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Couples' Urinary Concentrations of Benzophenone-Type Ultraviolet Filters and the Secondary Sex Ratio

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Abstract

The secondary sex ratio (SSR), defined as the ratio of males to females at birth, has been investigated in relation to endocrine disruptors to search for environmental toxicants perturbing human sex selection. Benzophenone (BP)-type ultraviolet (UV) filters, which are used in sunscreens and personal care products, have been reported to exert estrogenic and anti-androgenic activities. This study aimed to evaluate the association between maternal, paternal, and couple urinary concentrations of BP-type UV filters and the SSR, given the absence of previous investigation. The study cohort comprised 220 couples who were enrolled in the Longitudinal Investigation of Fertility and the Environment (LIFE) Study between 2005–2009 prior to conception and who had a singleton birth during the follow-up period. Couples' urinary concentrations of five BP-type UV filters (ng/mL) were measured using triple-quadrupole tandem mass spectrometry: 2,4-dihydroxybenzophenone (BP-1), 2,2',4,4'-tetrahydroxybenzophenone (BP-2), 2-hydroxy-4-methoxybenzophenone (BP-3), 2,2'-dihydroxy-4-methoxybenzophenone

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Ethical statement

This study was approved by the Institutional Review Boards at all collaborating institutions. Written informed consent was provided by all study participants before any data collection. This study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki; <http://www.wma.net/e/policy/b3.htm>) and Uniform Requirements for Manuscripts Submitted to Biomedical Journals (<http://www.nejm.org/general/text/requirements/1.htm>).

Conflict of interest: The authors declare no competing financial interest.

(BP-8), and 4-hydroxybenzophenone (4-OH-BP). Modified Poisson regression models were used to estimate the relative risks (RRs) of a male birth for each BP-type UV filter, after adjusting for potential confounders. When maternal and paternal urinary BP-type UV filter concentrations were modeled jointly, both maternal BP-2 (2nd vs 1st tertile, RR, 0.62, 95% confidence interval [CI], 0.43–0.91) and paternal BP-2 (3rd vs 1st tertile, RR, 0.67, 95% CI, 0.45–0.99; *p*-trend, 0.04) were significantly associated with an excess of female births. Contrarily, maternal 4-OH-BP was significantly associated with an excess of male births (2nd vs 1st tertile, RR, 1.87, 95% CI, 1.27–2.74; 3rd vs 1st tertile, RR, 1.80, 95% CI, 1.13–2.87; *p*-trend, 0.02). Our findings provide the first evidence suggesting that BP-type UV filters may affect the SSR. However, future corroboration is needed, given the exploratory design of this study.

Graphical abstract

Keywords

benzophenones; endocrine disruptors; fertility; sex ratio; sunscreen agents

1. Introduction

While the primary sex ratio is defined as the ratio of males to females at conception, the secondary sex ratio (SSR) is defined as the ratio of males to females at birth (Buck Louis and Platt, 2011). Due to difficulties in investigating conception at the population level (Orzack et al, 2015), the SSR, as an indicator of population health and fertility, has been explored in relation to endocrine disruptors to search for environmental toxicants perturbing human sex selection (Terrell et al., 2011). Deviations from an expected SSR, which is thought to range from 1.05 to 1.07 (Central Intelligence Agency; Mathews and Hamilton, 2005), can provide clues about underlying changes in population-wide environmental factors (Davis et al., 2007). Diverse classes of chemicals suspected of affecting endocrine function have been examined in relation to various reproductive and developmental outcomes including the SSR in humans. Specifically, persistent chemicals, which can accumulate in the environment and human body, have been reported to be associated with alterations in the SSR, exemplified by chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Mocarelli et al., 2000), polychlorinated biphenyls (PCBs) (Nieminen et al., 2013), and perfluoroalkyl and polyfluoroalkyl substances (PFASs) (Bae et al., 2015b). Although inconclusive with limited evidence, parental exposure to non-persistent chemicals with a

relatively short half-life and low bioaccumulative potential, such as bisphenol A (BPA) and phthalates, has been also reported to be associated with an excess of male or female births (Bae et al., 2015a).

Sunscreen agents are routinely applied to the skin by a large percentage of contemporary populations to provide protection against ultraviolet (UV) radiation, which is a well-known cause of skin photoaging and photocarcinogenesis (Pillai et al., 2005; Saraiya et al., 2004). These agents commonly contain a combination of several organic and inorganic UV filters for broad-spectrum protection against different types of UV light (Villalobos-Hernández and Müller-Goymann, 2006). Due to their photoallergic properties, organic or chemical UV filters such as para-aminobenzoic acid (PABA) and benzophenones (BPs) were first targeted by public health concern, while inorganic or mineral UV filters such as zinc oxide and titanium dioxide were then at the heart of scientific debates particularly because of their nanometric size (Gilbert et al., 2013). Along with acute toxic effects of sunscreen agents, interest in their chronic toxicity including reproductive and developmental effects has been growing, in light of their systemic absorption after topical application (Janjua et al., 2008; Jiang et al., 1999; Maier and Korting, 2005).

Although PABA was introduced as one of the first organic UV filters (Mackie and Mackie, 1999), 2-hydroxy-4-methoxybenzophenone (BP-3), also known as oxybenzone, is one of the most commonly-used chemical components in sunscreen formulations (Careghini et al., 2015; Kim and Choi, 2014; Maier and Korting, 2005). Indeed, different types of BP compounds from 2,4-dihydroxybenzophenone (BP-1) to 2-hydroxy-4-octyloxybenzophenone (BP-12) have been used in a variety of personal care products including sunscreens and cosmetics as well as plastic surface coatings for food packaging, because of their UV absorption properties (Kawamura et al., 2003; Liao and Kannan, 2014; Park et al., 2013; Schlecht et al., 2006). With diverse sources for human exposure, widespread occurrence of BP-3 as well as its metabolic derivatives such as BP-1, 2,2',4,4'-tetrahydroxybenzophenone (BP-2), 2,2'-dihydroxy-4-methoxybenzophenone (BP-8), and 4-hydroxybenzophenone (4-OH-BP) has been reported in many countries including the United States (Calafat et al., 2008; Philippat et al., 2015; Wang and Kannan, 2013; Wolff et al., 2008), China (Wang and Kannan, 2013; Zhang et al., 2013), and some European countries (Asimakopoulos et al., 2014; Philippat et al., 2012; Schlumpf et al., 2010). The reported urinary concentrations of BP-type UV filters, as commonly-used biomarkers of exposure to these chemicals, varied markedly across populations, with relatively scanty data on these chemical concentrations in other samples such as blood (Zhang et al., 2013) and breast milk (Schlumpf et al., 2010).

BP-type UV filters are an emerging class of endocrine disruptors, with various documented hormonal activities *in vitro* and *in vivo*. Specifically, BP-3 and its metabolic derivatives such as BP-1, BP-2, BP-8, and 4-OH-BP have been shown to elicit estrogenic activity in a variety of *in vitro* assays (Kawamura et al., 2003, 2005; Kunz and Fent, 2006; Morohoshi et al., 2005; Nakagawa and Suzuki, 2002; Nakagawa et al., 2000; Ogawa et al., 2006; Schlumpf et al., 2001, 2004; Schreurs et al., 2002, 2005; Suzuki et al., 2005). Of note, the estrogenic potency of some metabolic derivatives of BP-3 including BP-1, BP-2, and 4-OH-BP was found to be higher than that of their parent compound, BP-3 (Kunz and Fent, 2006;

Morohoshi et al., 2005). In acute *in vivo* models, the estrogenic activity of BP-type UV filters was confirmed by increased uterine weight following BP-3 (Schlumpf et al., 2001, 2004), BP-1 (Schlumpf et al., 2004), BP-2 (Schlumpf et al., 2004; Yamasaki et al., 2003), or 4-OH-BP (Nakagawa and Tayama, 2001; Yamasaki et al., 2003) exposure in immature rats, although the uterotrophic effect in immature rats conflicted with unchanged uterine weight following BP-3 exposure in oophorectomized rats (Schlecht et al., 2004; Suzuki et al., 2005). Another reported hormonal activity of BP-3 and its metabolic derivatives such as BP-1, BP-2, BP-8, and 4-OH-BP is *in vitro* anti-androgenic activity (Kawamura et al., 2005; Kunz and Fent, 2006; Ma et al., 2003; Schlumpf et al., 2004; Schreurs et al., 2005; Suzuki et al., 2005). Furthermore, some (Kunz and Fent, 2006; Morohoshi et al., 2005) but not all (Schreurs et al., 2002) experimental studies demonstrated that BP-3 exerted anti-estrogenic activity *in vitro*. Additionally, *in vitro* antagonistic action on human progesterone receptor (PR) was noted for BP-3 (Schreurs et al., 2005). BP-2 was also found to be androgenic *in vitro*, with the androgenic potency approximately 30,000-times less than that of 4,5-dihydrotestosterone (DHT) (Kunz and Fent, 2006).

Some (French, 1992; Hsieh et al., 2007; Weisbrod et al., 2007) but not all (Blüthgen et al., 2012) animal studies have shown that select BP-type UV filters may result in alterations in the reproductive system. Moreover, although preliminary in nature, results from recent human studies suggest that BP-type UV filters may be associated with couple fecundity (Buck Louis et al., 2014), semen quality (Buck Louis et al., 2015), birth outcomes (Wolff et al., 2008), and some estrogen-dependent gynecologic diseases such as uterine leiomyoma (Pollack et al., 2015) and endometriosis (Kunisue et al., 2012). In light of these emerging data on the reproductive and developmental toxicity of BP-type UV filters and the absence of previous investigation focusing on the SSR, we explored the association between maternal, paternal, and couple urinary concentrations of five BP-type UV filters (i.e., BP-3 and its metabolic derivatives, BP-1, BP-2, BP-8, and 4-OH-BP) and the SSR in a population-based preconception cohort.

2. Methods

2.1. Study population

The Longitudinal Investigation of Fertility and the Environment (LIFE) Study is a prospective cohort study designed to assess reproductive and developmental toxicity during sensitive windows of human reproduction and development (Buck Louis et al., 2011). Specifically, couples interested in becoming pregnant in the next two months were recruited from 16 counties in Michigan and Texas between 2005 and 2009 and prospectively followed until pregnant or 12 months of attempting pregnancy. The eligibility criteria for participation included: a) couples in a committed relationship; b) females aged 18–40 years and males aged 18 and older years; c) female partner's self-reported menstrual cycles ranging from 21 to 42 days; d) no use of injectable contraceptives in the past year; e) no history of physician-diagnosed infertility or sterilization procedures; and f) an ability to communicate in English or Spanish. Of the 501 couples enrolled in the LIFE Study, 235 (46.9 %) couples had a live singleton birth and two (0.4%) had live multiple births during the follow-up period. Of the 235 couples with a singleton birth, 15 (6.4%) couples missing both maternal and paternal

urinary concentrations of BP-type UV filters were excluded from the study cohort, leaving 220 couples for analysis.

2.2. Data collection

Research assistants visited couples' homes for consenting, enrolling, and interviewing couples in conjunction with biospecimen collection. Upon enrollment, a home pregnancy test was conducted to ensure that the female partner was not pregnant. During the baseline interview, information regarding socio-demographic characteristics (i.e., age, race/ethnicity, education, and annual household income) and reproductive history (i.e., parity and number of pregnancies fathered) was obtained from each partner of the couple. Urine samples (120 mL) were collected for the quantification of urinary concentrations of BP-type UV filters and creatinine. Couples with a live birth were asked to return standardized birth announcement following delivery that captured information regarding birth outcomes (i.e., infant sex, birth size, delivery mode, and date of birth). This study was approved by the Institutional Review Boards at all collaborating institutions. Written informed consent was provided by all study participants before any data collection. This study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki; <http://www.wma.net/e/policy/b3.htm>) and Uniform Requirements for Manuscripts Submitted to Biomedical Journals (<http://www.nejm.org/general/text/requirements/1.htm>).

2.3. Laboratory analysis

Five BP-type UV filters, BP-3 and its metabolic derivatives (i.e., BP-1, BP-2, BP-8, and 4-OH-BP), were determined using urine samples collected upon enrollment. Urinary concentrations of these chemicals (ng/mL) were quantified using isotopic dilution high performance liquid chromatography-triple quadrupole mass spectrometry. The recoveries of the target analytes ranged from 95 to 107%. Established standard operating procedures were used for the quantification of BP-type UV filters, inclusive of ongoing quality assurance and control procedures (Kunisue et al., 2010; Zhang et al., 2013). The limits of detection (LODs) of BP-type UV filters ranged from 0.01 to 0.02 ng/mL. All instrument measured concentrations were utilized for analysis without substituting for concentrations below the LOD to preclude bias introduced by this practice (Richardson and Ciampi, 2003; Schisterman et al., 2006). Urinary creatinine concentrations (mg/dL in 0.15 mL of urine) were determined by a Roche/Hitachi Model 912 clinical analyzer and the Creatinine Plus Assay.

2.4. Statistical analysis

The distributions of all variables were inspected, inclusive of missing data and influential observations. Differences in continuous and categorical characteristics by infant sex were assessed using the nonparametric Wilcoxon test and Fisher's exact test, respectively. Geometric means (GMs) and 95% confidence intervals (CIs) were estimated for BP-type UV filters by infant sex and assessed significance using the nonparametric Wilcoxon test. Using Poisson regression models with a robust error variance (Zou, 2004), the relative risks (RRs) of a male live birth and corresponding 95% CIs were estimated. Urinary BP-type UV filter concentrations were log-transformed and standardized by their standard deviations (SDs) to aid in the interpretation of results. Urinary BP-type UV filter concentrations were

also categorized into tertiles for analysis. Trend tests were performed to assess a linear trend in the RRs of a male live birth across tertiles. Separate models were first run for maternal and paternal urinary BP-type UV filter concentrations. We adjusted *a priori* for log-transformed urinary creatinine (continuous), research site (Michigan/Texas), age (continuous), annual household income (< \$70,000/ \$70,000), and maternal parity (nulliparous/parous) on the basis of our review of the literature (Chahnazarian, 1988; Grech et al., 2000; Jacobsen et al., 1999; Kolk and Schnettler, 2015; Mathews and Hamilton, 2005; Zhang et al., 2013). Subsequently, we modeled both partners' concentrations in the same model, given that there was no evidence suggestive of the multicollinearity between maternal and paternal urinary BP-type UV filter concentrations. Apart from a significant correlation between maternal and paternal urinary concentrations of 4-OH-BP (the correlation coefficient, 0.44), low correlations between maternal and paternal urinary concentrations of BP-3, BP-1, BP-2, and BP-8 were observed, with the correlation coefficients ranging from -0.02 to 0.12 (data not shown). Statistical significance was denoted by p -value < 0.05, given the exploratory nature of this work. In addition, considering the number of chemicals examined ($n=5$), we subsequently assessed the significance at the 0.01 level. All statistical analyses were performed by the SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

The study cohort comprised predominantly non-Hispanic white couples with a college education or higher educational attainment. The mean ages ($\pm$ SD) of female partners and male partners were 29.6 ($\pm$ 3.7) years and 31.4 ($\pm$ 4.6) years, respectively. Approximately half of the female partners (47.5%) were nulliparous and 40.9% of the male partners had not previously fathered a pregnancy upon enrollment. The numbers of male live births ($n=110$) and female live births ($n=110$) were equivalent, resulting in a SSR of 1.00 (95% CI, 0.76–1.31). No significant differences were observed for maternal and paternal baseline characteristics by infant sex (Table 1).

Most (> 90%) couples had urinary concentrations of BP-3, BP-1, and 4-OH-BP above the LOD. Approximately 70% of the couples had urinary concentrations of BP-2 and BP-8 above the LOD. The GMs of BP-type UV filter concentrations ranged from 0.05 ng/mL to 8.65 ng/mL, with BP-3 as the most predominant compound among the study population. Of note, paternal urinary BP-2 concentrations differed significantly by infant sex, indicating slightly higher chemical concentrations for female births than male births (Table 2).

Table 3 presents the RRs of a male birth by maternal and paternal BP-type UV filter concentrations when modeled separately. Neither maternal nor paternal BP-type UV filters were significantly associated with the SSR in any models. Meanwhile, when maternal and paternal chemical concentrations were modeled jointly, maternal 4-OH-BP was significantly associated with an excess of male births (multivariable-adjusted RR, 1.13 per 1 SD increase in log-transformed maternal 4-OH-BP; 95% CI, 1.00–1.26; p -value < 0.05) (Table 4).

When couples' categorized urinary UV filters were modeled individually (Table 5) or jointly (Table 6) in combination with other covariates, maternal categorized 4-OH-BP (2nd vs 1st

tertile, multivariable-adjusted RR, 1.87, 95% CI, 1.27–2.74; 3rd vs 1st tertile, multivariable-adjusted RR, 1.80, 95% CI, 1.13–2.87; *p*-trend, 0.02; Table 6) showed a consistently significant association with the SSR. On the contrary, both maternal and paternal categorized BP-2 were observed to be significantly associated with an excess of female births, when modeled individually or jointly (for maternal categorized BP-2, 2nd vs 1st tertile, multivariable-adjusted RR, 0.62, 95% CI, 0.43–0.91; for paternal categorized BP-2, 3rd vs 1st tertile, multivariable-adjusted RR, 0.67, 95% CI, 0.45–0.99, *p*-trend, 0.04; Table 6). However, when the significance was assessed at the 0.01 level, only the association observed for maternal categorized 4-OH-BP (2nd vs 1st tertile, multivariable-adjusted RR, 1.87, 95% CI, 1.27–2.74, *p*-value, 0.001) remained significant.

4. Discussion

In a population-based prospective cohort with the preconception enrollment of couples, we provide the first evidence suggesting that BP-type UV filters may affect human sex selection. Specifically, select metabolic derivatives of BP-3 (i.e., BP-2 and 4-OH-BP) rather than the parent compound itself were found to be associated with sex-biased birth outcomes. The effects of these chemicals on offspring sex determination appeared to be divergent, demonstrating opposite directions towards infant sex depending upon the type of chemicals. Namely, maternal but not paternal urinary 4-OH-BP concentrations were associated with an excess of male births; on the other hand, both maternal and paternal urinary BP-2 concentrations were associated with an excess of female births, when analyzed categorically. However, some of the findings no longer remained significant when adjusting for multiple comparisons, leaving only maternal urinary 4-OH-BP concentrations being associated with the SSR. Likewise, some of the findings no longer remained significant when restricting to couple conceiving within three months (*n*=135) in light of the relatively short half-lives of these chemicals, possibly due to a function of reduced power (Supplementary Table 1).

In spite of research efforts aiming to identify genetic (e.g., the SRY [sex-determining region Y] gene) and environmental determinants, the precise mechanism(s) for human sex selection remains elusive. However, the determination of infant sex is understood to be the result of complex paternal (e.g., sperm Y:X chromosome ratio) and maternal (e.g., sperm selection within the female reproductive tract and differential implantation and survival rates of embryos) factors (Almiñana et al., 2014; Davis et al., 2007). Although speculative, our findings on the association between select BP-type UV filters and the SSR may be due to the documented multiple hormonal activities of these chemicals, which have been postulated to be associated with alterations in the SSR (James, 2008, 2012; James, 2013). Specifically, it has been hypothesized that high maternal estrogen levels tend to be associated with male births, whereas low paternal testosterone levels tend to be associated with female births (James, 2013). The estrogenic activity of 4-OH-BP has been demonstrated in a variety of *in vitro* and *in vivo* assays including the MCF-7 cell assay, the yeast two-hybrid assay, the reporter gene assay, and the uterotrophic assay (Kawamura et al., 2003, 2005; Kunz and Fent, 2006; Nakagawa and Tayama, 2001; Nakagawa et al., 2000; Yamasaki et al., 2003). The anti-androgenic activity of 4-OH-BP has been also shown in some *in vitro* studies (Kawamura et al., 2005; Kunz and Fent, 2006). With regard to BP-2, *in vitro* and *in vivo* estrogenic (Kawamura et al., 2003, 2005; Kunz and Fent, 2006; Morohoshi et al., 2005;

Schlumpf et al., 2004; Yamasaki et al., 2003) as well as *in vitro* anti-androgenic (Kawamura et al., 2005; Kunz and Fent, 2006) and androgenic (Kunz and Fent, 2006) effects have been reported.

Regarding differences in hormonal potency by the chemical type of BPs, results from a study assessing BP and 16 of its derivatives showed that a 4-hydroxyl group on the phenyl ring of BP derivatives (e.g., BP-1, 4-OH-BP, and BP-2) was essential for high estrogenic and/or anti-androgenic activities, and the presence of other hydroxyl groups markedly altered these activities (Suzuki et al., 2005). In the yeast human estrogen receptor (ER) α transactivation assay, BP-1, which was reported to be approximately 5000-times less potent than 17 β -estradiol (E2), showed the highest estrogenic potency among 18 UV filters examined (Kunz and Fent, 2006). In addition, 4-OH-BP, BP-2, and BP-3 were reported to be approximately 16,000-times, 21,000-times, and 45,000-times less potent than E2, respectively (Kunz and Fent, 2006). In the yeast human androgen receptor (AR) transactivation assay of 18 UV filters, the highest anti-androgenic activity was observed with BP-1, which was even found to be four-fold more potent than flutamide, a known anti-androgen, followed by 4-OH-BP, BP-2, and BP-3 (Kunz and Fent, 2006). Meanwhile, in human ER α mediated reporter gene agonist assay, BP-2, whose estrogenic activity was found to be comparable to BPA, elicited luciferase induction much higher than did E2, which was referred to as super-agonism, although the underlying mechanism of this phenomenon was obscure (Kawamura et al., 2005). Of particular note is the absence of any significant associations for BP-1 as well as BP-3 and BP-8 in the present study, possibly reflecting other mechanisms by which BP-type UV filters affect the SSR independently of their hormonal potency. Likewise, the observed excess of female births following maternal exposure to BP-2 is difficult to explain, conflicting with the hormonal hypothesis which theorizes maternal estrogen levels being associated with an excess of male births (James, 2013).

To date, the reproductive and developmental toxicity of BP-type UV filters has been revealed in some animal and human studies. Previous animal studies have shown that select BP-type UV filters may result in alterations in the reproductive system. For instance, oral exposure to BP-3 was associated with a decrease in epididymal sperm density, an increase in abnormal spermatozoa, and an increase in estrous cycle length in adult mice (French, 1992). In utero exposure to BP-2 was associated with hypospadias, a birth defect postulated to be caused by estrogen-like compounds, in mice (Hsieh et al., 2007). In fathead minnows, exposure to BP-2 was associated with vitellogenin induction, secondary sex characteristics, gonadal development, and reproduction (Weisbrod et al., 2007). However, in zebrafish, neither vitellogenin induction nor histologic changes in the testes was observed after BP-3 treatment (Blüthgen et al., 2012). There is also a small but growing body of evidence suggestive of the reproductive and developmental toxicity of BP-type UV filters in humans. For instance, after topical application of cream containing BP-3, octyl-methoxycinnamate (OMC), and 3-(4-methylbenzylidene) camphor (4-MBC), differences in serum testosterone levels were observed in both men and women, with a minor difference in serum estradiol and inhibin B levels observed in men only (Janjua et al., 2004). In a study of 473 women who underwent laparoscopy/laparotomy for the diagnosis of fibroids at 14 clinical sites in

Utah and California during 2007–2009, significantly higher GM concentrations of BP-1 and BP-3 were observed in women with than without fibroids (BP-1, 11.1 µg/g vs 6.7 µg/g; BP-3, 11.3 µg/g vs 6.1 µg/g), although no significant associations were noted for any of these chemicals and the odds of a fibroid diagnosis when adjusting for relevant covariates (Pollack et al., 2015). In an extended study of 600 women who underwent laparoscopy/ laparotomy (n=473; operative cohort) or pelvic magnetic resonance imaging (n=127; population cohort), a significant association was observed for BP-1 and the odds of an endometriosis diagnosis in the operative cohort (Kunisue et al., 2012). In a study using data from the LIFE Study, male partners' BP-2 and 4-OH-BP were significantly associated with diminished couple fecundity, resulting in a longer time-to-pregnancy (Buck Louis et al., 2014). Among 413 men participating in the LIFE Study who provided semen samples, BP-2 and BP-8 were significantly associated with some semen quality endpoints (Buck Louis et al., 2015).

In light of this being the first investigation of BP-type UV filters and the SSR, we are unable to more fully interpret our findings in the context of previous literature. The opposing associations of maternal urinary 4-OH-BP and BP-2 concentrations with the SSR are interesting, but difficult to interpret, although these findings are possibly due to differences in endocrine-disrupting properties by the type of chemicals. Still, the underlying biological mechanisms responsible for the varying associations of specific BP-type UV filters with the SSR remain obscure. Furthermore, given our exploratory data analysis approach, cautious interpretation is needed, while accounting for the possibility of chance findings or potential residual confounding. We wish to stress the need for further confirmatory research before any conclusions can be drawn from our preliminary findings on the association between select BP-type UV filters and the SSR. Given that not all metabolic derivatives of BP-3 have been thoroughly evaluated for potential reproductive and developmental toxicity to date, our findings underscore the need for research beyond BP-3 to include various presumed metabolic derivatives of this parent compound. In addition, we believe that understanding the sources of exposure to various BP compounds and the metabolism of the parent compound, BP-3, may be helpful to more fully interpret our findings (Kim and Choi, 2014; Kunisue et al., 2010; Liao and Kannan, 2014; Wang and Kannan, 2013). We found a significant correlation of BP-3 with BP-1 (the correlation coefficient, 0.87 for females, 0.92 for males) or BP-8 (the correlation coefficient, 0.23 for females, 0.32 for males), but not with BP-2 and 4-OH-BP in our study cohort (Supplementary Table 2), although we assume that BP-2 and 4-OH-BP measured in urine are due to the metabolism of BP-3 (Kunisue et al., 2010; Liao and Kannan, 2014). While two metabolic derivatives of BP-3 (i.e., BP-2 and 4-OH-BP) were found to be associated with the SSR in the present study, the sum of BP-3 and all four metabolic derivatives of BP-3 measured in urine did not show a significant association with the SSR (Supplementary Table 3).

Our study is strengthened by the quantification of urinary BP-type UV filter concentrations prior to conception and the incorporation of both partners' chemical concentrations when assessing a couple-dependent outcome, along with the prospective cohort study design. Nevertheless, important study limitations should be taken into account, when interpreting our findings. These include our inability to measure the primary sex ratio and the absence of data on preconception hormone levels, which kept us from more fully explaining the impact

of BP-type UV filters on human sex selection. The sample size is relatively small with regard to the detection of variability in the SSR. In addition, our reliance on only one measurement of BP-type UV filters from a single spot urine sample may cause the misclassification of exposure status. The temporal variability of BP-3 has been assessed in some studies with a range (0.59 to 0.92) of intra-class correlation coefficients (Dewalque et al., 2015; Koch et al., 2014; Lassen et al., 2013; Meeker et al., 2013). However, information on the temporal variability of the other BP compounds is lacking. Lastly, given the sampling of couples planning pregnancies, our findings may not be generalizable to the general population or among couples with unplanned pregnancy.

5. Conclusions

This study provides the first report on the association between select metabolic derivative of BP-3 (i.e., BP-2 and 4-OH-BP) and the SSR. Our findings suggest that paternal preconception exposure to BP-2 may be associated with the reversal of the SSR resulting in an excess of female births, which is consistent with some but not all earlier findings on the association between paternal exposure to other estrogenic chemicals and the SSR. The varying effects of maternal preconception exposure to select BP-type UV filters on the SSR are noteworthy, but await future corroboration. Future research focusing on the underlying biologic mechanisms by which BP-type UV filters affect human sex selection is warranted to better understand the potential public health hazards of these ubiquitous chemicals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Several environmental chemicals are associated with the secondary sex ratio.
- Maternal urinary concentration of 4-OH-BP was associated with male excess of births.
- Maternal and paternal urinary BP-2 concentrations were associated with female excess.
- These findings need future corroboration, given the exploratory design of this study.

Table 1

Baseline characteristics of couples with a singleton birth by infant sex, 2005–2009

Characteristic	Male (n=110) n (%)	Female (n=110) n (%)	p-value
<i>Maternal characteristic</i>			
Age (year, mean $\pm$ SD)	29.7 $\pm$ 3.9	29.5 $\pm$ 3.4	0.74
Urinary creatinine (mg/dL, GM [95% CI])	61.2 (51.2–73.3)	67.3 (56.9–79.6)	0.62
Parity			0.34
Nulliparous	56 (50.9)	48 (44.0)	
Parous	54 (49.1)	61 (56.0)	
Education			0.96
High school graduate/GED	5 (4.6)	4 (3.6)	
Some college/technical school	13 (12.0)	13 (11.8)	
College graduate or higher	90 (83.3)	93 (84.6)	
Race/ethnicity			0.63
Non-Hispanic white	88 (81.5)	93 (84.6)	
Non-Hispanic black	2 (1.9)	1 (0.9)	
Hispanic	11 (10.2)	7 (6.4)	
Other	7 (6.5)	9 (8.2)	
<i>Paternal characteristic</i>			
Age (year, mean $\pm$ SD)	32.0 $\pm$ 5.1	30.8 $\pm$ 4.0	0.08
Urinary creatinine (mg/dL, GM [95% CI])	116 (101–134)	121 (105–140)	0.60
Number of pregnancies fathered			0.89
0	45 (44.6)	45 (42.9)	
1	56 (55.4)	60 (57.1)	
Education			0.18
High school graduate/GED	3 (2.8)	4 (3.7)	
Some college/technical school	33 (30.3)	21 (19.3)	
College graduate or higher	73 (67.0)	84 (77.1)	
Race/ethnicity			0.40

Characteristic	Male (n=110) n (%)	Female (n=110) n (%)	p-value
Non-Hispanic white	86 (78.9)	96 (87.3)	
Non-Hispanic black	3 (2.8)	2 (1.8)	
Hispanic	12 (11.0)	8 (7.3)	
Other	8 (7.3)	4 (3.6)	
<i>Couple characteristic</i>			
Research site			1.00
Michigan	20 (18.2)	20 (18.2)	
Texas	90 (81.8)	90 (81.8)	
Annual household income (\$)			0.66
< 70,000	32 (29.1)	36 (32.7)	
70,000	78 (70.9)	74 (67.3)	

CI, confidence interval; GED, General Educational Development; GM, geometric mean; SD, standard deviation.

Geometric means (95% confidence intervals) of maternal and paternal urinary concentrations of benzophenone-type ultraviolet filters by infant sex, 2005–2009

Table 2

UV filter (ng/mL)	Maternal concentration (n=213)				Paternal concentration (n=212)			
	% < LOD	Male GM (95% CI)	Female GM (95% CI)	% < LOD	Male GM (95% CI)	Female GM (95% CI)	% < LOD	Male GM (95% CI)
BP-1 (2,4-OH-BP)	<1	4.19 (2.78–6.31)	3.77 (2.64–5.39)	1	3.35 (2.21–5.07)	2.45 (1.70–3.53)		
BP-2 (2,2',4,4'-OH-BP)	28	0.05 (0.04–0.07)	0.07 (0.05–0.09)	28	0.05 (0.04–0.07)*	0.06 (0.05–0.08)*		
BP-3 (2-OH-4-MeO-BP)	<1	8.65 (5.66–13.2)	7.68 (5.08–11.6)	2	7.47 (4.94–11.3)	5.54 (3.86–7.95)		
BP-8 (2,2'-OH-4-MeO-BP)	29	0.19 (0.11–0.33)	0.18 (0.11–0.31)	27	0.18 (0.10–0.31)	0.17 (0.10–0.28)		
4-OH-BP	6	0.14 (0.11–0.19)	0.13 (0.10–0.16)	4	0.16 (0.13–0.20)	0.15 (0.12–0.20)		

CI, confidence interval; GM, geometric mean; LOD, limit of detection; UV, ultraviolet; 4-OH-BP, 4-hydroxybenzophenone; 2,4-OH-BP, 2,4-dihydroxybenzophenone; 2,2',4,4'-OH-BP, 2,2',4,4'-tetrahydroxybenzophenone; 2-OH-4-MeO-BP, 2-hydroxy-4-methoxybenzophenone; 2,2'-OH-4-MeO-BP, 2,2'-dihydroxy-4-methoxybenzophenone.

Note: Of 220 couples, 7 mothers and 8 fathers without urinary concentrations of benzophenone-type UV filters were excluded.

* Bolded values indicate p -value < 0.05.

Table 3

Urinary concentrations of benzophenone-type ultraviolet filters and the relative risks of a male birth by partner, 2005–2009

UV filter (ng/mL)	Maternal concentration (n=213)			Paternal concentration (n=212)		
	Unadjusted RR (95% CI)	Adjusted ^a RR (95% CI)	Adjusted ^b RR (95% CI)	Unadjusted RR (95% CI)	Adjusted ^a RR (95% CI)	Adjusted ^b RR (95% CI)
BP-1 (2,4-OH-BP)	1.06 (0.93–1.20)	1.07 (0.94–1.23)	1.07 (0.94–1.23)	1.09 (0.97–1.23)	1.10 (0.97–1.24)	1.10 (0.98–1.24)
BP-2 (2,2',4,4'-OH-BP)	0.93 (0.79–1.09)	0.94 (0.80–1.10)	0.94 (0.81–1.10)	1.01 (0.88–1.15)	1.01 (0.89–1.15)	1.00 (0.87–1.15)
BP-3 (2-OH-4-MeO-BP)	1.04 (0.91–1.19)	1.06 (0.92–1.22)	1.05 (0.91–1.21)	1.09 (0.96–1.23)	1.10 (0.97–1.24)	1.11 (0.98–1.24)
BP-8 (2,2'-OH-4-MeO-BP)	1.05 (0.94–1.18)	1.05 (0.93–1.19)	1.07 (0.95–1.20)	1.00 (0.88–1.15)	1.00 (0.87–1.14)	0.99 (0.87–1.13)
4-OH-BP	1.07 (0.95–1.20)	1.09 (0.96–1.24)	1.10 (0.97–1.24)	0.99 (0.86–1.13)	1.01 (0.88–1.16)	1.00 (0.86–1.15)

CI, confidence interval; RR, relative risk; UV, ultraviolet; 4-OH-BP, 4-hydroxybenzophenone; 2,4-OH-BP, 2,4-dihydroxybenzophenone; 2,2',4,4'-tetrahydroxybenzophenone; 2-OH-4-MeO-BP, 2-hydroxy-4-methoxybenzophenone; 2,2'-OH-4-MeO-BP, 2,2'-dihydroxy-4-methoxybenzophenone.

Note: Urinary concentrations of benzophenone-type UV filters were log-transformed and standardized by their standard deviations. Modified Poisson regression models were used to estimate the relative risks of a male live birth (Zou, 2004). All point and interval estimates were rounded to two decimal places.

^a Adjusted for log-transformed urinary creatinine (continuous).

^b Adjusted for log-transformed urinary creatinine (continuous), research site (Michigan/Texas), age (continuous), annual household income (< \$70,000/ \$70,000), and maternal parity (nulliparous/parous).

Couples' concentrations of benzophenone-type ultraviolet filters and the relative risks of a male birth, 2005–2009 (n= 205 couples)

Table 4

UV filter (ng/mL)	Maternal concentration			Paternal concentration		
	Unadjusted RR (95% CI)	Adjusted ^a RR (95% CI)	Adjusted ^b RR (95% CI)	Unadjusted RR (95% CI)	Adjusted ^a RR (95% CI)	Adjusted ^b RR (95% CI)
BP-1 (2,4-OH-BP)	1.03 (0.89–1.19)	1.04 (0.89–1.21)	1.03 (0.89–1.20)	1.09 (0.95–1.24)	1.09 (0.96–1.25)	1.10 (0.98–1.25)
BP-2 (2,2',4,4'-OH-BP)	0.93 (0.80–1.10)	0.95 (0.81–1.11)	0.93 (0.79–1.09)	0.99 (0.86–1.14)	1.00 (0.87–1.15)	1.01 (0.87–1.16)
BP-3 (2-OH-4-MeO-BP)	1.00 (0.86–1.16)	1.01 (0.86–1.19)	1.01 (0.86–1.18)	1.09 (0.95–1.26)	1.10 (0.96–1.27)	1.11 (0.97–1.26)
BP-8 (2,2'-OH-4-MeO-BP)	1.07 (0.92–1.25)	1.08 (0.92–1.26)	1.07 (0.91–1.24)	0.97 (0.81–1.16)	0.96 (0.80–1.15)	0.97 (0.81–1.15)
4-OH-BP	1.10 (0.98–1.24)	1.12 (0.99–1.27)	1.13 (1.00–1.26)*	0.92 (0.78–1.10)	0.93 (0.78–1.10)	0.93 (0.79–1.10)

CI, confidence interval; RR, relative risk; UV, ultraviolet; 4-OH-BP, 4-hydroxybenzophenone; 2,4-OH-BP, 2,4-dihydroxybenzophenone; 2,2',4,4'-tetrahydroxybenzophenone; 2-OH-4-MeO-BP, 2-hydroxy-4-methoxybenzophenone; 2,2'-OH-4-MeO-BP, 2,2'-dihydroxy-4-methoxybenzophenone.

Note: Couples' urinary concentrations of benzophenone-type UV filters were log-transformed and standardized by their standard deviations, and included simultaneously in the model. Modified Poisson regression models were used to estimate the relative risks of a male live birth (Zou, 2004). All point and interval estimates were rounded to two decimal places.

^a Adjusted for log-transformed urinary creatinine (continuous).

^b Adjusted for log-transformed urinary creatinine (continuous), research site (Michigan/Texas), age (continuous), annual household income (< \$70,000/ \$70,000), and maternal parity (nulliparous/parous).

* Bolded values indicate p -value < 0.05.

Categorized urinary concentrations of benzophenone-type ultraviolet filters and the relative risks of a male birth by partner, 2005–2009

Table 5

UV filter	Maternal concentration (n=213)			Paternal concentration (n=212)		
	Unadjusted RR (95% CI)	Adjusted ^a RR (95% CI)	Adjusted ^b RR (95% CI)	Unadjusted RR (95% CI)	Adjusted ^a RR (95% CI)	Adjusted ^b RR (95% CI)
BP-1 (2,4-OH-BP)						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	0.85 (0.59–1.22)	0.81 (0.56–1.18)	0.81 (0.56–1.17)	0.89 (0.63–1.26)	0.86 (0.60–1.24)	0.85 (0.59–1.21)
3rd tertile	1.24 (0.91–1.68)	1.25 (0.92–1.71)	1.28 (0.94–1.74)	1.11 (0.81–1.53)	1.11 (0.80–1.52)	1.10 (0.80–1.52)
<i>p</i> -trend	0.18	0.16	0.14	0.50	0.51	0.53
BP-2 (2,2',4,4'-OH-BP)						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	0.60 (0.42–0.86)*	0.61 (0.42–0.88)*	0.61 (0.42–0.89)*	0.90 (0.67–1.21)	0.95 (0.71–1.28)	0.98 (0.72–1.32)
3rd tertile	0.84 (0.62–1.12)	0.89 (0.64–1.23)	0.90 (0.65–1.25)	0.66 (0.46–0.94)*	0.67 (0.45–0.98)*	0.66 (0.45–0.96)*
<i>p</i> -trend	0.24	0.49	0.57	0.02 *	0.04 *	0.03 *
BP-3 (2-OH-4-MeO-BP)						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	0.78 (0.55–1.11)	0.74 (0.51–1.06)	0.73 (0.51–1.05)	0.94 (0.66–1.35)	0.96 (0.66–1.39)	0.96 (0.67–1.39)
3rd tertile	1.05 (0.78–1.43)	1.07 (0.79–1.45)	1.06 (0.78–1.45)	1.24 (0.91–1.71)	1.27 (0.91–1.77)	1.30 (0.93–1.82)
<i>p</i> -trend	0.74	0.66	0.68	0.18	0.14	0.11
BP-8 (2,2'-OH-4-MeO-BP)						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	0.96 (0.69–1.33)	0.94 (0.68–1.31)	0.94 (0.68–1.31)	1.00 (0.73–1.38)	0.98 (0.71–1.36)	0.98 (0.71–1.36)
3rd tertile	0.94 (0.68–1.32)	0.93 (0.66–1.32)	0.92 (0.65–1.31)	0.92 (0.65–1.28)	0.89 (0.63–1.27)	0.89 (0.62–1.26)
<i>p</i> -trend	0.74	0.68	0.65	0.61	0.52	0.50
4-OH-BP						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	1.47 (1.04–2.08)*	1.72 (1.21–2.45)*	1.70 (1.19–2.43)*	0.87 (0.62–1.21)	0.95 (0.67–1.35)	0.98 (0.69–1.38)
3rd tertile	1.25 (0.86–1.81)	1.58 (1.05–2.37)*	1.59 (1.04–2.41)*	0.97 (0.71–1.34)	1.05 (0.74–1.49)	1.03 (0.72–1.48)
<i>p</i> -trend	0.24	0.03 *	0.03 *	0.87	0.80	0.86

CI, confidence interval; RR, relative risk; UV, ultraviolet; 4-OH-BP, 4-hydroxybenzophenone; 2,4-OH-BP, 2,4-dihydroxybenzophenone; 2,2',4,4'-tetrahydroxybenzophenone; 2-OH-4-MeO-BP, 2-hydroxy-4-methoxybenzophenone; 2,2'-OH-4-MeO-BP, 2,2'-dihydroxy-4-methoxybenzophenone.

Note: Modified Poisson regression models were used to estimate the relative risks of a male live birth (Zou, 2004). All point and interval estimates were rounded to two decimal places.

^a Adjusted for log-transformed urinary creatinine (continuous).

^b Adjusted for log-transformed urinary creatinine (continuous), research site (Michigan/Texas), age (continuous), annual household income (< \$70,000/ \$70,000), and maternal parity (nulliparous/parous).

* Bolded values indicate *p*-value < 0.05.

Couples' categorized urinary concentrations of benzophenone-type ultraviolet filters and the relative risks of a male birth, 2005–2009 (n=205 couples)

Table 6

UV filter	Maternal concentration			Paternal concentration		
	Unadjusted RR (95% CI)	Adjusted ^a RR (95% CI)	Adjusted ^b RR (95% CI)	Unadjusted RR (95% CI)	Adjusted ^a RR (95% CI)	Adjusted ^b RR (95% CI)
BP-1 (2,4-OH-BP)						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	0.90 (0.59–1.36)	0.87 (0.57–1.34)	0.91 (0.60–1.38)	0.80 (0.53–1.20)	0.77 (0.51–1.18)	0.75 (0.50–1.14)
3rd tertile	1.32 (0.87–2.00)	1.36 (0.89–2.07)	1.39 (0.93–2.10)	0.99 (0.66–1.49)	0.98 (0.65–1.49)	0.98 (0.65–1.48)
<i>p</i> -trend	0.20	0.19	0.13	0.93	0.98	0.94
BP-2 (2,2',4,4'-OH-BP)						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	0.62 (0.43–0.88)*	0.62 (0.43–0.90)*	0.62 (0.43–0.91)*	0.91 (0.68–1.22)	0.96 (0.72–1.29)	1.00 (0.74–1.34)
3rd tertile	0.88 (0.65–1.18)	0.90 (0.65–1.26)	0.88 (0.63–1.23)	0.66 (0.45–0.95)*	0.65 (0.44–0.97)*	0.67 (0.45–0.99)*
<i>p</i> -trend	0.40	0.69	0.51	0.02 *	0.03 *	0.04 *
BP-3 (2-OH-4-MeO-BP)						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	0.77 (0.53–1.12)	0.72 (0.49–1.07)	0.74 (0.51–1.10)	0.99 (0.67–1.47)	1.03 (0.68–1.56)	1.06 (0.71–1.59)
3rd tertile	0.92 (0.64–1.32)	0.91 (0.63–1.33)	0.90 (0.62–1.29)	1.32 (0.91–1.92)	1.36 (0.91–2.03)	1.40 (0.95–2.06)
<i>p</i> -trend	0.67	0.72	0.56	0.16	0.13	0.08
BP-8 (2,2'-OH-4-MeO-BP)						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	0.93 (0.65–1.31)	0.88 (0.62–1.26)	0.88 (0.62–1.25)	1.04 (0.75–1.45)	1.03 (0.74–1.45)	1.04 (0.74–1.46)
3rd tertile	1.00 (0.68–1.48)	0.97 (0.65–1.44)	0.92 (0.63–1.36)	0.91 (0.61–1.36)	0.90 (0.59–1.36)	0.89 (0.59–1.35)
<i>p</i> -trend	0.92	0.79	0.63	0.69	0.68	0.63
4-OH-BP						
1st tertile	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
2nd tertile	1.57 (1.09–2.26)*	1.93 (1.31–2.85)*	1.87 (1.27–2.74)*	0.84 (0.60–1.19)	0.91 (0.64–1.30)	0.91 (0.63–1.31)
3rd tertile	1.44 (0.95–2.17)	1.90 (1.21–3.00)*	1.80 (1.13–2.87)*	0.87 (0.62–1.22)	0.84 (0.58–1.24)	0.90 (0.60–1.33)
<i>p</i> -trend	0.07	0.03 *	0.02 *	0.34	0.42	0.51

CI, confidence interval; RR, relative risk; UV, ultraviolet; 4-OH-BP, 4-hydroxybenzophenone; 2,4-OH-BP, 2,4-dihydroxybenzophenone; 2,2',4,4'-tetrahydroxybenzophenone; 2-OH-4-MeO-BP, 2-hydroxy-4-methoxybenzophenone; 2,2'-OH-4-MeO-BP, 2,2'-dihydroxy-4-methoxybenzophenone.

Note: Couples' urinary concentrations of benzophenone-type UV filters were included simultaneously in the model. Modified Poisson regression models were used to estimate the relative risks of a male live birth (Zou, 2004). All point and interval estimates were rounded to two decimal places.

^a Adjusted for log-transformed urinary creatinine (continuous).

^b Adjusted for log-transformed urinary creatinine (continuous), research site (Michigan/Texas), age (continuous), annual household income (< \$70,000/ \$70,000), and maternal parity (nulliparous/parous).

* Bolded values indicate p -value < 0.05.