 %–0.3 %. of this paraben are used in everyday life cosmetics. On the other hand, even though the use of parabens at concentrations used in commercially available formulations may impair sperm motility (*e.g.* enhance the generation of mitochondrial reactive oxygen species), underlining the potential cytotoxic and genotoxic effects of these compounds, methylparaben had no significant effect on DNA fragmentation in *in vitro* studies on human spermatozoa (Samarasinghe et al., 2018), as seen in our study population.

The BP-type UV filters selected in this study are the most common ones found in human population as previously noted. These compounds also have the potential to interfere with essential sperm function and may impair human fertilization (Rehfeld et al., 2016; Schiffer et al., 2014). In fact, the use of benzophenone-type chemicals found in sunscreen and personal care products has been related to diminished sperm endpoints, such as sperm concentration, sperm vitality, motility and morphology (Buck Louis et al., 2015). However, in the same study, Buck Louis et al. (2015) delineated an improbable correlation between preconception concentrations of urinary BP-type UV filters in male partners and their percentage of SDF, a finding that aligns and is consistent with our study results. Nonetheless, the effects of individual benzophenone-type chemicals on male reproductive parameters are still unclear (Adoamnei et al., 2018b).

In our work, an increase in the percentile concentration of 4OHBP contributed to a significant decrease in the SDF index, particularly in those with higher urinary levels (p50 and p75). It is known that 4OHBP is used in the field of organic synthesis for pharmacological use as a pharmacologic intermediate of clomiphene (Ruenitz et al., 1983). Clomiphene is used to induce ovulation in women and to treat oligospermia in male patients. It exerts an effect on the hypothalamic and pituitary systems, increasing the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn act on the testes, increasing testicular testosterone and sperm production (Huijben et al., 2022). This could be a possible explanation for our results, although we cannot rule out that this is a chance finding; therefore, it is necessary to confirm these results in other male study populations.

Regarding BPA, only a very few studies have investigated the potential relationship between urinary BPA concentrations and SDF within dissimilar male populations using different SDF analysis methodologies and reporting positive (Meeker et al., 2010; Radwan et al., 2018) or negative associations (Goldstone et al., 2015). More recently, Kiwitt-Cárdenas et al. (2021), in the same study population, found no association between urinary BPA concentrations and SDF index in a group of young men. However, positive and significant associations were observed in the subgroup of men with SDF index of >30 %. Similarly, in both, *in vivo* and *in vitro* rat models, BPA and its analogues were observed to increase sperm DNA fragmentation at higher concentration groups (Ullah et al., 2019).

Our current study investigated a chemical mixtures exposure approach rather than the association between single chemicals and SDF index. In the current scientific literature there is a notable dearth of research that systematically examines the collective effect of multiple chemicals on male reproductive outcomes, a more realistic context. That hampers our ability to discuss our findings with previous studies on the matter, particularly on the mixture approach. Moreover, this limitation poses challenges in understanding the synergistic or antagonistic interactions that may occur when individuals are exposed to a combination of environmental pollutants. Human exposure to pollutants is rarely limited to a single substance, and this absence may result in an incomplete understanding of the true reproductive health risks associated with environmental contaminants.

One of the main strength of the current study is that we provided clinical information and biological samples for our investigation. Moreover, the pre-designed research protocol ensured sufficient information to adjust for appropriate covariates and potential confounders in the process of statistical analysis. Finally, we assessed several chemicals, in a mixture approach, that were not evaluated in previous studies having a broad group of contaminants to which this young population was exposed. The current work has also several limitations. These correspond to those related to a cross-sectional design, not being able to establish if the exposure occurred before the outcome, leading to a limited causal inference. Other non-persistent EDCs, as well as some benzophenones (BP-2 and BP-8), were not studied or included in our study because of the low number of individuals above the LOD. Lastly, while single spot samples may not capture the full temporal variability of these biomarkers, they still offer valuable insights into exposure levels

at a specific point in time. Several studies reported that, despite variability in concentrations, a single spot urine measurement seems to correctly represent the long-term exposure of an individual, including relatively high intraclass correlation coefficients (Dewalque et al., 2015; Koch et al., 2014; Lassen et al., 2013; Meeker et al., 2013). Thus, some level of exposure misclassification may occur because of the short-lasting exposure to these non-persistent chemicals and their short biological half-lives (Perrier et al., 2016; Vernet et al., 2019). However, a non-differential misclassification error may have biased our results towards the null hypothesis, influencing the potential associations with SDF. Therefore, the potential effect of this bias in the observed null associations cannot be completely ruled out. Finally, several lifestyle factors were taken into account in our analyses, but other unmeasured factors might have influence our results. Thus, residual confounding or chance findings should always be considered.

5. Conclusions

Our findings support that urinary 4OHBP levels might be associated with a decrease in sperm DNA fragmentation index. Nonetheless the effects we found are likely to be small and of uncertain clinical significance. The replication of our findings across diverse male populations holds the key to clarifying the generalizability and practical implications of these associations. This, in turn, will contribute significantly to advancing our comprehension of modifiable factors that may influence male fertility.

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CRediT authorship contribution statement

Jonathan Kiwitt-Cárdenas: Formal analysis, Writing – original draft, Writing – review & editing, Data curation. **Julián J. Arense-Gonzalo:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Evdochia Adoamnei:** Formal analysis, Writing – original draft, Writing – review & editing. **Laura Sarabia-Cos:** Data curation, Investigation, Writing – review & editing. **Fernando Vela-Soria:** Formal analysis, Validation, Writing – review & editing. **Mariana F. Fernández:** Formal analysis, Validation, Writing – review & editing. **Jaime Gosálvez:** Conceptualization, Methodology, Validation, Writing – review & editing. **Jaime Mendiola:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. **Alberto M. Torres-Cantero:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.169314.

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