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Urinary bisphenol S concentrations: potential predictors of and associations with semen quality parameters among men attending a fertility center.

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Abstract

Background: Bisphenol S (BPS) was introduced in the market as a potentially safer alternative to bisphenol A (BPA). However, there are limited studies on health effects of BPS and no epidemiologic studies on its relationship with male reproductive health outcomes, specifically semen quality.

Objective: To investigate predictors of urinary BPS concentrations and its association with semen parameters among men attending a fertility center.

Methods: This cross-sectional analysis included 158 men of couples seeking fertility treatment (2011-2017) contributing 338 paired semen and urine samples. At the time of sample collection, men completed a questionnaire on self-reported use of household products and food intake within the previous 24 hours. Urinary concentrations of BPA, BPS and bisphenol F were quantified using

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isotope-dilution tandem mass spectrometry. Semen samples were analyzed following WHO guidelines. Multivariable mixed models were used to investigate predictors of urinary BPS concentrations and to evaluate associations between urinary BPS concentrations and semen parameters, using random intercept to account for correlation in outcomes across multiple observations per man and adjusting for abstinence time, specific gravity, age, body mass index (BMI), year of sample collection and BPA concentrations. Analyses were also stratified by BMI (≥25 vs <25 kg/m^2).

Results: Median (IQR) urinary BPS concentration was 0.30 (0.20, 0.90) μg/L, and 76% of samples had detectable (>0.1 μg/L) concentrations. Self-reported fabric softener and paint/solvent use as well as intake of beef and cheese within 24 hours before urine collection were positively associated with BPS concentrations. Men with higher BPS concentrations also had significantly higher BMI. Lower semen parameters were found among men with detectable BPS concentrations, compared to men with non-detectable BPS [2.66 vs. 2.91 mL for volume (p=0.03), 30.7 vs. 38.3 mil/mL for concentration (p=0.03), 76.8 vs. 90.0 mil for total count (p=0.09), 43.7 vs. 47.0% for motility (p=0.06), and 5.42 vs. 6.77% for morphologically normal sperm (p=0.24)]. Some associations of BPS with lower semen parameters were only found among men with a BMI≥25 kg/m^2.

Conclusions: We identified dietary and lifestyle factors associated with BPS exposure, suggesting potential avenues for reducing exposures. We also observed negative associations between BPS and semen parameters, especially among overweight and obese men.

Keywords

BPS; BPA; predictors; semen quality; infertility

Introduction

Bisphenol S (BPS), a structural analog of bisphenol A (BPA), had its first reported mass commercial use in paper receipts in 2005 (Glausiusz 2014; Rochester et al. 2015). BPS was introduced in the market as a potentially safer alternative to BPA, which had demonstrated endocrine disrupting activities (Matsushima et al. 2007; De Coster et al. 2012; Bonefeld-Jörgensen et al. 2007) and shown associations with adverse health outcomes in the general population (Rochester, 2013; Rezg et al., 2014). Similar to uses for BPA, BPS can be found in canned and other pre-packaged foods, as well as thermal receipts (Clark et al. 2012; Liao et al. 2012a; Lehmler et al. 2018). BPS, along with another bisphenol analog, bisphenol F (BPF), is currently unregulated and there are no identified tolerable dose intakes (Eladak et al. 2015). Their production and utilization have increased during recent years (Liao et al. 2012; Žalmanová et al. 2016), as reflected by detection in environmental samples and human biomonitoring studies (Jin et al. 1997, CDC 2019). BPS was detected in 81% of adults from eight countries including the USA, and 78% in samples collected solely from the USA (Liao et al. 2012b; Zhou et al 2014). Another study of U.S. adults reported an increase from 19% to 74% in urinary concentrations of BPS between 2000 and 2014, with corresponding declines in BPA over the same time (Ye et al. 2015). BPS has also been commonly detected in urine samples in other regions, including the Middle -East and East Asia (Liao et al.

2012b; Zhou et al 2014). It has been reported that BPS is systematically absorbed and excreted within hours following exposure, reflecting its short half-life (Oh et al, 2017).

Given its chemical structure, BPS has not surprisingly a similar toxicological profile to BPA based on *in vivo* and *in vitro* models (Rochester et al. 2015). For example, *in vivo* studies of BPA have demonstrated estrogenic activity in crustaceans (Chen et al. 2002), zebrafish and rats (Ji et al 2013; Naderi et al. 2014, Yamasaki et al. 2003). In vitro studies have confirmed estrogenic properties of BPS (Grignard et al. 2012; Rosenmai et al. 2014; Vinas and Watson 2013). Specifically, animal models have demonstrated that BPS has similar endocrine disruption mechanisms to BPA, and can affect ovarian follicles, oocyte quality, and testosterone levels (Ullah et al. 2016; Nevoral et al. 2018; Žalmanová et al. 2016). Mechanisms of BPS effects have been found to be similar to those of BPA, including oxidative stress, anti-androgenic activity, genotoxicity, and mutagenicity (Usman A and Ahmad M. 2016; Fic et al. 2013, Fic et al. 2015). For example, plasma levels of both FSH and LH were diminished proportionally in rats exposed to higher levels of either BPA or BPS, indicating similar endocrine disrupting potency (Ahsan et al. 2018).

It has been observed that exposure to BPA is associated with semen quality through germ cells and spermatogenesis derangement (Phillips et al. 2008). BPA has been also linked to blood-testis barrier disruption with a subsequent immunologic insult to the testicular germ cells *in utero* exposure (Salian et al.2009, Toyama et al. 2004) While effects of BPA on other male reproductive outcomes have been investigated (Minguez-Alarcon et al. 2016), there is still lack of epidemiological data on the potential associations of its analogs, BPS and BPF, with testicular endpoints. We provide the first epidemiologic study examining whether urinary BPS concentrations are associated with semen quality parameters among men attending a fertility center, and investigate predictors of urinary BPS concentrations in this cross-sectional study of men attending a fertility center.

Methods

Study population

Study participants were male partners of couples enrolled in the Environment and Reproductive Health (EARTH) Study, an ongoing prospective cohort of couples seeking fertility treatment at the Massachusetts General Hospital (MGH) Fertility Center aimed at evaluating environmental and dietary determinants of fertility (Messerlian et al. 2018). Men between the ages of 18–56 years and without a history of vasectomy were eligible to participate, and approximately 40% of those contacted by the research nurses were enrolled. This cross-sectional analysis included 158 men contributing 338 paired urine and semen samples (repeated measures); the semen sample was collected on the same day and at the same time as the urine sample for the analysis of bisphenol concentrations. Although the EARTH Study was established in 2004, urinary concentrations of the BPA analogs, BPS and BPF, were first evaluated starting in 2011. Thus, a total of 382 men were excluded because of lack of urinary BPS and BPF concentration data. After the study procedures were explained, participants signed an informed consent form. The participant's date of birth was collected at entry, and weight and height were measured by trained study staff. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

The participants completed a staff-administered questionnaire that contained additional questions on lifestyle factors, reproductive health, and medical history. The study was approved by the Human Subject Committees of the Harvard T.H. Chan School of Public Health, Partners Healthcare, and the Centers for Disease Control and Prevention (CDC).

Quantification of urinary concentrations of bisphenols

Men provided one spot urine sample per semen sample. Urine was collected in a sterile polypropylene specimen cup. Specific gravity (SG), which was used to correct bisphenol concentrations for urine dilution, was measured at room temperature using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA) calibrated with deionized water before each measurement. The urine was divided into aliquots, frozen, and stored at −80 °C. Samples were shipped on dry ice overnight to the CDC where they were stored at or below −40 °C until analysis. As previously described (Minguez-Alarcon et al. 2018b; Ye et al. 2005), online solid-phase extraction coupled with isotope dilution-high-performance liquid chromatography-tandem mass spectrometry was used to quantify the urinary concentrations of BPS, BPF and BPA. The limits of detection (LOD) were 0.1 μg/L for BPS, and 0.2 μg/L for BPF and BPA. Samples with bisphenols concentrations below the LOD were assigned a value equal to the LOD divided by the square root of 2.

At the time of the urine collection, men completed a household product use and food intake questionnaire that asked whether during the previous 24 hours they had used certain products or consumed specific foods. The percentages of use, for each group and the total cohort, were calculated as the number of times men reported having used/eaten that specific item per survey divided by the total of surveys (urines) collected.

Analysis of semen samples

Semen and urine samples were both collected at the same time with the majority of the samples collected during the morning (86%). Semen samples were collected on site at MGH in a sterile plastic specimen cup following a recommended 2-5 days abstinence period as previously described (Minguez-Alarcon et al. 2018a). Of the 158 men in the study, 69 (44%) contributed one semen sample, 51 (32%) contributed 2 samples, and 38 (24%) contributed 3 or more samples (range=1-8). Semen volume (mL) was measured by an andrologist using a graduated serological pipet. Sperm concentration (mil/mL) and motility (% motile) were assessed using a computer-aided semen analyzer (CEROS; software version 12.3; Hamilton Thorne Biosciences, 5 Beverly, MA, USA). To measure semen concentration and motility, 6 μL of semen was placed into a pre-warmed (37°C) and disposable Leja Slide (Spectrum Technologies, CA, USA). A minimum of 200 sperm cells from at least four different fields were analyzed from each specimen. Total sperm count (mil/ejaculate) was calculated by multiplying sperm concentration by semen volume. Motile spermatozoa were defined as according to the World Health Organization (WHO) four-category scheme: rapid progressive, slow progressive, non-progressive, and immotile (WHO 2010). Sperm morphology (% normal) was assessed on two slides per specimen (with a minimum of 200 cells assessed per slide) via a microscope with an oil-immersion 100× objective (Nikon, Tokyo, Japan). Strict Kruger scoring criteria was used to classify men as having normal or below normal morphology (Kruger et al. 1988). Andrologists were trained in semen analysis

and participated in rigorous daily and weekly internal quality control and external monitoring of within and between observer variation as required to maintain CLIA certification and accreditation by the College of American Pathologists. Infertility diagnosis was coded according to previously described definitions of the Society for Assisted Reproductive Technology (SART) including female, male and unexplained (SART 2015).

Statistical analysis

Demographic characteristics, semen quality parameters and frequency of household products use and food intake of the men were presented using median ± interquartile ranges (IQRs) or counts (%). Due to concern regarding potential non-linear relationships between BPS and semen parameters, urinary BPS concentrations were categorized into quartiles or into two groups, below and above the LOD (e.g., detectable vs non-detectable). Associations between demographic characteristics across quartiles of urinary BPS concentrations were evaluated using Kruskal– Wallis tests for continuous variables and Chi-squared tests for categorical variables (or Fisher's exact test where appropriate). Multivariable mixed models were used to investigate predictors of urinary BPS concentrations and to evaluate associations between urinary BPS concentrations and semen parameters, using random intercept to account for correlation in outcomes across multiple observations per man and adjusting for specific gravity and potential confounders. We evaluated the robustness of the BPS and semen parameters findings by restricting analyses to one semen sample (first sample) per man and also modeling the semen parameters as binary variables (above vs. below WHO reference limits). It may be possible that men who provided more semen samples had poorer semen quality and thus had female partners with more infertility treatment cycles (study visits).

Confounding was assessed using prior knowledge on biological relevance and descriptive statistics from our study population. The variables considered as potential confounders included factors previously related to male reproductive endpoints (Rooney and Domar 2014; Sharma et al. 2013), and factors associated with urinary BPS and semen parameters in this study. Fully adjusted models included abstinence time (days), specific gravity, age (years), BMI (kg/m^2), year of sample collection (year) and log-transformed bisphenol A concentrations (μg/L), To allow for better interpretation of the results, population marginal means (Searle et al. 1980) are presented adjusting for all the covariates in the model (at the mean level for continuous variables and for categorical variables at a value weighted according to their frequencies). Stratification of associations of BPS with semen parameters by BMI (≥25 vs <25 kg/m^2) was performed to evaluate modification by BMI. Statistical analyses were conducted with SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Statistical tests were two-tailed and all p-values<0.05 were regarded as statistically significant.

Results

Men included in this analysis had a median (IQR) age and BMI of 35.6 (32.6–39.0) years and 26.7 (24.1–30.1) kg/m^2, respectively (Table 1). Men were predominantly Caucasian (88%), highly educated (60% had a graduate degree) and 32% had ever smoked. Male factor infertility was diagnosed at enrollment among 36 men (23%). Men in the highest quartile of

urinary BPS concentrations were significantly heavier compared to men in the lowest quartile of BPS (mean BMI 27.0 vs 24.9 kg/m^2). No other demographic characteristics significantly differed across quartiles of urinary BPS concentrations (Table 1). For the 338 semen samples contributed by the 158 men, the median (IQR) values were 47.6 (22.4, 90.1) mil/mL for sperm concentration; 121 (58.0, 230) mil for total sperm count; 48 (27, 66) % for sperm motility; and 4 (3, 7) % for morphologically normal spermatozoa (Supplemental Table S1). Over one-third of semen samples (39%) were below the WHO 2010 lower reference limit for progressive sperm motility (> 32%) (WHO 2010). Men included in this analysis had slightly lower sperm concentration and total sperm count but were similar in their demographic characteristics and other semen parameters compared to men who were excluded from the analysis because of lack of measured urinary BPS concentrations (Supplemental Table S2).

In the 338 urine samples collected from the 158 men in the EARTH Study between 2011 and 2017, detection frequency was 76% (BPS) and 88% (BPA) (Table 2). We excluded BPF from further analysis because its detection frequency was 25%. The geometric mean (GM) BPS and BPA urinary concentrations were 0.37 and 0.77 μg/L, respectively. BPS concentrations did not significantly differ in samples collected between 2015 and 2017, compared to those collected between 2011 and 2014 (medians=0.40 vs. 0.30 μg/L, respectively). Urinary concentrations of BPA significantly decreased during the second part of the study (2015-2017) compared to those collected in earlier years (2011-2014) (medians=0.60 vs. 1.00 μg/L, respectively). The Spearman correlation for urinary concentrations of BPS and BPA was 0.45.

Self-reported use of fabric softener or paints/solvents during the 24 hours preceding urine collection was positively associated with urinary BPS concentrations (Table 3). Specifically, all six men who reported having used fabric softener in the 24 hours prior to urine collection had detectable urinary BPS concentrations (p-value=0.002). Similarly, a total of 10 men reported having used paints or solvents during the previous 24 hours to urine collection, and nine of those 10 men had detectable BPS concentrations in urine, compared to one man who had non-detectable concentrations (p-value=0.05). In addition, a higher percentage of men with detectable BPS had self-reported beef or cheese intake during the 24 hours prior to urine collection than those with non-detectable BPS (41% vs 19% with beef intake, 72% vs 59% with cheese intake) (Table 3). No other personal household products or food queried were significantly related to urinary concentrations of BPS.

Lower semen quality parameters with higher urinary BPS concentrations were observed in models adjusted for abstinence time and specific gravity, and in those further adjusted for age, BMI and year of sample collection after controlling for urinary BPA concentrations (Table 4). For example, men in the second, third and fourth quartile of urinary BPS concentrations had, respectively, 18% (p=0.05), 24% (p=0.03) and 20% (p=0.09) lower sperm concentration, compared to men in the first quartile of BPS, in the fully adjusted models. Similarly, 8% (p=0.06), 4% (0.33) and 9% (p=0.09) lower motility was found among men in quartiles 2, 3, and 4, respectively, compared to men in the lowest quartile of urinary BPS concentrations. Differences were also observed when comparing men with detectable urinary BPS concentrations to those with non-detectable BPS [2.66 vs. 2.91 mL

for volume (p=0.03), 30.7 vs. 38.3 mil/mL for concentration (p=0.03), 76.8 vs. 90.0 mil for total count (p=0.09), 43.7 vs. 47.0% for motility (p=0.06), and 5.42 vs. 6.77% for morphologically normal sperm (p=0.24)] (Table 4). Similar differences in semen quality parameters by urinary BPS concentrations were found when analyses were restricted to the first semen sample per man (Supplemental Table 3). However, results did not reach significant because of the smaller sample size (N=158). In addition, significantly higher probabilities of having low sperm concentration (<15 mil/mL) and motility (<40 %) were observed among men with detectable urinary BPS concentrations compared to those with non-detectable BPS (Supplemental Table S4).

Some of these inverse associations of BPS with semen parameters were only observed among overweight or obese men, but not among normal weight men (Figure 1). Specifically, among men with BMI>25 kg/m^2 (106 men contributing 225 semen samples), those with detectable concentrations of urinary BPS had significantly lower sperm concentration, total count and motility [26.7 vs. 42.3 mil/mL (p=0.007), 66.3 vs. 98.7 mil (p=0.02), and 41.9 vs. 46.9% (p=0.02), respectively] compared to men with non-detectable concentrations of urinary BPS. No significant associations of BPS with ejaculate volume or morphologically normal sperm were observed among overweight/obese men (data not shown). Among leaner men (<25 kg/m^2, 52 men contributing 113 semen samples), no significant differences in semen parameters were found when comparing men with detectable BPS concentrations versus men with non-detectable BPS [43.4 vs. 43.2 mil/mL for concentration (p=0.98), 110 vs. 104 mil for total count (p=0.74), and 48.1 vs. 49.9% for motility (p=0.51)].

Discussion

To our knowledge, this is the first study to investigate potential associations between urinary BPS concentrations and semen parameters among a group of men. We detected BPS in 76% of men, and found associations of urinary BPS concentrations with lower ejaculate volume, sperm concentration, total count and motility, in adjusted models and models further adjusted for urinary BPA concentrations. Some of these associations were only observed among overweight and obese men, but not among leaner men. In addition, urinary BPS concentrations were positively associated to use of fabric softener and paint/solvent as well as intake of beef and cheese within 24 hours before urine collection. Our results indicate that men are exposed to BPS and that BPS concentrations are associated with poorer semen quality in this study population of men who presented to the MGH Fertility Center.

Our results are in agreement with animal studies showing detrimental effects of BPS on the male reproductive system (Ji et al. 2013; Ullah et al. 2016). For example, BPS exposure has been associated with cellular oxidative stress and antiandrogenic activities (Fic et al. 2015; Fic et al. 2013). In an *in vitro* study, Eladak *et al* observed harmful effects of BPS exposure, similar to BPA exposure, on the physiologic function of human and mouse testes tissue (Eladak et al. 2015). They used a culture system of fetal testis assay and measured testosterone secretion in a dose-response curve for exposure of different bisphenol analogues at varying concentrations. A study by Kitamura *et al.* observed toxicological effects of bisphenol analogs on androgen activity alteration (Kitamura et al. 2004). It examined the role of BPA, BPS, and BPF on the androgen receptors, and found that BPS exhibited anti-

androgenic activities at concentrations between 1×10^{-6} to 1×10^{-4} M. These results are in agreement with another study indicating that all analogs for BPA have potentially toxic effects on human reproduction through the alteration of the endocrine activity (Rosenmai et al. 2014). Specifically, BPS showed an inhibition of testosterone secretion in human fetal testis and was found to have a more inhibitory potential effect on mouse fetal testis compared to BPA. Further cortisol and aldosterone secretion inhibition were shown to occur with all bisphenol analogs, including BPS.

Some of the inverse associations of urinary BPS concentrations with semen parameters were only observed among overweight and obese men. One possible hypothesis for this interesting finding is that overweight/obese men may be more sensitive to BPS exposure given that they are simultaneously exposed to a hyperestrogenic environment, since obesity increases circulating estrogen levels in men (Schneider et al, 1979), and an antiandrogenic signal, since BPS has antiandrogenic activity (Usman A & Ahmad M. 2016). Similar interpretation was given when we previously reported that the negative association between soy intake and semen parameters was stronger among overweight/obese men from the same study (Chavarro et al. 2008). This is particularly relevant since overweight and obesity have become a major public health concern worldwide especially among the U.S. adult population (Kelly et al. 2005; Wang et al. 2008; Flegal et al. 2012). Further studies are needed to corroborate this hypothesis.

Fabric softener and paint/solvent use, as well as consumption of beef and cheese within 24 hours before urine collection were associated with urinary BPS concentrations in the current study. In a study investigating bisphenols in consumer products collected in New York (USA), BPS was mainly detected in meat products (Liao and Kannan. 2013). However, urinary BPS concentrations were not associated with any self-reported product use (cleaning, personal care and pet products) among pregnant women in Northern Puerto Rico even though BPS was detected in 90% of urine samples (LOD=0.1 μg/L) with a median concentration of 0.5 μg/L (Ashrap et al. 2018). Because BPS is one of the replacements for BPA, co-exposure in humans is expected, as the Spearman correlation results between both bisphenols in our analysis indicated. We also found a trend of higher BPS concentration and lower BPA concentrations in recent years of the study (2015-2017), compared to earlier years (2011-2014). Other studies reported similar trends. For example, BPS concentrations in urine samples from U.S. adults have been increasing between 2000 and 2014 (Ye et al. 2015). Nevertheless, although BPA concentrations show a downward trend (CDC 2019; Ye et al. 2015; Ashrap et al. 2018), BPA concentrations are still higher compared to BPS (CDC 2019; Lehmler et al. 2018; Wu et al. 2018). Despite the negative associations between urinary BPS and certain semen parameters among men in this study, GM for urinary concentrations of BPS and BPA in this study were lower than those reported for males of all ages in the U.S. general population in 2013-2014: 0.46 μg/L for BPS and 1.43 μg/L for BPA (CDC 2019).

The current study has several limitations. First, it is uncertain whether our findings can be generalized to men in the general population and in non-Western countries. However, men in our study tended to have good semen quality compared to international reference standards (WHO 2010) and also fertile men (Levine et al. 2017). Second, exposure misclassification is

possible given the short biological half-lives of target bisphenols and the likely episodic nature of the exposures (Braun et al. 2012). However, 56% of the participants contributed more than one urine sample which would partially reduce exposure misclassification. Third, the cross-sectional design of this particular analysis limits our ability to infer causality. Last, some of the positive associations between urinary BPS and product use and food intake may be due to chance because of the low frequency of use for some items. Further studies are needed to corroborate these novel findings. The biggest strength of this study is the comprehensive adjustment for other demographic, reproductive and lifestyle factors that could result in residual confounding, such as co-exposure to BPA. Another important strength included the use of data on product use and food intake questionnaire

In conclusion, we identified some household products and foods as predictors of BPS exposure. Our findings also showed, for the first time, negative associations between urinary BPS concentrations and semen parameters, especially among overweight and obese men. Further studies are needed to replicate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- BPS was detected in 76% of the urine samples.
- Urinary BPS was positively associated to use of fabric softener and paint/solvent as well as intake of beef and cheese within 24 hours before urine collection.
- Urinary BPS was associated with lower semen parameters, and some associations were only observed among overweight and obese men.

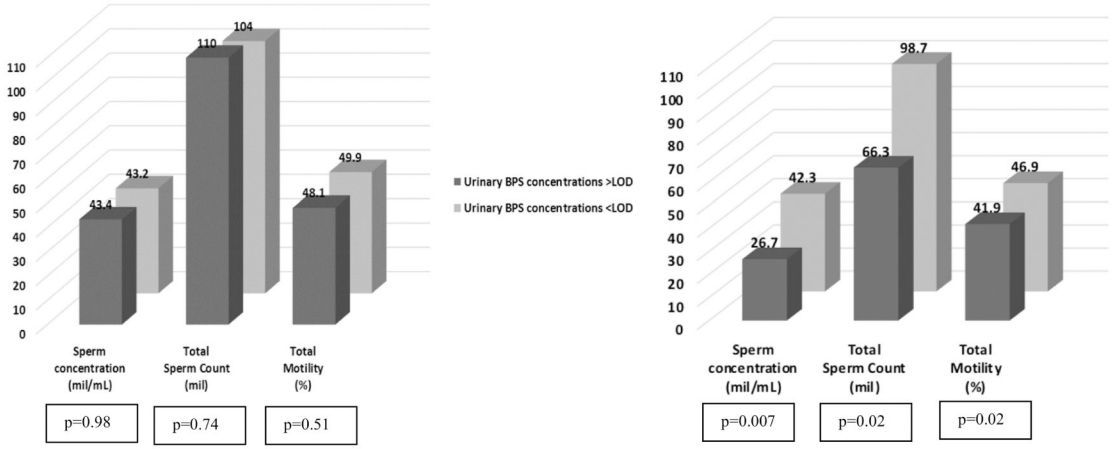

Figure 1.

Models are adjusted for abstinence time, specific gravity, age, BMI, year of sample collection and log-bisphenol A concentrations. P-interactions: 0.001 for sperm concentration, 0.05 for total sperm count and 0.15 for total motility. The limit of detection (LOD) was 0.1 μg/L. Medians (IQRs) of urinary BPS (μg/L) for leaner and obese/overweight men with concentrations above LOD were 0.50 (0.30, 1.00) and 0.50 (0.30, 1.10), respectively.

Table 1.

Demographic and reproductive characteristics [median (IQR) or counts (%)] across quartiles of urinary bisphenol S concentrations among 158 men in the EARTH Study.

		Quartiles of urinary bisphenol S concentrations				
	Total cohort N=158 men	**Q1 N=38 men**	**Q2 N=48 men**	**Q3 N=34 men**	**Q4 N=38 men**	**p-value[a] across quartiles**
Age, years	35.6 (32.6, 39.6)	35.6 (32.6, 39.0)	35.8 (32.7, 40.5)	35.7 (33.4, 39.5)	36.1 (31.8, 38.7)	0.81
Race, n (%)						0.62
White/Caucasian	139 (88)	33 (87)	43 (90)	28 (82)	35 (92)	
Black/Asian/Other	19 (22)	5 (13)	5 (10)	6 (18)	3 (8)	
Body Mass Index, kg/m^2	26.7 (24.1, 30.1)	24.9 (23.5, 26.8)	26.7 (25.3, 30.3)	28.7 (24.3, 31.6)	27.0 (24.2, 29.4)	0.008
Ever smoker, n (%)	51 (32)	13 (34)	16 (33)	10 (29)	12 (32)	0.97
Education, n (%)						0.48
High school/some college	15 (10)	3 (8)	4 (8)	3 (9)	5 (13)	
College graduate	49 (31)	7 (18)	17 (36)	11 (32)	14 (37)	
Graduate degree	94 (60)	28 (74)	27 (56)	20 (59)	19 (50)	
Total physical activity (hrs/week)	4.02 (0, 10.7)	3.6 (0, 7.9)	4.7 (0, 8.4)	3.7 (0, 11.7)	6.9 (0.8, 12.5)	0.48
Infertility diagnosis, n (%)						0.82
Male	36 (23)	8 (21)	9 (19)	8 (24)	11 (29)	
Female	57 (36)	12 (32)	19 (40)	11 (32)	15 (39)	
Unexplained	65 (41)	18 (47)	20 (42)	15 (44)	12 (32)	

Table 2.

Distribution of urinary concentrations (μg/L) of bisphenol S, bisphenol F and bisphenol A among 158 men contributing 338 semen samples in the EARTH Study.

	Entire study period, 2011-2017 N=338						2011-2014 N=186			2015-2017 N=152			p-value comparing both periods
	N urines	Detection Frequency %	GM (SD)	25th	50th	75th	25th	50th	75th	25th	50th	75th	
Bisphenol S	338	76	0.37 (0.03)	0.20	0.30	0.90	0.20	0.30	0.80	<LOD	0.40	0.90	0.92
Bisphenol F	181	25	<LOD	<LOD	<LOD	0.30	<LOD	<LOD	0.40	<LOD	<LOD	0.30	-
Bisphenol A	338	88	0.77 (0.05)	0.40	0.80	1.60	0.50	1.00	1.80	0.30	0.60	1.15	.0001

Note: The limit of detection (LOD) for bisphenol S is 0.1 μg/L. and bisphenol F and bisphenol A are 0.2 μg/L.

Table 3.

Household product use [n (%)] and food consumption [n (%)] within the previous 24 hours of urine collection by urinary bisphenol S concentrations among 158 men in the EARTH Study.

			Quartiles of urinary bisphenol S concentrations, µg/L (range)					
	N urines	**Total cohort**	**Q1 (<LOD)**	**Q2 (0.20, 0.30)**	**Q3 (0.40, 0.80)**	**Q4 (0.90, 52.0)**	**Q2-4 (>LOD)**	**p-value Q1 vs. Q2-4**
Fabric softener	239	6 (3)	0 (0)	1 (2)	3 (5)	2 (3)	6 (3)	0.002
Laundry detergent	238	33 (14)	6 (11)	8 (13)	10 (18)	9 (14)	27 (15)	0.87
Dishwashing liquid	238	150 (63)	34 (63)	38 (60)	35 (63)	43 (66)	116 (63)	0.68
Paints/solvents	239	10 (4)	1 (2)	3 (5)	2 (4)	4 (6)	9 (5)	0.05
Gum	238	34 (14)	10 (19)	12 (19)	9 (16)	3 (5)	24 (10)	0.42
Mints	234	30 (13)	6 (11)	10 (16)	6 (11)	8 (13)	24 (13)	0.23
Had eaten in stored/heated plastic container	238	98 (41)	18 (33)	26 (42)	27 (47)	27 (42)	80 (44)	0.28
Canned beverages	238	20 (8)	4 (7)	6 (10)	4 (7)	6 (9)	16 (9)	0.81
Canned food	239	62 (26)	13 (24)	16 (25)	15 (26)	18 (28)	49 (27)	0.76
Beef	239	94 (40)	10 (19)	33 (52)	22 (39)	29 (45)	84 (41)	0.0006
Lamb	239	9 (4)	1 (2)	2 (3)	1 (2)	5 (8)	8 (4)	0.18
Pork	239	71 (30)	14 (26)	17 (27)	18 (32)	22 (34)	57 (31)	0.17
Chicken	239	112 (47)	22 (41)	32 (51)	29 (51)	29 (45)	90 (49)	0.59
Fish	239	41 (17)	11 (20)	5 (8)	16 (28)	9 (14)	30 (16)	0.26
Other poultry	239	30 (13)	4 (7)	10 (16)	6 (11)	10 (15)	26 (14)	0.12
Cold cuts	238	49 (21)	9 (17)	14 (22)	9 (16)	17 (26)	40 (22)	0.60
Hot dogs	238	11 (5)	1 (2)	3 (5)	1 (2)	6 (9)	10 (5)	0.23
Milk	238	142 (60)	27 (51)	38 (60)	39 (68)	38 (58)	115 (62)	0.20
Sausage	238	28 (12)	6 (11)	7 (11)	9 (16)	6 (9)	22 (12)	0.20
Cheese	238	164 (69)	31 (59)	43 (68)	44 (77)	46 (71)	133 (72)	0.05
Ice-cream	238	44 (19)	13 (25)	13 (21)	6 (11)	12 (19)	31 (17)	0.45

Note: Floor wax, car wax, pesticides, flea/tick prevention products, laundry starch, vinyl boots, vinyl gloves, vinyl raincoat, grooming products, mothballs and intake of veal have been excluded from analysis because of low frequency (<3%). The limit of detection (LOD) was 0.1 µg/L.

Table 4.

Semen quality parameters (adjusted mean, 95% CI) by urinary bisphenol S concentrations among 158 men contributing 338 semen samples in the EARTH Study.

	Ejaculate volume (mL)	Sperm concentration (mil/mL)	Total sperm count (mil)	Total motility (%)	Total motile count (mil/ejaculate)	Normal Morphology[a] (%)	Normal morphology count[a] (mil/ejaculate)
Adjusted for abstinence time and specific gravity							
Q1	2.67 (2.41, 2.94)	38.5 (27.9, 53.1)	91.3 (66.2, 126)	47.0 (42.7, 51.3)	26.5 (14.3, 49.1)	6.90 (6.09, 7.72)	6.85 (5.05, 9.29)
Q2	2.99 (2.75, 3.25) *	31.5 (22.9, 43.4) ǂ	83.4 (60.8, 114)	43.2 (39.0, 47.4) ǂ	21.0 (11.2, 39.3)	6.18 (5.59, 6.78)	5.80 (4.43, 7.58)
Q3	2.88 (2.60, 3.15)	29.0 (20.4, 41.2) *	71.2 (49.7, 102) ǂ	44.8 (39.8, 49.8)	19.0 (9.6, 37.9)	6.75 (6.01, 7.49)	5.02 (3.40, 7.41) ǂ
Q4	2.82 (2.55, 3.10)	30.6 (21.7, 43.1) ǂ	73.4 (51.5, 105) ǂ	43.2 (38.6, 47.7) ǂ	17.4 (8.8, 34.5)	6.69 (5.87,7.50)	5.23 (3.71, 7.39)
Adjusted for abstinence time, specific gravity, age, BMI and year of sample collection							
Q1	2.68 (2.41, 2.94)	38.5 (28.0, 52.8)	91.0 (66.2, 125)	47.0 (42.7, 51.2)	26.3 (14.2, 48.8)	6.91 (6.08, 7.75)	6.84 (5.05, 9.28)
Q2	3.00 (2.76, 3.26) *	31.4 (22.8, 43.1) ǂ	82.7 (60.0, 114)	43.2 (39.0, 49.8) ǂ	20.6 (10.9, 39.0)	6.12 (5.52, 6.71) ǂ	5.72 (4.34, 7.53)
Q3	2.89 (2.62, 3.15)	29.1 (20.6, 41.1) *	71.7 (50.3, 102) ǂ	44.8 (40.0, 49.8)	19.2 (9.80, 37.7)	6.78 (6.06, 7.50)	5.15 (3.57, 7.45) ǂ
Q4	2.82 (2.56, 3.08)	30.8 (22.0, 43.0) ǂ	74.1 (52.5, 105) ǂ	43.2 (38.7, 47.7) ǂ	17.7 (9.10, 34.7)	6.71 (5.92, 7.51)	5.31 (3.80, 7.43)
Adjusted for abstinence time, specific gravity, age, BMI, year of sample collection and log-bisphenol A concentrations							
Q1	2.68 (2.41, 2.95)	38.4 (28.0, 52.7)	91.1 (66.4, 125)	46.9 (42.6, 51.3)	26.3 (14.3, 48.7)	6.91 (6.09, 7.73)	6.84 (5.05, 9.26)
Q2	3.00 (2.75, 3.25) *	31.4 (22.8, 43.3) ǂ	82.5 (59.8, 114)	43.2 (39.0, 47.5) ǂ	20.6 (10.9, 39.0)	6.11 (5.51, 6.71) ǂ	5.69 (4.30, 7.53)
Q3	2.87 (2.61, 3.14)	29.2 (20.6, 41.5) *	71.4 (49.9, 102) ǂ	44.9 (39.9, 49.9)	19.2 (9.70, 38.0)	6.77 (6.06, 7.49)	5.12 (3.53, 7.43) ǂ
Q4	2.81 (2.55, 3.08)	30.9 (22.0, 43.4) ǂ	74.0 (52.2, 105) ǂ	43.3 (38.7, 47.8) ǂ	17.7 (9.00, 34.8)	6.71 (5.91, 7.51)	5.29 (3.78, 7.41)
<LOD[b]	2.91 (2.69, 3.12)	38.3 (28.0, 52.4)	90.0 (65.7, 123)	47.0 (42.6, 41.3)	25.9 (14.1, 47.7)	6.99 (6.16, 7.82)	6.77 (4.99, 9.19)
>LOD	2.66 (2.39, 2.92) *	30.7 (22.4, 42.0) *	76.8 (56.2, 105) ǂ	43.7 (40.0, 47.4) ǂ	19.3 (10.4, 35.7)	6.46 (5.94, 7.00)	5.42 (4.16, 7.06)

[a] A total of 10 semen samples (3%) had missing data for normal sperm morphology and thus total normal morphology count, resulting in N=328 semen samples.

[b] The limit of detection (LOD) was 0.1 μg/L.

* p-value <0.05 when compared that group with the lowest group of exposure.

ǂ p-value <0.10 when compared that group with the lowest group of exposure.