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