Urinary concentrations of benzophenone-type ultraviolet light filters and semen quality

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Objective: To assess benzophenone-type ultraviolet (UV) filter concentrations, chemicals used in sunscreen and personal care products, and semen endpoints.
Design: Cohort.
Setting: Not applicable.
Patient(s): A total of 413 men provided semen and urine samples, 2005–2009. Five UV filters were quantified (ng/mL) in urine using liquid chromatography–triple quadrupole mass spectrometry: BP-1 (2,4-dihydroxybenzophenone), BP-2 (2,2′,4,4′-tetrahydroxybenzophenone), BP-3 (2-hydroxy-4-methoxybenzophenone), BP-8 (2,2′-dihydroxy-4-methoxybenzophenone), and 4-OH-BP (4-hydroxybenzophenone). Using linear regression, β-coefficients (β) and 95% confidence intervals (CIs) for each chemical dichotomized at the 75th percentile and Box-Cox transformed semen endpoint were estimated, after adjusting for age, body mass index, cotinine, season, and site.
Intervention(s): None.
Main Outcome Measure(s): Thirty-five semen endpoints.
Result(s): BP-2 was associated with diminished sperm concentration ($\beta = -0.74$; 95% CI −1.41, −0.08), straight ($\beta = -4.57$; 95% CI −8.95, −0.18) and linear movement ($\beta = -3.15$; 95% CI −6.01, −0.30), more immature sperm ($\beta = 0.38$; 95% CI 0.15, 0.62), and a decreased percentage of other tail abnormalities ($\beta = -0.16$; 95% CI −0.31, −0.01). BP-8 was associated with decreased hypo-osmotic swelling ($\beta = -2.57$; 95% CI −4.86, −0.29) and higher acrosome area ($\beta = 1.14$; 95% CI 0.01, 2.26). No associations were observed for BP-1, BP-3, or 4OH-BP.
Conclusion(s): The findings suggest that specific UV filters may be associated with some aspects of semen endpoints, but await future corroboration. (Fertil Steril® 2015;104:989–96. ©2015 by American Society for Reproductive Medicine.)
Key Words: Benzophenones, fecundity, semen, sperm, sunscreens

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Various classes of persistent environmental chemicals or those that resist degradation and bioaccumulate and biomagnify within food chains, such as dichlorodiphenyldichloroethylene (*p,p'*-DDE), perfluorinated alkyl acids, or polychlorinated biphenyls, have been associated with changes in semen quality in some study populations, suggesting possible implications for male fecundity (1–3). Interest in nonpersistent chemicals, or those compounds with short half-lives ranging from hours to days, is growing in light of their ubiquitous sources of exposure for contemporary populations and reported association with semen quality. For example, both bisphenol A (BPA) and phthalates, or chemicals used in the manufacture of polycarbonate plastics

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and to enhance the flexibility of plastics among other uses, respectively, have been associated with diminished semen quality in some (4, 5) but not all (6, 7) study populations.

Recently, concern has arisen about benzophenone (BP)-type ultraviolet (UV) light filters, given the detection of one such compound in 97% of the US population during 2003–2004 (8), and a comparable percentage in Chinese adults and children during 2010–2012 (9). With increasing recognition of the harmful human health effects attributed to UV radiation, BP-type UV filters have been added to personal care products, insect repellents, and sunscreens to block or minimize the harmful effects of UV light on human skin and hair. These chemicals are also used to coat surfaces exposed to sunlight, including some food packaging (10), where they can migrate to food (11).

Humans are exposed to BP-type UV filters largely through dermal absorption, with evidence that reapplication of certain products may further increase systematic absorption (12, 13).

Benzophenone-type UV filters represent approximately 29 compounds, though the sources for some are unknown, and not all are in commercial use. In recent years, a few BP-type UV filters have been reported to have various hormonal activities, including in vitro and in vivo estrogenic, antiestrogenic, and antiandrogenic effects (14–16). For example, the UV filter BP-2 (2,2′,4,4′-tetrahydroxybenzophenone) has been shown to be capable of binding to estrogen (E) receptors and exerting E-agonistic activity (16). Only minimal research has focused on human health endpoints. A recent article reported that BP-1 (2,4-dihydroxybenzophenone) was associated with endometriosis, an E-dependent gynecologic disease (17). Additionally, urinary concentration of specific BP-type UV filters in men were associated with diminished couple fecundity, manifesting in a longer time required to achieve pregnancy (18). In light of these emerging data, we explored the relation between five BP-type UV filters and semen quality among men recruited from the general population who were not seeking clinical care.

MATERIAL AND METHODS

Study Population

Male partners of couples participating in the Longitudinal Investigation of Fertility and the Environment (LIFE) Study comprised the study population for this work. Briefly, 501 couples discontinuing contraception and trying for pregnancy were recruited from 16 counties in Michigan and Texas between 2005 and 2009 (19). Eligibility criteria for participation included age $\geq$ 18 years, in a committed relationship, no history of clinical diagnosis of infertility, and an ability to communicate in English or Spanish.

Data Collection

Upon enrollment into the cohort, male partners completed baseline interviews followed by a standardized anthropometric assessment to determine body mass index (BMI; weight in kg/height in m^2). Men provided urine and blood specimens for the quantification of urinary UV-filters and serum cotinine, respectively. In addition, 473 men (94%) provided a semen sample, of whom 378 (80%) provided a second sample approximately 1 month later using specifically designed at-home collection kits. Semen samples were mailed overnight to a centralized andrology laboratory where analyses were performed within 24 hours. Among the 501 participating men, 413 had provided semen samples and had sufficient urine available for the quantification of BP-filters and comprise the study population for this work. Human subjects' approval was obtained from all collaborating institutions, and all men provided informed consent before any data collection.

Toxicologic Analysis

Five UV filters were quantified: BP-1, BP-2, BP-3 (2-hydroxy-4-methoxybenzophenone), BP-8 (2,2′-dihydroxy-4-methoxy benzophenone), and 4-OH-BP (4-hydroxybenzophenone). Of note, BP-3 is metabolized by phase 1 and 2 reactions, resulting in its conjugation and urinary excretion (8, 20). BP-1, BP-2, BP-8, and 4OH-BP are metabolic derivatives of BP-3, as generated in phase 1 and 2 reactions (21, 22). As such, urine is an appropriate matrix for quantifying these chemicals.

Urinary quantification of the five UV-filters were determined using established standard operating procedures (21, 23), and performed using isotopic dilution high-performance liquid chromatography–triple quadrupole tandem mass spectrometry with recoveries ranging from 95% to 107%. All laboratory analyses included ongoing quality assurance and quality control procedures inclusive of procedural blanks. The limits of detection (LOD) for the five UV filters in urine ranged from 0.01 to 0.02 ng/mL. All machine-measured concentrations were reported without substituting for concentrations below the LOD to avoid introducing bias associated with this practice (24, 25). Concentrations of UV filters are presented as ng/mL of urine or μg/g creatinine. Urinary creatinine was quantified (mg/dL) in 0.15 mL of urine using the Roche/Hitachi Model 912 clinical analyzer and the Creatinine Plus Assay. Serum cotinine concentration was quantified (ng/mL) in 1 mL of serum using liquid chromatography–isotope dilution tandem mass spectrometry (26).

Semen Collection and Analysis

Males collected up to two semen samples approximately 1 month apart using an established at-home collection protocol (27). Briefly, men were asked to abstain from intercourse for 2 days and to collect the sample by masturbation without the use of any lubricants. A glass collection jar was provided for collection, to which a temperature data logger (I-Button, Maxim Integrated) was attached to record temperature during the 24-hour interval from collection to laboratory analysis. Men were asked to place a specifically prepared sperm migration straw filled with hyaluronic acid and plugged at one end (Vitrotubes #3520, VitroCom) into the ejaculate after collection to capture sperm motility at the time the specimen was collected. Men recorded the last day of ejaculation and any spillage

on the container's label. Semen samples were shipped overnight, allowing for analysis within 24 hours by established andrology laboratories.

Semen samples were quantified for 35 semen endpoints: 5 general characteristics (volume, straw distance, sperm concentration, total sperm count, hypo-osmotic swollen), 8 motility measures, 8 morphology measures, 12 morphometry measures, and 2 sperm chromatin stability assay measures. Sperm motility was quantified using the HTM-IVOS (Hamilton Thorne) computer-assisted semen analysis system, whereas sperm viability was measured using the hypo-osmotic swelling assay and sperm concentration using the IVOS system and the IDENT stain. Sperm morphometry was performed using the IVOS METRIX system. The sperm chromatin stability assay was used according to the methods of Evenson et al. (28) to quantify DNA fragmentation and the percentage of high stainable sperm. The distance traveled by the vanguard sperm in the migration straw was measured to the nearest mm. The above semen endpoints were measured by a single andrology laboratory at the National Institute for Occupational Safety and Health. Sperm morphology was performed by Fertility Solutions using both the traditional and strict morphology techniques (29, 30). Ongoing quality assurance and quality control procedures were in place throughout analysis, inclusive of the use of Westgard Rules and monitoring for drift. All data were inspected to ensure the absence of batch-related differences and/or laboratory drift. None were detected. Analysis of the second semen sample was restricted to general characteristics, motility, and sperm head measurements, largely for budgetary reasons and to verify azoospermia in the first sample. Distributions for all semen quality endpoints have been previously published (27).

Statistical Analysis

We assessed the completeness of data and used χ^2 and nonparametric Wilcoxon tests to assess differences in categorical and continuous socio-demographic characteristics, respectively, with regard to BP-filter concentrations. The distributional properties of all chemicals and semen endpoints were assessed. Specific semen endpoints were transformed using Box-Cox procedures and the Shapiro-Wilk *W* statistics (31).

Specifically, we observed that 14 endpoints (i.e., swollen, average path velocity, straight line velocity, curvilinear velocity, amplitude head displacement, beat cross frequency, straightness, linearity, area, width, perimeter, elongation factor, acrosome area of head, and traditional normal) required no transformation, 14 required natural logarithm transformation (i.e., length, straw distance, round, pyriform, bicephalic, taper, megalo head, micro head, neck or mid-piece abnormalities, coiled tail, other tail abnormalities, immature sperm, DNA fragmentation index, and high DNA stainability), and 7 required cubic root transformation (i.e., volume, total count, sperm concentration, percent motility, strict criteria, amorphous, and cytoplasmic droplet). Detailed information on the varying transformation procedures is published elsewhere (32).

We used linear mixed models with fixed and random effects to assess changes in semen endpoints associated with BP-type UV filters. Specifically, we estimated the change (β-coefficients and accompanying 95% confidence intervals [CIs]) in semen endpoints for men above the 75th percentile for each chemical concentration relative to men below. We selected this dichotomy to differentiate men who were more highly exposed from less-exposed men, and in light of few data to help inform the modeling of chemical distributions relative to semen quality. A random intercept was used in the mixed models to account for the correlation arising from the use of two semen samples for outcomes measured in both samples (i.e., volume, sperm concentration, total sperm count, hypo-osmotic swollen, next-day motility, and sperm head morphology). Regression models were first run including only the chemical and creatinine (natural log-transformed, mg/dL) concentrations and, subsequently, to adjust for a priori specified covariates in light of the dearth of information about these chemicals and male fecundity: age (years), body mass index (kg/m^2), active smoking status (serum cotinine >40.35 ng/mL) (33), creatinine (log-transformed, left continuous), season (spring, summer, winter, fall), and research site (Michigan/Texas). Our rationale for modeling creatinine continuously was to account for the interindividual variation in concentration to more closely reflect men's urinary dilution while preserving statistical power. Selection was based on factors associated with distributions of other nonpersistent chemicals, observed associations with exposures, and to account for any residual confounding by site. Separate models were run for each chemical and semen endpoint. In light of this exploratory analysis, we did not adjust for multiple comparisons. All analyses were performed with SAS version 9.3 (SAS Institute).

RESULTS

As reflected in Table 1, the cohort comprised mostly non-Hispanic white (81%), college educated (99%) men who had health insurance (92%) and who had not previously fathered a pregnancy (53%). With regard to lifestyle, most men reported some weekly alcohol consumption (52%), though fewer reported regular exercise (42%) or actively smoking cigarettes (14%). Among men providing semen samples, the average abstinence period was 4 days, and most men reported no spillage (89%). Eighty-eight percent (n = 413) of men had both urine and semen samples available for analysis and comprise the final sample for analysis, none of which were found to be azoospermic.

Geometric means and accompanying 95% CIs are provided in Table 2 and reflect a range of exposures, with the highest detectable urinary concentrations for BP-1, followed by BP-3, 4OH-BP, BP-8, and BP-2. Of note is the observation that 28.1% of the concentrations for BP-2 was <LOD, and 27.4% for BP-8. Correlation coefficients for the five BP-type UV filters were low, ranging from −0.03 (BP-8 and BP-2) to 0.46 (BP-1 and BP-8). However, BP-3 and BP-1 were highly correlated (0.92, $P<.0001$), consistent with BP-1 being considered a metabolite of BP-3 (data not shown).

TABLE 1

Description of male partners, LIFE Study (n = 413).

Characteristic	n	%
Age (y)		
≤24	14	3
25–29	128	31
30–34	158	38
35–39	83	20
≥40	30	7
Mean (SD)	31.8 (4.8)	
Self-identified race/ethnicity		
White, non-Hispanic	334	81
Black, non-Hispanic	18	4
Hispanic	33	8
Other	28	7
Education		
≤High school education	3	1
Some college/college graduate	30	7
Graduate/professional school	378	92
Health insurance		
No	35	8
Yes	378	92
Previously fathered a pregnancy		
No	219	53
Yes	193	47
Cigarette smoker at enrollment		
No	354	86
Yes	59	14
Mean (SD) serum cotinine (ng/mL)[a]	54.5 (135.7)	
Drinking alcoholic beverages at enrollment		
No	58	14
Yes, sporadic (≤3 drinks/mo)	130	31
Yes, regular (≤6 drinks/wk)	213	52
Yes, daily	12	3
Body mass index (kg/m^2)		
Thin (<25.0)	69	17
Normal (25.0–29.9)	169	43
Overweight (30.0–34.9)	105	27
Obese (≥35.0)	54	14
Regular exercise		
No	239	58
Yes	174	42
Abstinence (no. days)		
1	1	<1
2	165	41
≥3	236	59
Mean (SD)	4.03 (5.0)	
Reported spillage of semen		
No	359	89
Yes	43	11
Geometric mean (95% CI) creatinine (μg/g)	113.96 (105.77, 122.8)	

Note: Restricted to male partners with available urine and semen for analysis.
[a] $P \leq .01$ with BP-1 and BP-3.

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TABLE 2

Distribution of BP-type UV light filter concentrations by creatinine adjustment status.

		Unadjusted (ng/mL)		Creatinine adjusted (μg/g)	
UV filter	% < LOD	Geometric mean	95% CI	Geometric mean	95% CI
BP-1	1.21	1.89	1.54, 2.31	1.79	1.44, 2.22
BP-2	28.09	0.05	0.04, 0.06	0.05	0.04, 0.06
BP-3	1.69	4.42	3.63, 5.38	4.13	3.35, 5.09
BP-8	27.36	0.11	0.09, 0.15	0.12	0.09, 0.16
4OH-BP	4.36	0.14	0.13, 0.16	0.13	0.11, 0.14

Note: All data were rounded to two decimal places.

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Two of the five BP-filters were observed to be significantly associated with one or more semen endpoints in the adjusted analyses (Table 3), with specific associations observed for each of the two chemicals. BP-2 was associated with five semen endpoints, including diminished sperm concentration ($\beta = -0.74$; 95% CI −1.41, −0.08), a lower percentage of straight ($\beta = -4.57$; 95% CI −8.95, −0.18) and linear moving sperm ($\beta = -3.15$; 95% CI −6.01, −0.30), and an increased number of immature sperm ($\beta = 0.38$; 95% CI 0.15, 0.62), but a decreased percentage of other tail abnormalities ($\beta = -0.16$; 95% CI −0.31, −0.01). BP-8 was associated with two sperm characteristics, including a decreased percentage of hypo-osmotic swollen sperm ($\beta = -2.57$; 95% CI −4.86, −0.29) and an increased percentage of acrosome area ($\beta = 1.14$; 95% CI 0.01, 2.26). No significant associations were observed for either BP-1, BP-3, or 4OH-BP.

DISCUSSION

We found some evidence suggesting that two of five measured BP-type UV filters were associated with one or more semen endpoints, with some estimates suggestive of reductions in select semen quality endpoints. Specifically, men above the 75th percentile for BP-2 and BP-8 had more changes in semen quality in comparison with men below this cut point. BP-2 was significantly associated with five semen quality endpoints: decreased sperm concentration, decreased percentage of straight and linear movement, increased number of immature sperm, and a decreased percentage of other tail anomalies. BP-8 was negatively associated with hypo-osmotic swelling but positively associated with acrosome area. Of note is the absence of any significant associations for BP-1, BP-3, or 4OH-BP. Collectively, these findings suggest that the metabolic derivatives (BP-2 and BP-8) may be more relevant for semen quality than their parent compound, BP-3. The extent to which these observations may reflect higher reported estrogenic activity for the derivative BP-2 relative to its parent compound, BP-3 (3, 34), remains to be established in light of more signals observed for the former vs. the latter compound. Still, the estrogenic potencies of BP-3 are 1,000–100,000 times lower than 17-β-estradiol, yet higher than other xenoestrogens such as BPA (35), and the potencies for BP-2 await further research. Another mode of action may be via antiandrogenic pathways (14, 15). Of note, 4OH-BP is a pharmaceutical intermediate of clomiphene citrate, a selective E receptor modulator, underscoring its hormonal properties (36).

In light of this being the first investigation of BP-type UV filters and semen quality, we are unable to more fully interpret our findings in the context of previous literature

TABLE 3

Benzophenone-type UV filter concentrations (dichotomized at the 75th percentile) and semen quality endpoints: adjusted linear mixed modeling results.

	BP-1		BP-2		BP-3		BP-8		4OH-BP	
Semen quality endpoint	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**	**β**	**95% CI**
General characteristics										
Volume (mL)	0.13	−0.04, 0.29	0.04	−0.13, 0.22	0.12	−0.04, 0.28	0.09	−0.08, 0.26	0.04	−0.13, 0.21
Sperm concentration ($\times 10^6$/mL)	−0.05	−0.69, 0.59	−0.74[b]	−1.41, −0.08[b]	0.11	−0.53, 0.74	−0.03	−0.68, 0.61	−0.49	−1.16, 0.18
Total sperm count ($\times 10^6$/ejaculate)	0.41	−0.55, 1.36	−0.91	−1.91, 0.09	0.59	−0.36, 1.55	0.22	−0.75, 1.18	−0.40	−1.40, 0.61
Hypo-osmotic swollen (%)	0.22	−2.05, 2.50	−1.75	−4.14, 0.63	−0.13	−2.40, 2.14	−2.57[b]	−4.86, −0.29[b]	−0.34	−2.73, 2.05
Straw distance (mm)	0.01	−0.13, 0.15	0.02	−0.13, 0.17	0.00	−0.13, 0.14	−0.06	−0.20, 0.08	−0.01	−0.15, 0.14
Sperm motility (24 h)										
Average path velocity (μm/s)	0.72	−2.05, 3.49	−0.62	−3.53, 2.30	0.33	−2.44, 3.10	−0.63	−3.43, 2.16	1.29	−1.63, 4.20
Straight line velocity (μm/s)	0.12	−2.15, 2.40	−0.71	−3.10, 1.69	−0.37	−2.64, 1.91	−1.00	−3.30, 1.30	0.78	−1.61, 3.18
Curvilinear velocity (μm/s)	1.92	−2.91, 6.75	−0.27	−5.35, 4.80	1.10	−3.73, 5.93	−1.18	−6.06, 3.70	3.83	−1.24, 8.90
Amplitude head displacement (μm)	0.01	−0.29, 0.32	0.03	−0.29, 0.35	0.04	1.29, −1.63	−0.02	−0.33, 0.29	0.29	−0.03, 0.61
Beat cross frequency (Hz)	1.01	−0.52, 2.54	−0.47	−2.08, 1.14	0.67	−0.86, 2.20	−0.98	−2.52, 0.56	0.50	−1.11, 2.11
Straightness (%)	0.30	−3.89, 4.50	−4.57[b]	−8.95, −0.18[b]	−0.19	1.29, −1.63	−3.51	−7.72, 0.71	−0.89	−5.29, 3.52
Linearity (%)	0.05	−2.68, 2.78	−3.15[b]	−6.01, −0.30[b]	−0.19	−2.92, 2.54	−2.25	−4.99, 0.49	−1.56	−4.42, 1.30
Percent motility (%)	−0.23	−0.87, 0.40	−0.31	−0.98, 0.36	−0.36	−1.00, 0.27	−0.37	−1.01, 0.27	−0.30	−0.97, 0.37
Sperm head measurements										
Length (μm)	−0.01	−0.02, 0.01	0.01	−0.01, 0.02	−0.01	−0.02, 0.00	0.00	−0.01, 0.02	0.00	−0.02, 0.01
Area (μm^2)	−0.12	−0.32, 0.08	−0.07	−0.28, 0.14	−0.13	−0.33, 0.07	−0.04	−0.24, 0.16	−0.06	−0.27, 0.15
Width (μm)	−0.02	−0.06, 0.02	−0.04	−0.08, 0.00	−0.01	−0.05, 0.03	−0.03	−0.08, 0.01	0.00	−0.05, 0.04
Elongation factor (%)	−0.02	−1.27, 1.23	−1.29	−2.60, 0.01	0.41	−0.84, 1.66	−1.13	−2.39, 0.14	0.00	−1.32, 1.32
Perimeter (μm)	−0.07	−0.19, 0.05	0.02	−0.10, 0.15	−0.08	−0.20, 0.03	0.01	−0.10, 0.13	−0.04	−0.16, 0.08
Acrosome area of head (%)	0.59	−0.53, 1.70	−0.82	−1.99, 0.35	0.88	−0.24, 1.99	1.14[b]	0.01, 2.26[b]	−0.01	−1.19, 1.17
Morphology										
Strict criteria (%)[a]	0.59	−0.47, 1.64	−0.85	−1.99, 0.30	0.40	−0.66, 1.45	−0.08	−1.16, 1.00	0.72	−0.41, 1.86
Traditional normal (%)[a]	1.92	−1.18, 5.02	−2.64	−6.00, 0.71	1.46	−1.63, 4.56	−0.14	−3.31, 3.03	1.35	−1.98, 4.68
Amorphous (%)	−0.13	−0.37, 0.12	0.23	−0.04, 0.50	−0.15	−0.40, 0.09	−0.06	−0.32, 0.19	0.02	−0.25, 0.28
Round (%)	−0.02	−0.15, 0.11	0.09	−0.05, 0.23	0.02	−0.11, 0.15	−0.01	−0.15, 0.12	−0.04	−0.18, 0.10
Pyriform (%)	0.03	−0.17, 0.22	0.11	−0.10, 0.32	−0.02	−0.22, 0.17	0.15	−0.05, 0.35	−0.01	−0.23, 0.20
Bicephalic (%)	−0.04	−0.17, 0.10	0.12	−0.03, 0.27	−0.04	−0.17, 0.10	0.00	−0.14, 0.13	−0.03	−0.18, 0.11
Taper (%)	−0.06	−0.22, 0.11	0.09	−0.09, 0.26	−0.09	−0.25, 0.07	0.05	−0.11, 0.22	−0.01	−0.18, 0.17
Megalo head (%)	0.02	−0.10, 0.14	0.11	−0.02, 0.24	−0.02	−0.14, 0.10	0.03	−0.09, 0.15	0.07	−0.06, 0.19
Micro head (%)	−0.02	−0.13, 0.09	0.00	−0.12, 0.12	−0.03	−0.14, 0.08	0.05	−0.06, 0.17	−0.04	−0.16, 0.08
Neck/mid-piece abnormalities (%)	−0.05	−0.14, 0.04	0.05	−0.04, 0.15	−0.02	−0.11, 0.06	0.00	−0.09, 0.09	−0.05	−0.15, 0.05
Coiled tail (%)	0.05	−0.06, 0.15	−0.01	−0.12, 0.11	0.02	−0.09, 0.13	−0.01	−0.12, 0.10	−0.02	−0.13, 0.10
Other tail abnormalities (%)	−0.11	−0.24, 0.03	−0.16[b]	−0.31, −0.01[b]	−0.08	−0.22, 0.06	−0.03	−0.17, 0.11	−0.07	−0.21, 0.08
Cytoplasmic droplet (%)	0.09	−0.17, 0.35	0.09	−0.19, 0.37	0.07	−0.19, 0.33	−0.03	−0.29, 0.24	0.10	−0.18, 0.38
Immature sperm (#)	0.08	−0.14, 0.30	0.38[b]	0.15, 0.62[b]	0.05	−0.17, 0.27	0.01	−0.21, 0.24	0.16	−0.08, 0.40
Sperm chromatin stability assay										
DNA fragmentation index (%)	−0.02	−0.15, 0.11	−0.01	−0.14, 0.13	0.00	−0.13, 0.12	0.09	−0.04, 0.22	−0.04	−0.18, 0.09
High DNA stainability (%)	−0.08	−0.21, 0.06	0.13	−0.01, 0.27	−0.09	−0.22, 0.04	−0.09	−0.23, 0.04	0.01	−0.13, 0.15

Note: Models include each UV-filter dichotomized at the 75th percentile, creatinine (log-transformed), age, body mass index, active smoking (serum cotinine >43.5 ng/mL), season of enrollment (winter, spring, summer, fall), and research site. Various semen endpoints were transformed as stated in methods.

[a] Traditional and strict criteria; differentials were conducted using the traditional morphology.

[b] Significant finding.

Buck Louis. Benzophenones and semen quality. Fertil Steril 2015.

and wish to stress the preliminary nature of these findings and the need for cautious interpretation of the findings. We are aware of one previous article reporting that BP-3 can be detected and quantified in semen (37). In our previous article (18) focusing on these same five BP-type UV filters as measured in both partners of the couples participating in the LIFE Study, we found that male partners' BP-2 concentrations were associated with a significant 31%

reduction in couple fecundity, resulting in a longer time to pregnancy even after adjusting for the female partners concentrations. Although speculative, it remains possible that BP-2 may account for the longer observed time to pregnancy, perhaps through subtle alterations in semen endpoints. Further investigation of these findings is needed. In light of our exploratory analytic plan consistent with this work representing one of the earliest undertakings for this class of environmental chemicals and male fecundity as measured by semen quality, we refrain from further interpretation of the point estimates (i.e., β-coefficients) and emphasize that they are not directly comparable, given the number of independent models run. Rather, we summarize the results as supporting the need for additional research to corroborate (or not) these early findings. Our findings underscore the need for research beyond BP-3 to include other chemicals in this class, including its presumed derivatives, to understand any potential implications for human fecundity.

There is a small body of animal evidence focusing on BP-type UV filters and male reproduction. For example, BP-2 has been associated with hypospadias in mice (38). The estrogenic effects of BP-2 have been demonstrated in fathead minnows, where dose-dependent relationships were observed with gonad histology (testes had fewer spermatocytes), secondary sex characteristics, and reproduction (39). In zebrafish, low levels of BP-3 concentrations inhibited steroidogenesis and affected the hormonal milieu at different developmental stages (40). The relevance of these findings for human populations awaits future investigation.

In light of this study representing an initial attempt to assess BP-type UV filters and semen quality, as globally measured by various endpoints, cautious interpretation of the findings is needed. Most notably, our research relied on a single preconception measurement of BP-type UV filters. The degree to which this timing is relevant for the period of spermatogenesis remains to be established. Other research focusing on nonpersistent chemicals such as BPA and phthalates and semen quality has also relied on a single spot urine, which has prompted investigators to assess the reliability of a single measurement. We are aware of three publications reporting intraclass correlations (ICCs) for BP-3. Among 105 pregnant women in Puerto Rico, the ICC was 0.62 (41). Higher ICCs ranging from 0.80 to 0.92 were reported for a sample of 33 young Danish men (42) and in a sample of four Flemish couples (43).

Our modeling approach for this exploratory analysis was designed to differentiate men with higher vs. lower concentrations of UV filters in the context of a priori–specified covariates. As such, our findings need to be interpreted with these intentions, recognizing that we cannot eliminate potential residual confounding and that our findings may be to model specification. Our findings are based on a dichotomized exposure at the 75th percentile for each chemical, which mitigates the influence of nonlinear relations between exposures and semen endpoints. Further work, including various modeling options based on reported exposure distributions, will help to delineate potential associations meaningful for human fecundity. Although our models appropriately accommodated repeated semen samples per male, we cannot rule out chance findings because we did not control for multiple comparisons in light of our efforts to fully explore the BP-type UV filters and semen quality beyond the semen endpoints typically reported.

Other important limitations include the absence of a urologic examination that might have identified factors associated with semen quality. However, the extent to which such pathology might also be associated with UV-filters is unknown. Use of a 24-hour semen analysis is in keeping the population-based sampling framework used in the LIFE Study and reliance on next day analysis, which has been used in previous studies focusing on environmental chemicals and semen quality (44). Previous authors have compared at-home with clinic-based semen collection with regard to a range of semen endpoints save for DNA fragmentation, and reported no clinically significant effect of at-home collection on semen quality endpoints, including morphology (45). In fact, some authors report that at-home collection may be associated with higher-quality semen endpoints than clinic-based collection (46, 47). Still, we recognize that our semen analysis is not interchangeable with a clinical diagnostic analysis. We also recognize that motility is sensitive to time, underscoring the need for cautious interpretation of the motility findings. Additionally, there are some data suggesting that sperm DNA fragmentation increases over time (48, 49), though it is unlikely to be systematically associated with male partners' preconception concentrations of urinary BP-type UV filters. Finally, careful interpretation of our findings is needed, given that most of the signals were for the two BP-type UV filters with the highest percentage of measurements <LOD. Still, we know of no data to support a systematic difference in urine concentrations above or below the LOD, because the analytic laboratory was blinded to study participants' semen quality.

In sum, we observed that two of five measured BP-type UV filters were associated with changes in semen endpoints, including sperm concentration, sperm viability, motility, sperm head, and morphology. Whether such changes are sufficient to affect couple fecundity, as measured by the time needed to achieve pregnancy, or other couple-dependent fertility outcomes, remains to be established, as do underlying mechanisms.

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