Published in final edited form as:

Environ Res. 2015 February ; 137: 450–457. doi:10.1016/j.envres.2014.11.011.

Couples' Urinary Bisphenol A and Phthalate Metabolite Concentrations and the Secondary Sex Ratio

Jisuk Bae, M.D., Ph.D.^{a,b,*}, Sungduk Kim, Ph.D.^a, Kurunthachalam Kannan, Ph.D.^c, and Germaine M. Buck Louis, Ph.D.^a

Sungduk Kim: kims2@mail.nih.gov; Kurunthachalam Kannan: kkannan@wadsworth.org; Germaine M. Buck Louis: louisg@mail.nih.gov

^aDivision of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, 6100 Executive Boulevard, Rockville, Maryland 20852, USA

^bDepartment of Preventive Medicine, Catholic University of Daegu School of Medicine, 33 Duryugongwon-ro 17-gil Nam-gu, Daegu, 705-718, Republic of Korea

^cWadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, USA

Abstract

With limited research focusing on non-persistent chemicals as exogenous factors affecting human sex selection, this study aimed to evaluate the association of urinary bisphenol A (BPA) and phthalate metabolite concentrations with the secondary sex ratio (SSR), defined as the ratio of male to female live births. The current analysis is limited to singleton live births (n=220, 43.9%) from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, which enrolled couples upon discontinuing contraception and followed while trying for pregnancy and through delivery those achieving pregnancy. Using modified Poisson regression models accounting for potential confounders, we estimated the relative risks (RRs) of a male birth per standard deviation change in the log-transformed maternal, paternal, and couple urinary BPA and 14 phthalate metabolite concentrations (ng/mL) measured upon enrollment. When maternal and paternal

© 2014 Published by Elsevier Inc.

*Correspondence: Jisuk Bae, M.D., Ph.D., Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, 6100 Executive Boulevard, Rockville, Maryland 20852, USA. Tel: +1-301-496-1073; Fax: +1-301-402-2084; jisuk.bae@nih.gov. Department of Preventive Medicine, Catholic University of Daegu School of Medicine, 33 Duryugongwon-ro 17-gil Nam-gu, Daegu, 705-718, Republic of Korea, Tel: +82-53-650-3190; Fax: +82-53-654-3881; jialove@cu.ac.kr.

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.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

chemical concentrations were modeled jointly, paternal BPA (RR, 0.77; 95% confidence interval [CI], 0.62–0.95) and mono-isobutyl phthalate (RR, 0.82; 95% CI, 0.67–1.00) were significantly associated with a female excess. Contrarily, maternal BPA (RR, 1.16; 95% CI, 1.03–1.31), mono-isobutyl phthalate (RR, 1.28; 95% CI, 1.06–1.54), mono-benzyl phthalate (RR, 1.31; 95% CI, 1.08–1.58), and mono-n-butyl phthalate (RR, 1.24; 95% CI, 1.01–1.51) were significantly associated with a male excess. These findings underscore varying patterns for the SSR in relation to parental exposures. Given the absence of previous investigation, these partner-specific associations of non-persistent chemicals with the SSR need future corroboration.

Keywords

bisphenol A; endocrine disruptors; fertility; phthalates; sex ratio

1. Introduction

Environmental chemicals that interfere with the endocrine system can adversely affect population health including human reproduction and development (Casals-Casas and Desvergne, 2011). Toxicological and epidemiological studies on endocrine-disrupting chemicals (EDCs) have suggested that persistent chemicals with varying physiochemical properties can bioaccumulate in humans and cause reproductive and developmental toxicity with epigenetic and transgenerational effects, exemplified by research on dioxins, polychlorinated biphenyls (PCBs), and pesticides (Guerrero-Bosagna and Skinner, 2009; Mrema et al., 2013; Younglai et al., 2007). Non-persistent chemicals, which have relatively short half-lives in the human body and do not bioaccumulate significantly, have also been reported to have endocrine-disrupting properties and are suspected to affect human reproduction and development (Frederiksen et al., 2014; Diamanti-Kandarakis et al., 2009). Among the non-persistent chemicals of particular focus to date are bisphenol A (BPA) and phthalates.

BPA and phthalates are ubiquitous environmental toxicants. BPA is mainly used in the manufacture of polycarbonate plastics and epoxy resin, comprising a large variety of consumer products such as food and beverage containers, medical and dental devices, and thermal paper (Geens et al., 2012; Liao and Kannan, 2013, 2014; Rubin, 2011). Phthalates are primarily used in polyvinyl chloride plastics, including a broad range of phthalates-containing products from medical devices and pharmaceuticals to adhesives, detergents, packaging, household applications, and personal-care products (Wittassek et al., 2011). Human exposures to BPA and phthalates are believed to occur mainly through ingestion of contaminated water and food and possibly through non-oral routes such as inhalation and dermal absorption (Geens et al., 2011; Heudorf et al., 2007; Liao and Kannan, 2013, 2014). Toxicokinetic studies have suggested that absorbed BPA undergoes hepatic glucuronidation and renal excretion within 24 hours in the human body, implying that urinary BPA concentrations are reflective of all routes of exposure (Teeguarden et al., 2011). Likewise, phthalate diesters are hydrolyzed in the intestine to the corresponding monoesters, which are further oxidized depending on the monoester and excreted in the urine in humans (Frederiksen et al., 2007). Thus, urinary concentrations of phthalate monoesters are

considered as biomarkers with different sensitivity to assess recent exposures to the parent phthalate diesters (Barr et al., 2003; Guo et al., 2012).

Concerns have been raised about the potential adverse health effects of ubiquitous exposures to BPA and phthalates in the general population not only based on experimental studies but also in consideration of recently published human data. However, human evidence is still limited in comparison with the large body of *in vitro* and *in vivo* experimental evidence documenting the reproductive and developmental toxicity of BPA and phthalates (Fowler et al., 2012; Knez, 2013). Epidemiologic studies have reported possible associations between BPA and male reproductive function, primarily focusing on reproductive hormones and semen quality in both fertile and infertile males (Lassen et al., 2014; Manfo et al., 2014). With scarce available evidence, BPA has been also demonstrated to be related to reproductive outcomes especially in females undergoing *in-vitro* fertilization, such as diminished ovarian response or blastocyst formation and implantation failure (Ehrlich et al., 2012a; Ehrlich et al., 2012b; Mok-Lin E et al., 2010). Potential toxic effects of a variety of phthalates, including commonly used di-2-ethylhexyl phthalate (DEHP), in humans have been reported in relation to markers of male reproductive function such as reproductive hormones and semen quality including DNA integrity and sperm motility (Hauser et al., 2008; Joensen et al., 2012; Jurewicz et al., 2013; Specht et al., 2014). Previous studies also provide limited evidence on the association of pregnancy loss and couple fecundity with select phthalates (Buck Louis et al., 2014; Toft et al., 2012; Tranfo et al., 2012).

The secondary sex ratio (SSR) is defined as the ratio of male to female live births, whereas the primary sex ratio is the ratio of male to female conceptions (Buck Louis and Platt, 2011). The SSR, which is typically estimated restricting to singleton births, has been monitored to assess population health and fertility, despite controversy on its meaningfulness (Davis et al., 1998; James, 2008a). In recent decades, the SSR has been decreasing notably in developed countries including the United States, Canada, Japan, and some northern and western European countries (Davis et al., 2007; Grech et al., 2003; Mathews and Hamilton, 2005). In the United States, for instance, the SSR generally declined between 1942 and 1959, increased between 1959 and 1971, and declined from 1971 to 2002 (Mathews and Hamilton, 2005). During the time period, the SSR of the United States ranged from 1.046 to 1.059, indicative of a slight excess of male births.

To date, there has been no established explanation for the stability and variability of the SSR observed at the population level. However, the SSR has been reported to be associated with a variety of factors, such as parental ages (Chahnazarian, 1988; Juntunen et al., 1997), race/ethnicity (Davis et al., 2007; Mathews and Hamilton, 2005), birth order (Biggar et al., 1999; Mathews and Hamilton, 2005), follicular phase length (Weinberg et al., 1995), timing of conception during the menstrual cycle (James, 1987), stress (Fukuda et al., 1998; Zorn et al., 2002), and endocrine and immunological effects (James, 2008a; Ober, 1992). Specifically, it has been suggested that exposures to EDCs may have contributed to the recent decline in the SSR observed in some developed countries. A review article (Terrell et al., 2011) investigated the effects of maternal and paternal exposures to a variety of environmental hazards such as dioxins, PCBs, pesticides, metals, and radiation. The authors summarized that there had been relatively consistent evidence that paternal dioxins were associated with

an excess of female births, while some of paternal PCBs were associated with an excess of male births. As for the maternal exposures, there have been limited or inconsistent evidence on any hazards (Terrell et al., 2011).

To our knowledge, however, there have been no studies focusing on the association between nonpersistent chemicals such as BPA and phthalates and the SSR. BPA and phthalates are identified as EDCs according to an Endocrine Society scientific statement (Diamanti-Kandarakis, 2009). Although mechanisms by which EDCs affect the SSR may be divergent, the effects of EDCs on parental hormones around the time of conception have been hypothesized to be associated with alterations in the SSR (James, 2008b, 2012, 2013). With the lack of research on non-persistent chemicals as exogenous factors affecting human sex selection and the importance of couple-based approaches when assessing couple-dependent outcomes, our study sought to evaluate the association of couples' preconception urinary BPA and phthalate metabolite concentrations with the SSR.

2. Methods

2.1. Study population

The Longitudinal Investigation of Fertility and the Environment (LIFE) Study is a cohort study, in which couples from Michigan and Texas were enrolled prior to conception between 2005 and 2009 and prospectively followed until pregnant or 12 months of attempting pregnancy (Buck Louis et al., 2011). The aim of this cohort study was to elucidate reproductive and developmental toxicity of various environmental chemicals during sensitive windows of human reproduction and development. The inclusion criteria were used to determine the eligibility for the study participants: a) in a committed relationship; b) women aged 18–40 years and men aged 18 and older years; c) female partner's menstrual cycle length 21–42 days; d) no injectable contraceptives in the past 12 months; e) no physician-diagnosed infertility or sterilization procedures; and f) couples able to communicate in English or Spanish. Of the 501 couples recruited in the study, 237 (47.3 %) couples had a live birth during the follow-up period and reported birth outcomes. Seventeen couples were excluded, including 2 with multiple births and 15 missing both maternal and paternal urinary chemical measurements, leaving 220 couples with a singleton birth for analysis.

2.2. Data collection

Baseline interviews were conducted in the couples' home after ensuring that the female partner was not pregnant as evident by a negative home pregnancy test. Information on socio-demographic characteristics (i.e., age, race/ethnicity, education, and annual income) and reproductive history (i.e., parity and number of pregnancies fathered) was obtained by a trained research nurse and assistant. Upon enrollment, urine samples (120 mL) were collected from each partner of the couple and used to quantify urinary concentrations of BPA, phthalate metabolites, and creatinine. Couples who had a live birth during the follow-up period were asked to report birth outcomes including 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 assessment

Both maternal and paternal urinary concentrations of total BPA (ng/mL in 0.5 mL of urine) and phthalate metabolites (ng/mL in 0.5 mL of urine) were quantified by enzymatic deconjugation, followed by solid phase extraction (SPE), and high-performance liquid chromatography (HPLC)-isotope dilution-tandem mass spectrometry (MS/MS) according to published methods, inclusive of ongoing quality assurance and control procedures (Guo et al., 2011; Zhang et al., 2011). The limitation of detection (LOD) of BPA was 0.05 ng/mL, twice that of the lowest valid acceptable calibration standard. The LOD of phthalate metabolites ranged from 0.2 to 1.0 ng/mL, which was determined as the lowest point of the calibration standard and a nominal urine volume of 0.5 mL. A total of 14 phthalate metabolites were measured in urine collected upon enrollment: mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-isononyl phthalate (MiNP), mono-benzyl phthalate (MBzP), mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), mono-cyclohexyl phthalate (MCHP), mono-ethyl phthalate (MEP), mono-methyl phthalate (MMP), mono-(3-carboxypropyl) phthalate (MCP), mono-n-octyl phthalate (MOP), and mono-[(2-carboxymethyl) hexyl] phthalate (MCMHP). Creatinine (mg/dL in 0.15 mL of urine) was quantified using a Roche/Hitachi Model 912 clinical analyzer and the Creatinine Plus Assay. No concentrations below the LOD were substituted to prevent introducing bias (Richardson and Ciampi, 2003; Schisterman et al., 2006); rather, all instrument measured concentrations were used in the analysis.

2.4. Statistical analysis

Descriptive analysis included the inspection of missing data and influential observations. The study cohort was assessed by select characteristics for couples. Differences in characteristics in couples were assessed using the nonparametric Wilcoxon test and Fisher's exact test (Table 1). The distribution of each chemical concentration was assessed, and geometric mean (GM) concentrations and 95% confidence intervals (CIs) were calculated by infant's sex and assessed significance using the nonparametric Wilcoxon test (Table 2). Each chemical concentration was log-transformed and divided by its standard deviation (SD) to rescale concentrations for biologic interpretation. We estimated the relative risks (RRs) of a male birth and corresponding 95% CIs using Poisson regression models with a robust error variance (Zou, 2004). Separate models were run for maternal and paternal urinary BPA and phthalate metabolite concentrations. We adjusted *a priori* for urinary creatinine (continuous), research site (Michigan/Texas), age (continuous), annual income (< \$30,000, 30,000–49,999, 50,000–69,999, and 70,000), and maternal parity (nulliparous/parous). To check the multicollinearity between maternal and paternal chemical concentrations for couple-based models, we assessed correlation coefficients, the eigenvalue and condition index. Low correlations between maternal and paternal concentrations of BPA, MiBP, MBzP and MOP were observed with the correlation coefficients ranging from

0.15 to 0.25. In addition, we noted small eigenvalues and all condition indices < 30 reflecting the absence of multicollinearity (data not shown). Based on the lack of multicollinearity, we modeled both partners' concentrations in the same model. Significance was set to p -values < 0.05 . 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 and college-educated couples. The mean ages ($\pm$ SD) of mothers and fathers were 29.6 ($\pm$ 3.7) years and 31.4 ($\pm$ 4.6) years, respectively. Approximately half of the mothers (47.5%) were nulliparous and 40.9% of the fathers had never fathered a pregnancy upon enrollment. An equivalent number of births were males and females ($n=110$) resulting in a SSR of 1.00 (95% CI, 0.76–1.31) (Table 1).

Most couples had BPA concentrations $>$ LOD (99%) and 9/14 phthalate metabolite concentrations $>$ LOD (95%) (Table 2). The 5 remaining phthalate metabolites had almost more than half of the concentrations $<$ LOD, ranging from 49% to 97%. The GMs of BPA and phthalate metabolites among the study population ranged from 0.01 ng/mL to 103 ng/mL, which were relatively lower than those among participants in the National Health and Nutrition Examination Survey (Centers for Disease Control and Prevention, 2014). No significant differences were observed between the GMs of any chemical by infant sex irrespective of parent. Although not statistically significant, fathers of female infants had higher concentrations of BPA and 12/14 phthalate metabolites in comparison with fathers of male infants.

Table 3 presents the RRs of a male birth by maternal and paternal urinary BPA and phthalate metabolite concentrations when modeled separately. Neither BPA nor any of the phthalate metabolites were significantly associated with the SSR in any models, irrespective of parent. Though the CIs included 1, most RRs were > 1 for maternal BPA and phthalate metabolite concentrations, suggestive of a male excess; whereas, most RRs were < 1 for paternal BPA and phthalate metabolite concentrations, suggestive of a female excess.

As Table 4 reflects, when couples' chemical concentrations were jointly modeled in combination with other covariates, significant findings were noted for both maternal and paternal BPA and select phthalate metabolites. In the multivariable-adjusted model, the RR of a male live birth was 1.16 (95% CI, 1.03–1.31) per 1 SD increase in log-transformed maternal BPA, reflecting a male excess. Maternal MiBP (RR, 1.28; 95% CI, 1.06–1.54), MBzP (RR, 1.31; 95% CI, 1.08–1.58), and MnBP (RR, 1.24; 95% CI, 1.01–1.51) were also significantly associated with an increased SSR in the multivariable-adjusted model. On the contrary, the RR of a male live birth was 0.82 (95% CI, 0.67–1.00) per 1 SD increase in log-transformed paternal MiBP in the multivariable-adjusted model, reflecting a female excess. In addition, paternal BPA was observed to be significantly associated with a decreased SSR in all models (RRs, range 0.77–0.84).

4. Discussion

This prospective cohort study suggests that paternal preconception exposure to BPA and phthalates may decrease the SSR, whereas maternal preconception exposure to these non-persistent chemicals may increase the SSR. Of note is that the opposing associations of urinary BPA and phthalate metabolite concentrations with the SSR were only observed when both partners' concentrations were modeled jointly, highlighting the importance of couple-based approach for assessing couple-dependent outcomes. Nevertheless, caution should be exercised when interpreting our results, given the lack of robustness in the significant findings depending upon model specification as well as the possibility of chance findings due to our exploratory data analysis approach. The fragility of models and/or the small sample size may have resulted in relatively few significant findings in this exploratory work, some of which no longer remained significant when restricting to couples conceiving within three months in light of the short half-lives of BPA and phthalates (data not shown). Further confirmatory research is needed before any conclusions can be drawn from our preliminary findings on the association between non-persistent chemicals and the SSR.

The observed gender-specific effects of BPA and phthalates on the SSR are difficult to explain, given our inability to find previous studies focusing on both partners' concentrations, rather than maternal or paternal, and the SSR. Our findings on paternal BPA and MiBP appear to be consistent with previous findings on persistent chemicals and the SSR. Although existing evidence is limited, paternal exposures to persistent chemicals including dioxins have been reported to be associated with an excess of female births, with the exception of PCBs, of which the effects on the SSR differed by individual congener (Taylor et al., 2007; Terrell et al., 2011). Meanwhile, Taylor et al. (2007) reported that the direction of the odds of a male birth varied by the purported hormonal activity of PCB congeners. Specifically, maternal preconception exposure to estrogenic PCBs but not anti-estrogenic PCBs was associated with an excess of male births, although none of the findings achieved significance. Still, the biological mechanisms responsible for the varying relations between BPA or particular phthalates and the SSR remain unclear. However, it is known that phthalates are predominantly anti-androgenic compounds (Carbone et al., 2013; Albert and Jégou, 2014), whereas BPA is an estrogenic compound. Gender-specific changes in steroid hormone levels in rats exposed to DEHP have been shown (Carbone et al., 2013). Gender differences in hypothalamic-pituitary-adrenal axis responses, which are known to be related to gonadal steroid hormones, have been reported in rats exposed to BPA (Chen et al, 2014; Handa & Weiser, 2014).

BPA and phthalates are suspected to produce anti-androgenic activity by reducing testosterone production (Akingbemi et al., 2004; Hu et al., 2009) and possibly weak estrogenic or anti-estrogenic activity (Moore, 2000; Okubo et al., 2003; vom Saal and Hughes, 2005), despite inconsistency between *in vitro* and *in vivo* studies. These hormonal effects are thought to be mediated by nuclear estrogen receptors (α and β) and possibly by other pathways (Casals-Casas and Desvergne, 2011). One of the major hypotheses that explain the determinants of sex-selection in humans and other mammals is the hormonal hypothesis, which postulates that the SSR may be affected by parental hormones around the time of conception (James, 2008b, 2012, 2013). According to the hypothesis, low paternal

testosterone and high follicle-stimulating hormone levels tend to be associated with female births, whereas high maternal estrogen levels tend to be associated with male births (James, 2013). Exposure to environmentally relevant doses of BPA has been associated with reduction in testosterone in males (Mendiola et al., 2010; Joensen et al., 2012). The ‘over-ripeness ovopathy’ theory also links human sex selection to oocyte maturation and cervical mucus liquefaction mediated by estrogen (Jongbloet, 2004). This theory focuses on the characteristics of smaller Y-bearing sperm in comparison with X-bearing sperm, which may be more capable of navigating in the female reproductive tract. If hormonal imbalance caused by environmental factors generates non-optimally matured oocytes with coexisting non-optimally liquefied cervical mucus, preferential fertilization of the oocyte by Y-bearing sperm may be facilitated, which, in turn, may result in the selective loss of male embryos (Jongbloet, 2004). Our findings and scarce experimental evidence on the effects of BPA and phthalates on sex ratio may be a consequence of hormonal imbalance, though our findings are limited by the absence of preconceptional hormonal data (Izumi et al., 2008; Jarmołowicz et al., 2014).

To date, not all phthalates and their metabolites have been thoroughly evaluated for potential reproductive and/or developmental toxicity either in animals or in humans. MnBP, MiBP and MBzP are phthalate metabolites which reflect recent exposure to the parent phthalate diesters, dibutyl phthalate (DBP), di-isobutyl phthalate (DiBP) and benzylbutyl phthalate (BzBP), respectively. Previous studies have demonstrated that DBP, a substitute for DiBP with similar properties, and BzBP may produce reproductive and developmental toxicity, with the possibility of species-related differences (McKee et al., 2004). In multi-generation rodent studies, dietary exposure to DBP or BzBP resulted in a decrease in serum testosterone level in the parent animals and reduced anogenital distance and undescended testes in the male offspring (Kavlock et al., 2002; Struve et al., 2009; Tyl et al., 2004). These genital morphological changes have been also revealed in boys whose mothers had elevated prenatal phthalate exposure (Swan et al., 2005). Here, we first report the effects of MnBP, MiBP and MBzP on the SSR, but the findings need future corroboration.

There are important study limitations that should be taken into account when interpreting our findings. We could not measure the primary sex ratio due to our reliance on births rather than conceptions. The disproportionate loss of males relative to females from conception to birth has been previously summarized (Buck Louis and Platt, 2011). The effects of non-persistent chemicals on this differential remain obscure, given our inability to measure conceptions. Although the strength of this study includes our prospective cohort with preconception chemical measurements, the sample size is relatively small with regard to the detection of variability in the SSR. We also relied on only one measurement of total BPA and phthalate metabolites, despite their reported short half-lives. Thus, the temporal variability of these chemicals and possible misclassification of exposure status should be considered (Lassen et al., 2013; Meeker et al., 2013; Koch et al., 2014). Among the study participants, the levels of BPA and phthalate metabolites were relatively lower in comparison with those in the U.S. general population. This may be due to differences in age distribution among the two populations, given that the LIFE study cohort comprises only couples of reproductive age. In addition, our analysis was restricted to couples with a live birth, possibly excluding couples exposed to the highest levels of the chemicals and

consequently experienced adverse reproductive outcomes. Due to the lack of data on hormone levels of each partner, we could not prove underlying mechanisms in relation to hormonal imbalance caused by the chemicals. Lastly, other selective factors or the possibility of residual confounding need to be considered.

5. Conclusion

This study provides new evidence on the effects of non-persistent chemicals on the SSR. While not inconsistent with data from persistent chemicals, this study suggests that paternal preconception exposure to BPA and MiBP may be associated with the reversal of the SSR resulting in an excess of female births. The opposing associations observed for male and female partners' BPA and select phthalate metabolite concentrations are interesting and challenging, as yet, to explain, but underscore the importance of assessing couples in relation to the SSR. Further studies with the incorporation of both partners' hormonal profiles and other relevant reproductive outcomes are warranted to elucidate the effects of non-persistent chemicals on human sex selection, as a parentally-mediated reproductive event.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This study was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (contracts #N01-HD-3-3355, N01-HD-3-3356, N01-HD-3-3358). Dr. Bae was supported by the Korea-US Visiting Scientist Training Award (VSTA) of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (award #VFTB057303).

List of abbreviations

BPA	bisphenol A
BzBP	benzylbutyl phthalate
CI	confidence interval
DBP	dibutyl phthalate
DEHP	di-2-ethylhexyl phthalate
DiBP	di-isobutyl phthalate
EDC	endocrine-disrupting chemical
GED	General Educational Development
GM	geometric mean
LIFE	Longitudinal Investigation of Fertility and the Environment
LOD	limit of detection

MBzP	mono-benzyl phthalate
MCHP	mono-cyclohexyl phthalate
MCMHP	mono-[(2-carboxymethyl) hexyl] phthalate
MCPP	mono-(3-carboxypropyl) phthalate
MECPP	mono-(2-ethyl-5-carboxypentyl) phthalate
MEHHP	mono-(2-ethyl-5-hydroxyhexyl) phthalate
MEHP	mono-2-ethylhexyl phthalate
MEOHP	mono-(2-ethyl-5-oxohexyl) phthalate
MEP	mono-ethyl phthalate
MiBP	mono-isobutyl phthalate
MiNP	mono-isononyl phthalate
MMP	mono-methyl phthalate
MnBP	mono-n-butyl phthalate
MOP	mono-n-octyl phthalate
PCB	polychlorinated biphenyl
RR	relative risk
SD	standard deviation
SSR	secondary sex ratio

References

- Akingbemi BT, Sottas CM, Koulova AI, Kliinfelter GR, Hardy MP. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology*. 2004; 145:592–603. [PubMed: 14605012]
- Albert O, Jégou B. A critical assessment of the endocrine susceptibility of the human testis to phthalates from fetal life to adulthood. *Hum Reprod Update*. 2014; 20:231–249. [PubMed: 24077978]
- Barr DB, Silva MJ, Kato K, Reidy JA, Malek NA, Hurtz D, et al. Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. *Environ Health Perspect*. 2003; 111:1148–1151. [PubMed: 12842765]
- Biggar RJ, Wohlfahrt J, Westergaard T, Melbye M. Sex ratios, family size, and birth order. *Am J Epidemiol*. 1999; 150:957–962. [PubMed: 10547141]
- Buck Louis, GM.; Platt, RW. *Reproductive and perinatal epidemiology*. 1. Oxford University Press; New York: 2011. p. 50-51.
- Buck Louis GM, Schisterman EF, Sweeney AM, Wilcosky TC, Gore-Langton RE, Lynch CD, et al. Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development – the LIFE Study. *Paediatr Perinat Epidemiol*. 2011; 25:413–424. [PubMed: 21819423]
- Buck Louis GM, Sundaram R, Sweeney AM, Schisterman EF, Maisog J, Kannan K. Urinary bisphenol A, phthalates, and couple fecundity: the Longitudinal Investigation of Fertility and the Environment (LIFE) Study. *Fertil Steril*. 2014; 101:1359–1366. [PubMed: 24534276]

- Carbone S, Ponzo OJ, Gobetto N, Samaniego YA, Reynoso R, Scacchi P, et al. Antiandrogenic effect of perinatal exposure to the endocrine disruptor di-(2-ethylhexyl) phthalate increases anxiety-like behavior in male rats during sexual maturation. *Horm Behav.* 2013; 63:692–699. [PubMed: 23399322]
- Casals-Casas C, Desvergne B. Endocrine disruptors: from endocrine to metabolic disruption. *Annu Rev Physiol.* 2011; 73:135–162. [PubMed: 21054169]
- Centers for Disease Control and Prevention. Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables. Aug. 2014 Available from: <http://www.cdc.gov/exposurereport/>
- Central Intelligence Agency. The world factbook. Available from: https://www.cia.gov/library/publications/the-world-factbook/fields/print_2018.html
- Chahnazarian A. Determinants of the sex ratio at birth: review of recent literature. *Soc Biol.* 1988; 35:214–235. [PubMed: 3071849]
- Chen F, Zhou L, Bai Y, Zhou R, Chen L. Sex differences in the adult HPA axis and affective behaviors are altered by perinatal exposure to a low dose of bisphenol A. *Brain Res.* 2014; 1571:12–24. [PubMed: 24857958]
- Davis DL, Gottlieb MB, Stampnitzky JR. Reduced ratio of male to female births in several industrial countries: a sentinel health indicator? *JAMA.* 1998; 279:1018–1023. [PubMed: 9533502]
- Davis DL, Webster P, Stainthorpe H, Chilton J, Jones L, Doi R. Declines in sex ratio at birth and fetal deaths in Japan, and in U.S. whites but not African Americans. *Environ Health Perspect.* 2007; 115:941–946. [PubMed: 17589604]
- Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev.* 2009; 30:293–342. [PubMed: 19502515]
- Ehrlich S, Williams PL, Missmer SA, Flaws JA, Berry KF, Calafat AM, et al. Urinary bisphenol A concentrations and implantation failure among women undergoing in vitro fertilization. *Environ Health Perspect.* 2012a; 120:978–983. [PubMed: 22484414]
- Ehrlich S, Williams PL, Missmer SA, Flaws JA, Ye X, Calafat AM, et al. Urinary bisphenol A concentrations and early reproductive health outcomes among women undergoing IVF. *Hum Reprod.* 2012b; 27:3583–3592. [PubMed: 23014629]
- Fowler PA, Bellingham M, Sinclair KD, Evans NP, Pocar P, Fischer B. Impact of endocrine-disrupting compounds (EDCs) on female reproductive health. *Mol Cell Endocrinol.* 2012; 355:231–239. [PubMed: 22061620]
- Frederiksen H, Jensen TK, Jørgensen N, Kyhl HB, Husby S, Skakkebaek NE, et al. Human urinary excretion of non-persistent environmental chemicals: an overview of Danish data collected between 2006 and 2012. *Reproduction.* 2014; 147:555–565. [PubMed: 24395915]
- Frederiksen H, Skakkebaek NE, Andersson AM. Metabolism of phthalates in humans. *Mol Nutr Food Res.* 2007; 51:899–911. [PubMed: 17604388]
- Fukuda M, Fukuda K, Shimizu T, Moller H. Decline in sex ratio at birth after Kobe earthquake. *Hum Reprod.* 1998; 13:2321–2322. [PubMed: 9756319]
- Geens T, Aerts D, Berthot C, Bourguignon JP, Goeyens L, Lecomte P, et al. A review of dietary and non-dietary exposure to bisphenol-A. *Food Chem Toxicol.* 2012; 50:3725–3740. [PubMed: 22889897]
- Geens T, Goeyens L, Covaci A. Are potential sources for human exposure to bisphenol-A overlooked? *Int J Hyg Environ Health.* 2011; 214:339–347. [PubMed: 21570349]
- Grech V, Vassallo-Agius P, Savona-Ventura C. Secular trends in sex ratios at birth in North America and Europe over the second half of the 20th century. *J Epidemiol Community Health.* 2003; 57:612–615. [PubMed: 12883068]
- Guerrero-Bosagna CM, Skinner MK. Epigenetic transgenerational effects of endocrine disruptors on male reproduction. *Semin Reprod Med.* 2009; 27:403–408. [PubMed: 19711250]
- Guo Y, Alomirah H, Cho HS, Minh TB, Mohd MA, Nkata H, et al. Phthalates metabolites in human urine from several Asian countries. *Environ Sci Technol.* 2011; 45:3138–3144. [PubMed: 21395215]

- Guo Y, Zhang Z, Liu L, Li Y, Ren N, Kannan K. Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure. *J Agric Food Chem*. 2012; 60:6913–6919. [PubMed: 22703192]
- Handa RJ, Weiser MJ. Gonadal steroid hormones and the hypothalamo-pituitary-adrenal axis. *Front Neuroendocrinol*. 2014; 35:197–220. [PubMed: 24246855]
- Hauser R. Urinary phthalate metabolites and semen quality: a review of a potential biomarker of susceptibility. *Int J Androl*. 2008; 31:112–117. [PubMed: 18067563]
- Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. *Int J Hyg Environ Health*. 2007; 210:623–634. [PubMed: 17889607]
- Hu GX, Lian QQ, Ge RS, Hardy DO, Li XK. Phthalate-induced testicular dysgenesis syndrome: Leydig cell influence. *Trends Endocrinol Metab*. 2009; 20:139–145. [PubMed: 19278865]
- Izumi N, Yanagibori R, Shigeno S, Sajiki J. Effects of bisphenol A on the development, growth, and sex ratio of the housefly *Musca domestica*. *Environ Toxicol Chem*. 2008; 27:1343–1353. [PubMed: 18211122]
- James WH. Evolution and the variation of mammalian sex ratios at birth: reflections on Trivers and Willard (1973). *J Theor Biol*. 2013; 334:141–148. [PubMed: 23810932]
- James WH. Hypotheses on the stability and variation of human sex ratios at birth. *J Theor Biol*. 2012; 310:183–186. [PubMed: 22776504]
- James WH. Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels around the time of conception. *J Endocrinol*. 2008a; 198:3–15. [PubMed: 18577567]
- James WH. The variations of human sex ratio at birth with time of conception within the cycle, coital rate around the time of conception, duration of time taken to achieve conception, and duration of gestation: a synthesis. *J Theor Biol*. 2008b; 255:199–204. [PubMed: 18687340]
- James WH. The human sex ratio. Part 1: a review of the literature. *Hum Biol*. 1987; 59:721–752. [PubMed: 3319883]
- Jarmołowicz S, Demska-Zak K, Zak Z. Impact of butyl benzyl phthalate on development of the reproductive system of European pikeperch, *Sander lucioperca* (L.). *Acta Vet Hung*. 2014:1–11. Epub ahead of print. [PubMed: 24334080]
- Joensen U, Frederiksen H, Jensen MB, Lauritsen MP, Olesen IA, Lassen TH, et al. Phthalate excretion pattern and testicular function: a study of 881 healthy Danish men. *Environ Health Perspect*. 2012; 120:1397–1403. [PubMed: 22832070]
- Jongbloet PH. Over-ripeness ovopathy: a challenging hypothesis for sex ratio modulation. *Hum Reprod*. 2004; 19:769–774. [PubMed: 15033945]
- Juntunen KS, Kvist AP, Kauppila AJ. A shift from a male to a female majority in newborns with the increasing age of grand grand multiparous women. *Hum Reprod*. 1997; 12:2321–2323. [PubMed: 9402303]
- Jurewicz J, Radwan M, Sobala W, Ligocka D, Radwan P, Bochenek M, et al. Human urinary phthalate metabolites level and main semen parameters, sperm chromatin structure, sperm aneuploidy and reproductive hormones. *Reprod Toxicol*. 2013; 42:232–241. [PubMed: 24140385]
- Kavlock R, Boekelheide K, Chapin R, Cunningham M, Faustman E, Foster P, et al. NTP Center for the Evaluation of Risks to Human Reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di-n-butyl phthalate. *Reprod Toxicol*. 2002; 16:489–527. [PubMed: 12406493]
- Knez J. Endocrine-disrupting chemicals and male reproductive health. *Reprod Biomed Online*. 2013; 26:440–448. [PubMed: 23510680]
- Koch HM, Aylward LL, Hays SM, Smolders R, Moos RK, Cocker J, et al. Inter-and intra-individual variation in urinary biomarker concentrations over a 6-day sampling period. Part 2: Personal care product ingredients. *Toxicol Lett*. 2014 (Available online ahead of print on June 20 2014. 10.1016/j.toxlet.2014.06.023
- Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Joensen UN, Main KM, et al. Urinary bisphenol A levels in young men: association with reproductive hormones and semen quality. *Environ Health Perspect*. 2014; 122:478–484. [PubMed: 24786630]

- Liao C, Kannan K. Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. *J Agric Food Chem*. 2013; 61:4655–4662. [PubMed: 23614805]
- Liao C, Kannan K. A survey of alkylphenols, bisphenols, and triclosan in personal care products from China and the United States. *Arch Environ Contam Toxicol*. 2014; 67:50–59. [PubMed: 24639116]
- Manfo FP, Jubendradass R, Nantia EA, Moundipa PF, Mathur PP. Adverse effects of bisphenol A on male reproductive function. *Rev Environ Contam Toxicol*. 2014; 228:57–82. [PubMed: 24162092]
- Mathews TJ, Hamilton BE. Trend analysis of the sex ratio at birth in the United States. *Natl Vital Stat Rep*. 2005; 53:1–17.
- McKee RH, Butala JH, David RM, Gans G. NTP center for the evaluation of risks to human reproduction reports on phthalates: addressing the data gaps. *Reprod Toxicol*. 2004; 18:1–22. [PubMed: 15013060]
- Meeker JD, Cantonwine DE, Rivera-González LO, Ferguson KK, Mukherjee B, Calafat AM, et al. Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico. *Environ Sci Technol*. 2013; 47:3439–3447. [PubMed: 23469879]
- Mendiola J, Jørgensen N, Andersson AM, Calafat AM, Ye X, Redmon JB, et al. Are environmental levels of bisphenol A associated with reproductive function in fertile men? *Environ Health Perspect*. 2010; 118:1286–1291. [PubMed: 20494855]
- Mok-Lin E, Ehrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, et al. Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. *Int J Androl*. 2010; 33:385–393. [PubMed: 20002217]
- Moore NP. The oestrogenic potential of the phthalate esters. *Reprod Toxicol*. 2000; 14:183–192. [PubMed: 10838119]
- Mrema EJ, Rubino FM, Brambilla G, Moretto A, Tsatsakis AM, Colosio C. Persistent organochlorinated pesticides and mechanisms of their toxicity. *Toxicology*. 2013; 307:74–88. [PubMed: 23219589]
- Ober C. The maternal-fetal relationship in human pregnancy: an immunogenetic perspective. *Exp Clin Immunogenet*. 1992; 9:1–14. [PubMed: 1642900]
- Okubo T, Suzuki T, Yokoyama Y, Kano K, Kano I. Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. *Biol Pharm Bull*. 2003; 26:1219–1224. [PubMed: 12913283]
- Richardson DB, Ciampi A. Effects of exposure measurement error when an exposure variable is constrained by a lower limit. *Am J Epidemiol*. 2003; 157:355–363. [PubMed: 12578806]
- Rubin BS. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. *J Steroid Biochem Mol Biol*. 2011; 127:27–34. [PubMed: 21605673]
- Schisterman EF, Vexler A, Whitcomb BW, Liu A. The limitations due to exposure detection limits for regression models. *Am J Epidemiol*. 2006; 163:374–383. [PubMed: 16394206]
- Specht IO, Toft G, Hougaard KS, Lindh CH, Lenters V, Jönsson BA. Associations between serum phthalates and biomarkers of reproductive function in 589 adult men. *Environ Int*. 2014; 66:146–156. [PubMed: 24583187]
- Struve MF, Gaido KW, Hensley JB, Lehmann KP, Ross SM, Sochaski MA, et al. Reproductive toxicity and pharmacokinetics of di-n-butyl phthalate (DBP) following dietary exposure of pregnant rats. *Birth Defects Res B Dev Reprod Toxicol*. 2009; 86:345–354. [PubMed: 19585553]
- Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect*. 2005; 113:1056–1061. [PubMed: 16079079]
- Taylor KC, Jackson LW, Lynch CD, Kostyniak PJ, Buck Louis GM. Preconception maternal polychlorinated biphenyl concentrations and the secondary sex ratio. *Environ Res*. 2007; 103:99–105. [PubMed: 16780830]
- Teeguarden JG, Calafat AM, Ye X, Doerge DR, Churchwell MI, Gunawan R, et al. Twenty-four hour human urine and serum profiles of bisphenol A during high-dietary exposure. *Toxicol Sci*. 2011; 123:48–57. [PubMed: 21705716]

- Terrell ML, Hartnett KP, Marcus M. Can environmental or occupational hazards alter the sex ratio at birth. *Emerg Health Treats J.* 2011; 4:7109.
- Toft G, Jönsson BA, Lindh CH, Jensen TK, Hjøllund NH, Vested A, et al. Association between pregnancy loss and urinary phthalate levels around the time of conception. *Environ Health Perspect.* 2012; 120:458–463. [PubMed: 22113848]
- Tranfo G, Caporossi L, Paci E, Aragona C, Romanzi D, De Carolis C, et al. Urinary phthalate monoesters concentration in couples with infertility problems. *Toxicol Lett.* 2012; 213:15–20. [PubMed: 22197707]
- Tyl RW, Myers CB, Marr MC, Fail PA, Seely JC, Brine DR, et al. Reproductive toxicity evaluation of dietary butyl benzyl phthalate (BBP) in rats. *Reprod Toxicol.* 2004; 18:241–264. [PubMed: 15019722]
- vom Saal FS, Hughes C. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. *Environ Health Perspect.* 2005; 113:926–933. [PubMed: 16079060]
- Weinberg CR, Baird DD, Wilcox AJ. The sex of the baby may be related to the length of the follicular phase in the conception cycle. *Hum Reprod.* 1995; 10:304–307. [PubMed: 7769053]
- Wittassek M, Koch HM, Angerer J, Brüning T. Assessing exposure to phthalates -the human biomonitoring approach. *Mol Nutr Food Res.* 2011; 55:7–31. [PubMed: 20564479]
- Younglai EV, Wu YJ, Foster WG. Reproductive toxicology of environmental toxicants: emerging issues and concerns. *Curr Pharm Des.* 2007; 13:3005–3012. [PubMed: 17979743]
- Zhang Z, Alomirah H, Cho HS, Li Y, Liao C, Minh TB, et al. Urinary bisphenol A concentrations and their implications for human exposure in several Asian countries. *Environ Sci Technol.* 2011; 45:7044–7050. [PubMed: 21732633]
- Zorn B, Sucur V, Stare J, Meden-Vrtovec H. Decline in sex ratio at birth after 10-day war in Slovenia: brief communication. *Hum Reprod.* 2002; 17:3173–3177. [PubMed: 12456620]
- Zou G. A modified Poisson regression approach to prospective studies with binary data. *Am J Epidemiol.* 2004; 159:702–706. [PubMed: 15033648]

Highlights

- Several chemicals have been reported to be associated with the SSR.
- The effects of BPA and phthalates on the SSR have not been explored.
- Paternal BPA and MiBP were associated with an excess of female births.
- Maternal BPA, MnBP, MiBP, and MBzP were associated with an excess of male births.
- These partner-specific associations need future corroboration.

Table 1

Baseline characteristics of mothers and fathers with a singleton birth, LIFE Study, Michigan and Texas, 2005–2009

Characteristic	Mothers (n=220) N (%)	Fathers (n=220) N (%)
Parity		
Nulliparous	104 (47.5)	
Parous	115 (52.5)	
Previously fathered a pregnancy		
No		90 (40.9)
Yes		130 (59.1)
Annual income (\$)		
< 30,000	4 (1.9)	4 (1.8)
30,000–49,999	25 (11.6)	20 (9.2)
50,000–69,999	28 (13.0)	33 (15.2)
70,000	158 (73.5)	160 (73.7)
Education **		
High school graduate/GED	9 (4.1)	7 (3.2)
Some college/technical school	26 (11.9)	54 (24.8)
College graduate or higher	183 (83.9)	157 (72.0)
Race/ethnicity		
Non-Hispanic white	181 (83.0)	182 (83.1)
Non-Hispanic black	3 (1.4)	5 (2.3)
Hispanic	18 (8.3)	20 (9.1)
Other	16 (7.3)	12 (5.5)
Infant sex		
Boy		110 (50.0)
Girl		110 (50.0)
	Mean (±SD)	Mean (±SD)
Age (year) *	29.6 (±3.7)	31.4 (±4.6)
	Geometric mean (95% CI)	Geometric mean (95% CI)
Urinary creatinine (mg/dL) ***	64.1 (56.8–72.4)	118.5 (107.2–131.1)

GED, General Educational Development; SD, standard deviation; CI, confidence interval

*
p-value < 0.05;

**
p-value < 0.01;

p-value < 0.0001

Geometric means (95% confidence intervals) of maternal and paternal urinary bisphenol A and phthalate metabolite concentrations by infant sex, LIFE Study, Michigan and Texas, 2005–2009

Table 2

Chemical (ng/mL)	Maternal concentration (n=213)			Paternal concentration (n=212)		
	% < LOD	Boy GM (95% CI)	Girl GM (95% CI)	% < LOD	Boy GM (95% CI)	Girl GM (95% CI)
BPA	1	0.34 (0.25–0.46)	0.41 (0.32–0.52)	1	0.53 (0.41–0.67)	0.70 (0.53–0.92)
MMP	70	0.61 (0.40–0.94)	0.57 (0.40–0.82)	63	0.67 (0.47–0.97)	1.01 (0.70–1.45)
MEP	<1	59.4 (42.9–82.3)	65.9 (49.0–88.7)	<1	95.5 (68.4–113)	103 (75.4–140)
MCPP	5	2.96 (2.17–4.03)	3.22 (2.39–4.35)	4	5.33 (4.02–7.06)	5.71 (4.25–7.68)
MnBP	<1	6.20 (4.77–8.05)	6.11 (4.84–7.71)	1	6.43 (4.93–8.37)	7.50 (5.93–9.49)
MtBP	3	3.30 (2.51–4.34)	3.33 (2.63–4.21)	2	3.71 (2.95–4.66)	4.12 (3.24–5.24)
MECPP	1	14.4 (10.9–19.2)	12.4 (9.19–16.6)	<1	17.5 (13.0–23.6)	20.5 (15.7–26.9)
MCMHP	<1	9.32 (7.06–12.3)	9.48 (7.18–12.5)	1	17.1 (12.6–23.2)	21.0 (15.9–27.9)
MEHHP	1	9.00 (6.83–11.9)	9.30 (7.03–12.3)	1	13.6 (9.88–18.7)	16.0 (12.0–21.5)
MEOHP	4	5.51 (4.05–7.49)	4.79 (3.47–6.61)	1	6.17 (4.44–8.58)	8.10 (6.16–10.7)
MCHP	95	0.02 (0.01–0.03)	0.01 (0.01–0.02)	96	0.01 (0.01–0.02)	0.01 (0.01–0.02)
MBzP	4	2.90 (2.15–3.90)	2.91 (2.22–3.82)	4	3.12 (2.36–4.13)	3.80 (2.97–4.85)
MEHP	60	3.46 (2.30–5.20)	4.53 (2.88–7.11)	49	4.45 (2.92–6.79)	4.62 (3.09–6.91)
MOP	97	0.06 (0.04–0.09)	0.07 (0.03–0.13)	97	0.06 (0.03–0.12)	0.07 (0.04–0.12)
MtNP	95	0.05 (0.03–0.08)	0.08 (0.05–0.14)	95	0.09 (0.07–0.13)	0.07 (0.03–0.13)

LIFE, Longitudinal Investigation of Fertility and the Environment; LOD, limitation of detection; GM, geometric mean; CI, confidence interval; BPA, bisphenol A; MMP, mono-methyl phthalate; MEP, mono-ethyl phthalate; MCP, mono-(3-carboxypropyl) phthalate; MnBP, mono-n-butyl phthalate; MtBP, mono-isobutyl phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MCMHP, mono-[(2-carboxymethyl) hexyl] phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MCHP, mono-cyclohexyl phthalate; MBzP, mono-benzyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MOP, mono-n-octyl phthalate; MtNP, mono-isononyl phthalate

Note: Of 220 couples, 7 mothers and 8 fathers without urinary BPA and phthalate metabolite concentrations were excluded. The LODs ranged from 0.02 to 1 ng/mL. None of the GMs differed significantly by infant sex (all *p*-values > 0.05).

Urinary bisphenol A and phthalate metabolite concentrations and the relative risks of a male birth by partner, LIFE Study, 2005–2009

Table 3

Chemical (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)
BPA	1.00 (0.88–1.15)	1.05 (0.92–1.20)	1.04 (0.91–1.18)	0.85 (0.72–1.01)	0.84 (0.69–1.01)	0.82 (0.67–1.02)
MMP	0.99 (0.86–1.13)	1.02 (0.88–1.18)	1.04 (0.89–1.23)	0.88 (0.76–1.02)	0.87 (0.74–1.03)	0.87 (0.74–1.02)
MEP	0.97 (0.85–1.11)	0.99 (0.86–1.15)	0.99 (0.85–1.14)	0.98 (0.85–1.12)	0.99 (0.85–1.15)	1.00 (0.85–1.17)
MCP	0.97 (0.85–1.12)	1.02 (0.84–1.23)	0.99 (0.82–1.20)	0.95 (0.83–1.10)	0.93 (0.78–1.10)	0.91 (0.75–1.10)
MnBP	1.01 (0.89–1.16)	1.11 (0.93–1.32)	1.14 (0.95–1.37)	0.95 (0.81–1.10)	0.90 (0.72–1.13)	0.84 (0.66–1.06)
MtBP	1.03 (0.91–1.18)	1.13 (0.96–1.33)	1.19 (0.99–1.43)	0.94 (0.82–1.08)	0.91 (0.75–1.11)	0.87 (0.71–1.07)
MECPP	1.05 (0.92–1.20)	1.12 (0.96–1.30)	1.12 (0.96–1.31)	0.95 (0.83–1.09)	0.95 (0.81–1.13)	0.94 (0.79–1.13)
MCMHP	0.99 (0.87–1.14)	1.03 (0.86–1.22)	1.04 (0.86–1.25)	0.93 (0.81–1.07)	0.92 (0.78–1.08)	0.87 (0.73–1.05)
MEHHP	1.00 (0.87–1.14)	1.03 (0.88–1.20)	1.05 (0.89–1.24)	0.95 (0.82–1.09)	0.95 (0.81–1.11)	0.92 (0.78–1.09)
MEOHP	1.04 (0.92–1.19)	1.10 (0.95–1.28)	1.12 (0.95–1.32)	0.94 (0.81–1.08)	0.94 (0.80–1.11)	0.93 (0.78–1.11)
MCHP	1.01 (0.89–1.16)	1.02 (0.89–1.16)	1.01 (0.89–1.16)	0.94 (0.79–1.11)	0.94 (0.79–1.11)	0.94 (0.78–1.12)
MBzP	1.02 (0.89–1.16)	1.13 (0.95–1.34)	1.15 (0.96–1.37)	0.95 (0.82–1.09)	0.92 (0.76–1.12)	0.94 (0.77–1.16)
MEHP	0.99 (0.86–1.13)	1.00 (0.87–1.15)	1.01 (0.87–1.17)	0.92 (0.78–1.07)	0.93 (0.79–1.08)	0.92 (0.78–1.09)
MOP	0.97 (0.85–1.11)	0.97 (0.84–1.11)	0.97 (0.84–1.12)	1.03 (0.93–1.15)	1.03 (0.92–1.14)	0.98 (0.82–1.19)
MtNP	0.93 (0.82–1.06)	0.93 (0.82–1.05)	0.94 (0.82–1.08)	1.00 (0.87–1.14)	1.00 (0.87–1.14)	1.01 (0.88–1.16)

LIFE, Longitudinal Investigation of Fertility and the Environment; RR, relative risk; CI, confidence interval; BPA, bisphenol A; MMP, mono-methyl phthalate; MEP, mono-ethyl phthalate; MCP, mono-(3-carboxypropyl) phthalate; MnBP, mono-n-butyl phthalate; MtBP, mono-isobutyl phthalate; MECPP, mono-(2-ethyl-5-carboxyphenyl) phthalate; MCMHP, mono-[(2-carboxymethyl) hexyl] phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MCHP, mono-cyclohexyl phthalate; MBzP, mono-benzyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MOP, mono-n-octyl phthalate; MtNP, mono-isononyl phthalate

Note: Chemicals 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 urinary creatinine (continuous)

^b Adjusted for urinary creatinine (continuous), research site (Michigan/Texas), age (continuous), annual income (< \$30,000, 30,000–49,999, 50,000–69,999, and 70,000), and maternal parity(nulliparous/parous)

Couples' urinary bisphenol A and phthalate metabolite concentrations and the relative risks of a male birth, LIFE Study, Michigan and Texas, 2005–2009 (n= 205 couples)

Table 4

Chemical (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)
BPA	1.06 (0.93–1.22)	1.15 (1.02–1.31)*	1.16 (1.03–1.31)*	0.84 (0.71–1.00)*	0.79 (0.65–0.95)*	0.77 (0.62–0.95)*
MMP	1.04 (0.91–1.19)	1.08 (0.93–1.25)	1.12 (0.93–1.35)	0.87 (0.75–1.02)	0.86 (0.73–1.01)	0.87 (0.73–1.05)
MEP	0.99 (0.86–1.13)	1.02 (0.87–1.18)	1.03 (0.88–1.20)	0.98 (0.85–1.13)	0.98 (0.84–1.15)	0.98 (0.83–1.17)
MCPP	0.99 (0.86–1.14)	1.04 (0.85–1.28)	1.03 (0.84–1.27)	0.97 (0.84–1.11)	0.93 (0.78–1.10)	0.90 (0.73–1.11)
MnBP	1.06 (0.92–1.22)	1.18 (0.97–1.43)	1.24 (1.01–1.51)*	0.94 (0.80–1.10)	0.88 (0.68–1.12)	0.80 (0.63–1.02)
MtBP	1.08 (0.94–1.24)	1.22 (1.03–1.44)*	1.28 (1.06–1.54)*	0.92 (0.79–1.06)	0.84 (0.69–1.03)	0.82 (0.67–1.00)*
MECPP	1.09 (0.95–1.24)	1.16 (1.00–1.35)	1.17 (0.98–1.39)	0.95 (0.82–1.09)	0.92 (0.78–1.09)	0.93 (0.77–1.12)
MCMHP	1.03 (0.90–1.19)	1.11 (0.91–1.35)	1.15 (0.92–1.44)	0.94 (0.81–1.08)	0.89 (0.75–1.07)	0.85 (0.69–1.04)
MEHHP	1.02 (0.89–1.18)	1.06 (0.90–1.25)	1.06 (0.88–1.29)	0.95 (0.82–1.10)	0.94 (0.80–1.10)	0.92 (0.77–1.10)
MEOHP	1.08 (0.94–1.23)	1.15 (0.99–1.34)	1.17 (0.98–1.39)	0.93 (0.80–1.08)	0.91 (0.77–1.08)	0.92 (0.76–1.11)
MCHP	1.01 (0.89–1.15)	1.01 (0.89–1.15)	1.02 (0.89–1.17)	0.94 (0.78–1.12)	0.93 (0.78–1.11)	0.94 (0.78–1.13)
MBzP	1.08 (0.94–1.23)	1.24 (1.06–1.46)*	1.31 (1.08–1.58)*	0.94 (0.82–1.09)	0.89 (0.74–1.07)	0.90 (0.73–1.11)
MEHP	1.01 (0.88–1.16)	1.01 (0.88–1.17)	1.04 (0.87–1.23)	0.92 (0.78–1.09)	0.93 (0.80–1.09)	0.94 (0.78–1.12)
MOP	0.95 (0.82–1.10)	0.94 (0.81–1.09)	0.94 (0.80–1.11)	1.04 (0.93–1.16)	1.04 (0.94–1.16)	0.97 (0.80–1.18)
MtNP	0.92 (0.80–1.06)	0.93 (0.81–1.06)	0.94 (0.79–1.11)	1.01 (0.89–1.15)	1.01 (0.89–1.14)	1.02 (0.88–1.17)

LIFE, Longitudinal Investigation of Fertility and the Environment; RR, relative risk; CI, confidence interval; BPA, bisphenol A; MMP, mono-methyl phthalate; MEP, mono-ethyl phthalate; MCP, mono-(3-carboxypropyl) phthalate; MnBP, mono-n-butyl phthalate; MtBP, mono-isobutyl phthalate; MECPP, mono-(2-ethyl-5-carboxyphenyl) phthalate; MCMHP, mono-[(2-carboxymethyl) hexyl] phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MCHP, mono-cyclohexyl phthalate; MBzP, mono-benzyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MOP, mono-n-octyl phthalate; MtNP, mono-isononyl phthalate

Note: Couples' chemical concentrations 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 urinary creatinine (continuous)

^b Adjusted for urinary creatinine (continuous), research site (Michigan/Texas), maternal age (continuous), difference in couples' ages (continuous), annual income (< \$30,000, 30,000–49,999, 50,000–69,999, and 70,000), and maternal parity (nulliparous/parous)

* *p*-value < 0.05 before rounding