Published in final edited form as:
Fertil Steril. 2014 May ; 101(5): 1359–1366. doi:10.1016/j.fertnstert.2014.01.022.

Urinary Bisphenol A, Phthalates and Couple Fecundity, The LIFE Study

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Abstract

Objective—Assess the relation between environmental chemicals and couple fecundity or time-to-pregnancy (TTP).

Design—Prospective cohort.

Setting—Couples completed interviews and anthropometric assessments and provided urine specimens for quantification of bisphenol A (BPA) and 14 phthalate metabolites using high-performance liquid chromatography with electrospray triple-quadrupole mass spectrometer. Women recorded menstruation and pregnancy test results in daily journals. Couples were followed until a positive hCG pregnancy test or 12 cycles without pregnancy.

Patients—501 couples recruited upon discontinuing contraception to become pregnant, 2005–2009.

Interventions—None

Main Outcome—Fecundability odds ratios (FORs) and 95% confidence intervals (CIs) were estimated for each partner's chemical concentrations adjusting for age, body mass index, cotinine, creatinine, and research site while accounting for time off contraception.

Results—Neither female nor male BPA concentration was associated with TTP (FOR 0.98; 95% CI 0.86, 1.13 and FOR 1.04; 95% CI 0.91, 1.18, respectively). Men's urinary concentrations of

monomethyl, mono-*n*-butyl and monobenzyl phthalates were associated with a longer TTP (FOR=0.80, 95% CI 0.70, 0.93; FOR=0.82, 0.70, 0.97; and FOR=0.77, 0.65–0.92, respectively).

Conclusions—Select male but not female phthalate exposures were associated with an approximately 20% reduction in fecundity underscoring the importance of assessing both partners' exposure to minimize erroneous conclusions.

Keywords

Bisphenol A; endocrine disrupting chemicals; fecundity; phthalates; reproduction

Introduction

During the past few decades, considerable evidence has arisen suggesting that exogenous chemicals may interfere with hormonal homeostasis resulting in a spectrum of adverse reproductive and/or developmental outcomes (1). At least one professional society has concluded that sufficient evidence now exists linking endocrine disrupting chemicals (EDCs) to adverse human reproductive effects, including possible epigenetic and trans-generational effects (2, 3). Much of the available EDC literature has focused on persistent chemicals such as dioxins or polychlorinated biphenyls that resist degradation and biaccumulate and biomagnify in the ecosystem, serving as a route of exposure for human and wildlife populations (4–6).

Recent findings suggest that chemicals with short half-lives, or non-persistent chemicals, also may impact human fecundity, defined as the biologic capacity of men and women for reproduction irrespective of pregnancy intentions (7). Much of the available human research conducted to date utilizes couples seeking assisted reproductive technologies (ART). The unique feature of this evolving body of evidence reflects the ability to measure sensitive fecundity endpoints, from oocyte retrieval through implantation, in keeping with the highly timed and conditional nature of human reproduction. Bisphenol A (BPA) and phthalates are ubiquitous environmental chemicals that have been linked to reproductive outcomes among couples seeking infertility treatment including ART. For example, an inverse relation between urinary BPA and serum inhibin and estradiol:testosterone ratio in men attending an infertility clinic was recently reported (8).

Free testosterone, but not other reproductive hormone concentrations or semen parameters, was negatively associated with urinary BPA concentrations among 375 male partners of pregnant women (9). In women, other findings include an inverse relation between BPA and peak estradiol and the number of oocytes retrieved in women (10, 11), the absence of pregnancy (12), and implantation failure, particularly for women with diminished ovarian reserve (13). While reasons for implantation failure were not reported in the latter study, follow on work reported higher BPA concentrations in female partners as being associated with lower serum estradiol, oocyte yield, mature oocyte count, and the number of normally fertilizing oocytes (14). Perhaps the evidence most suggestive of BPA's potential reproductive toxicity in females stems from recently completed experimental research using discarded human oocytes. Specifically, BPA was negatively associated with the percentage

of oocytes progressing to metaphase II along with a positive relation with the percentage of oocytes that either degenerated or underwent spontaneous activation (15).

With regard to phthalates, much of the research on human fecundity focuses on semen quality with little study of other reproductive endpoints. A landmark paper reported that environmentally relevant concentrations of monoethyl phthalate (MEP) but not seven other phthalates was associated with increased sperm DNA damage among men attending an andrology clinic as a part of an infertility evaluation (16). Additional investigation of these and other men identified a dose response relation between mono-n-butyl phthalate (MBP), and sperm motility and concentration, and monomethyl phthalate (MMP) with abnormal semen morphology (17). However, no significant associations were observed between any of the eight phthalates and sperm movement characteristics, as determined using computer-aided sperm analysis (CASA) (18). In an occupational cross-sectional study involving 45 workers in a polyvinyl chloride plant where phthalates are added to enhance the flexibility of plastics, ambient air concentrations of di-2-ethylhexyl phthalate (DEHP) were adversely associated with sperm motility and chromatin DNA integrity (19). Adverse effects for some but not all phthalates and select semen quality parameters have been assessed in two samples of men from the general population. Specifically, MEP was negatively associated with motile sperm and luteinizing hormone concentration, though positively associated with immotile sperms in 234 Swedish men undergoing their military conscript medical examination (20). Among 881 Danish men participating in a semen quality study, 14 phthalate metabolites were assessed and only the primary metabolites of DEHP and diisononyl phthalate (DiNP) excreted as mono (2-ethylhexyl) phthalate (mEHP), and monoisononyl phthalate (MiNP) were associated with compromised testosterone production along with pituitary-hypothalamic inhibition of gonadotropin release (21).

Two previous novel papers offer some insight regarding male mediated effects and time-to-pregnancy (TTP). In a small case control study comprising 56 couples with and 56 without infertility, infertile partners excreted higher concentrations of five phthalate metabolites in comparison to fertile partners (22). A cohort study comprising couples planning pregnancy and prospectively followed for six cycles reported that mEHP but not five other phthalates was significantly associated with approximately a three-fold increased odds of pregnancy loss (23).

These early findings are consistent with a considerable body of experimental animal evidence suggesting that BPA and phthalates disrupt oocyte maturation (24, 25), impair steroidogenesis (26, 27) and alter development of reproductive organs (28, 29) among other pathways. The ability of BPA and phthalates to reach sensitive targeted tissues such as follicular fluid and semen (30, 31) coupled with their high volume production (32, 33) and ubiquitous source of human exposure (34, 35) underscores the need for purposeful investigation. We utilized data and biospecimens from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study to assess urinary BPA and phthalate concentrations and couple fecundity as measured by TTP.

Materials and Methods

Study Design and Population

We recruited 501 couples discontinuing contraception and attempting to become pregnant using state-specific population-based sampling frameworks from 16 targeted counties in Michigan and Texas during 2005–2009 with reported exposure to persistent environmental chemicals (as described elsewhere) (36). Briefly, couples were screened for eligibility: females aged 18–44 and males aged ≥ 18 years; female's menstrual cycle 21–42 days; in a committed relationship; no injectable hormonal contraceptives in the past year or currently lactating; no physician diagnosed infertility/sterility; and an ability to communicate in English or Spanish. Our cohort size was powered for detection of significant differences in the concentration of persistent chemicals and TTP.

Data Collection and Operational Definitions

Baseline interviews with each partner of the couple were conducted in the home followed by standardized anthropometric assessment for the calculation of body mass index (BMI), along with the collection of urine and blood specimens for chemical and cotinine quantification (100% participation). Women completed daily journals to capture information on intercourse, menstruation and home pregnancy test results, while men completed daily journals on lifestyle. Women were trained in the use of the Clearblue® Easy Fertility Monitor, which is a urinary-based kit that tracks the rise in estrone-3-glucuronide (E_3G) and luteinizing hormone (LH). The monitor displays low, high or peak fertility to aid couples in timing intercourse, and is reported to be 99% accurate in detecting the LH surge when compared to vaginal ultrasonology (37). Women also were instructed in the accurate use of digital Clearblue® Easy home pregnancy kits commencing on the day of expected menstruation. Study participants were remunerated $75 for complete participation. Human subjects' approval was received from all collaborating institutions, and participants were fully consented before data collection.

TTP was used to assess couple fecundity and denotes the number of prospectively observed menstrual cycles required for an hCG pregnancy. We utilized fertility monitor and daily journal information to define a menstrual cycle, i.e., interval (in days) from the onset of bleeding with 2+ bleeding days of increasing intensity to the onset of the next similar bleeding episode. Because of our design, we were able to differentiate couples becoming pregnant within the first few weeks of enrollment, or before a fully observed menstrual cycle (TTP=0), from those becoming pregnant in the first fully observed cycle (TTP=1). Definitions of relevant covariates included: age (years), BMI (weight in kg/ height in m^2), gravidity (# pregnancies), parity (# live births), serum cotinine (ng/ml), and urine creatinine (mg/dl) concentrations.

Toxicologic Analysis

Urinary total BPA concentrations were quantified (ng/mL) using high-performance liquid chromatography (HPLC) coupled with API 2000 electrospray triple-quadrupole mass spectrometer (MS/MS) using an established protocol (38). The laboratory limit of quantitation (LOQ) was 0.05 ng/mL, as calculated from twice that of the lowest valid

acceptable calibration standard. Phthalate metabolites **w**ere analyzed in 0.5 mL of urine after enzymatic deconjugation followed by solid phase extraction and HPLC-MS/MS detection using an established protocol (39). Fourteen phthalate metabolites were quantified (ng/mL): mono (3-carboxypropyl) phthalate (mCPP), monomethyl phthalate (mMP), monoethyl phthalate (mEP), mono (2-isobutyl phthalate) (miBP), mono-*n*-butyl phthalate (mBP), mono (2-ethyl-5-carboxyphentyl) phthalate (mECPP), mono-[(2-carboxymethyl) hexyl] phthalate (mCMHP), mono (2-ethyl-5-oxohexyl) phthalate (mEOHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), monocyclohexyl phthalate (mCHP), monobenzyl phthalate (mBzP), mono (2-ethylhexyl) phthalate (mEHP), mono-isononyl phthalate (mNP), and monooctyl phthalate (mOP).

Laboratory operating procedures included ongoing quality assurance and quality control procedures, including participation in proficiency testing programs. A method blank, a spiked blank and a pair of matrix-spiked sample/duplicates were processed for each batch comprising 25 samples. Trace levels of mBP, miBP, and mEHP were detected in procedural blanks necessitating the subtraction of blank values. The regression coefficient of calibration standards, injected at concentrations ranging from 0.05 to 20 ng/mL, was > 0.999. The limit of quantitation (LOQ) for phthalate metabolites ranged from 0.2 to 1.0 ng/mL, as determined from the lowest point of the calibration standard and a nominal sample volume of 0.5 mL.

Creatinine was quantified (mg/dl) in 0.15 ml of urine using a Roche/Hitachi Model 912 clinical analyzer (Dallas, TX) and the Creatinine Plus Assay. Cotinine concentration was quantified (ng/ml) in 1 ml of serum using liquid chromatography-isotope dilution tandem mass spectrometry (40).

Statistical Analysis

A variety of descriptive statistical analyses were undertaken to explore the completeness of data and to evaluate the distributions of BPA and phthalates. Statistical significance was formally assessed using either the Chi-square statistic, t-test or Wilcoxon nonparametric test for categorical and continuous data, respectively. Fecundability odds ratios (FORs) and 95% confidence intervals (CIs) were estimated for each chemical and initially for each partner adjusting for *a priori* established potential confounders: age, BMI, and cigarette smoking (41–46). Also, all models adjusted for research site to account for any residual confounding and creatinine to account for urinary volume. Models accounted for left truncation or time couples were off contraception. Lastly, we jointly modeled both partners' exposures given their low correlations to assess associations between female chemical concentrations and fecundity when adjusting for the male partner's concentrations, as well as the reverse. FORs were estimated using Cox models for discrete survival time that allows for a cycle-varying intercept (47). Couples were censored in analyses upon withdrawal or upon 12 months of trying. Chemicals were log transformed and rescaled by their standard deviations to estimate the odds of becoming pregnant per one standard deviation increase in chemical concentration conditional on not achieving pregnancy in the previous cycle. Consistent with contemporary analytical practice, we used all machine observed concentrations and did not substitute concentrations <LOQ nor creatinine standardized (48–50). Rather, creatinine was

included as a covariate in the model. All testable modeling assumptions were evaluated, namely proportional hazards and covariates' linearity (51). Diminished fecundity denotes FORs <1 or a longer TTP, while FORs >1 denote a shorter TTP. Consistent with our exploratory analytic plan, findings were considered significant if p-values were <0.05 or CIs excluded one without adjusting for multiple comparisons. We report p-values with CIs to aid in the interpretation of findings.

Results

The study cohort comprised mostly white, college-educated couples with health insurance (Table 1). Female and male partners were on average 30.0 (±4.1) and 31.8 (±4.9) years of age, with mean BMIs of 27.6 (±7.3) and 29.8 (±5.6), respectively. Among couples, 47% of women and 48% of men reported a previous live birth, with an average of 1.1 (standard deviation ±1.3) pregnancies. During the period of follow-up, 347 (69%) couples became pregnant.

As Table 2 reflects, most study participants had BPA concentrations >LOQ (98%) and also for 9/14 phthalates (94%–99%). The five remaining phthalates (i.e., mMP, mCHP, mEHP, mOP, and mNP) had half or more concentrations <LOQ, ranging from 58% to 98%. Among women, geometric mean concentrations for mEP were significantly higher in women not becoming pregnant in comparison to women becoming pregnant (103.5 and 93.8 ng/mL, respectively). Four phthalates were significantly higher in men whose partners did not achieve pregnancy in comparison to those who did. These included: mMP (0.80 and 0.63 ng/mL), mBP (7.09 and 5.94 ng/mL), mECPP (19.49 and 17.19 ng/mL), and mBzP (3.84 and 2.79 ng/mL), respectively. Of note are the low correlations between partners' chemical concentrations for either BPA (r=0.14) or phthalates (r=0 to 0.3) diminishing concerns about possible collinearity in joint models with couples' exposures. Reasons for the lack or correlations for couples' concentrations are unknown, but may reflect varying behaviors or lifestyle.

Neither female BPA nor any phthalate concentrations were significantly associated with diminished couple fecundity in (un)adjusted models (Table 3). One noted exception was for phthalate mCPP, which was significantly ($p < 0.05$) associated with a shorter TTP (FOR = 1.20; 95% CI 1.00, 1.43). Male BPA concentration was positively associated with TTP, though the CI included one (FOR = 1.04; 95% CI 0.91, 1.18). For males, three phthalates were significantly associated with approximately a 20% reduction in couple fecundity per standard deviation change in concentration reflecting in a longer TTP. They are: mMP (FOR = 0.80; 95% CI 0.70, 0.93), mBP (FOR=0.82; 95% CI 0.70. 0.97) and mBzP (FOR = 0.77; 95% CI = 0.65, 0.92).

As Table 4 reflects when both partners' chemical concentrations were jointly modeled along with relevant covariates, female mCPP and mOP were observed to be associated with a significantly shorter TTP (FOR = 1.22; 95% CI 1.02, 1.47 and FOR = 1.18; 95% CI 1.03, 1.35, respectively). Among male partners, mMP and mBzP remained significantly associated with diminished couple fecundity (FOR = 0.81; 95% CI 0.70, 0.94 and FOR = 0.80; 95% CI 0.67, 0.97, respectively) in adjusted models that also included the female's

concentration. When all four significant phthalates listed on Table 3 were included in one model along with covariates, male mMP remained significant (FOR = 0.83; 95% CI 0.72, 0.96 data not shown).

Discussion

In this prospective cohort study with preconception recruitment of couples discontinuing contraception for purposes of becoming pregnant and followed until a hCG pregnancy or a year of trying, we found that select phthalates as measured in the urine of male but not female partners were associated with a longer TTP. BPA was not associated with TTP irrespective of partner's urinary concentration, though inspection of dose dependency corroborated the consistent negative and positive associations with female and male concentrations and TTP (data not shown). The FORs for mMP, mBP and mBzP reflected approximately a 20% reduction in fecundity, comparable in magnitude to effects reported for cigarette smoking or BMI (43, 52). A salient finding is the importance of quantifying exposures in both partners of the couple when assessing couple dependent reproductive outcomes such as time to pregnancy. Our findings would essentially be null or in an opposing direction had we only considered the female partner.

Interpreting our findings within the context of the available literature is challenging, as we are unaware of any published work focusing on either BPA or phthalates and couple fecundity. As noted above, previous research has utilized couples undergoing ART for the assessment of BPA or clinical samples of men for the assessment of phthalates and reproductive hormones or semen quality. We are unaware of any research focusing on couple fecundity or TTP for these chemicals. Still, the evolving ART research is suggestive of BPA adversely affecting ovarian response, possibly through estrogenic and anti-androgenic mechanisms (53, 54) resulting in a range of adverse outcomes as reported by the infertility and ART literature on this topic (8–13). In fact, BPA has long been reported to have implications for reproductive and developmental outcomes (55). One possible explanation for the lack of an association between BPA and couple fecundity may reflect our population- rather than clinic-based sampling framework. While speculative, our findings suggest that BPA in females may be negatively associated with TTP in fecund couples. While FORs were <1.0 for female BPA, CIs included one. This observation suggests that higher exposures or a larger cohort may be needed for detection of statistically significant findings at ubiquitous environmentally relevant concentrations.

Interpreting our phthalate findings to address the observed partner specific associations with TTP is even more challenging, but assisted by the novel work focusing on men that suggests alterations in hormonal milieu and semen quality (56, 57), possibly indicative of their antiandrogenic mechanisms (58, 59). The absence of any significant negative associations between phthalates and TTP when based upon female exposures suggests that male exposure may be driving the reduction in couple fecundity reflecting in a longer TTP. Still, we are unaware of any toxicologic mechanisms responsible for the varying patterns between phthalates and TTP. A key limitation of TTP is that it is a functional measure of couple fecundity and does not determine whether reductions are female, male or couple mediated. As such, our future research plans include assessing exposures in relation to a spectrum of

male (e.g., semen quality) and female (e.g., menstruation and ovulation) fecundity and fertility endpoints.

There are important limitations that accompany this observational cohort study. While we have prospectively measured TTP, we have only one measurement of unconjugated BPA and phthalates despite their short life and excretion from the body. However, this measurement was at baseline or upon recruitment of the couple into the cohort and before observed pregnancies. Also, a single spot urine sample is reported to adequately characterize average exposure for BPA and phthalates (60, 61). The geometric mean concentrations for BPA and most phthalates observed in the LIFE Study were generally lower than those reported for U.S. biomonitoring data among participants in the NHANES Survey (http://www.cdc.gov/exposurereport/pdf/FourthReport.pdf). Also, we enrolled couples without known infertility or sterility diagnoses despite the possibility that such individuals may have the highest exposures. Other important limitations include the lack of hormonal data for either partner. Lastly, we recognize that specification of models for estimating FORs are inexact, largely a reflection of relatively limited empirical data on the determinants of human fecundity to aid model specification. While lifestyle factors are commonly assumed to be important determinants, at the population level only 14% of the variance in TTP was explained by age, menstrual cycle length, oral contraception use, and parity (62). Common lifestyle factors provided no added contribution.

In summary, select phthalates in male partners were associated with a longer TTP underscoring the importance of continued investigation of ubiquitous environmental chemicals and human reproduction and development. Such work will help inform the extent to which such exposures might adversely impact population health.

Acknowledgments

Funding: Funded by the Intramural Research Program, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (NICHD; contracts #N01-HD-3-3355; N01-HD-3-3356; NOH-HD-3-3358; HHSN27500001).

References

1. Crews D, McLachlan JA. Epigenetics, evolution, endocrine disruption, health and disease. Endocrinol. 2006; 147:S4–10.
2. Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-Disrupting chemicals: an Endocrine Society Scientific Statement. Endocrine Rev. 2009; 30(4):293–342. [PubMed: 19502515]
3. Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, et al. Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. Endocrinol. 2012; 153(9):4097–4110.
4. Fowler PA, Bellingham M, Sinclair KD, Evans NP, Pocar P, Fischer B, et al. Impact of endocrine-disrupting compounds (EDCs) on female reproductive health. Mol Cell Endocrinol. 2012; 355:231–9. [PubMed: 22061620]
5. Gregoraszczuk EL, Ptak A. Endocrine-disrupting chemicals: some actions of POPs on female reproduction. Inter J Endocrinol. 201310.1155/2013/828532
6. Balabanič D, Rupnik M, Klemenčič AK. Negative impact of endocrine-disrupting compounds on human reproductive health. Reprod Fertil Dev. 2011; 23(3):403–16. [PubMed: 21426858]

7. Buck Louis, GM. Fecundity and fertility. In: Buck Louis, GM.; Platt, RW., editors. Reproductive and perinatal epidemiology. New York: Oxford University Press; 2011. p. 16
8. Meeker JD, Calafat AM, Hauser R. Urinary bisphenol A concentrations in relation to serum thyroid and reproductive hormones levels in men from an infertility clinic. Environ Sci Technol. 2010; 44:1458–63. [PubMed: 20030380]
9. Mendiola J, Jørgensen N, Andersson A-M, 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(9):1286–91. [PubMed: 20494855]
10. Mok-Lin E, Shrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, Ye X, Hauser R. Urinary bisphenol a concentrations and ovarian response among women undergoing IVF. Int J Androl. 2010; 33(2):385–93. [PubMed: 20002217]
11. Bloom MS, Kim D, vom Saal FS, Taylor JA, Cheng G, Lamb JD, Fujimoto VF. Bisphenol A exposure reduces the estradiol response to gonadotropin stimulation during in vitro fertilization. Fertil Steril. 2011; 96(3):672–7. [PubMed: 21813122]
12. Lamb, JD.; Bloom, MS.; vom Saal, FS.; Taylor, JA.; Sandler, JR.; Fujimoto, VF. Fertil Steril; Serum bisphenol A (BPA) and reproductive outcomes in couples undergoing IVF. American Society of Reproductive Medicine Annual Meeting; San Francisco, CA. November 8–12; 2008. p. s186
13. Ehrlich S, Williams PL, Missmer SA, Flaws JA, Berry KF, Calafat AM, Ye X, Petrozza JC, Wright D, Hauser R. Urinary bisphenol A concentrations and implantation failure among women undergoing in vitro fertilization. Environ Health Perspect. 2012; 120(7):978–83. [PubMed: 22484414]
14. 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. 2012; 27(12):3583–92. [PubMed: 23014629]
15. Machtinger R, Combelles CMH, Missmer SA, Correia KF, Williams P, Hauser, et al. Bisphenol-A and human oocyte maturation in vitro. Hum Reprod. 201310.1093/humrep/det312
16. Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, Ryan L, et al. The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. Environ Health Perspect. 2003; 111(9):1164–9. [PubMed: 12842768]
17. Duty SM, Silva MJ, Barr DB, Brock JW, Ryan L, Chen Z, et al. Phthalate exposure and human semen parameters. Epidemiol. 2003; 14(3):269–77.
18. Duty SM, Calafat AM, Silva MJ, Brock JW, Ryan L, Chen Z, Overstreet J, Hauser R. The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. J Androl. 2004; 25(2):293–302. [PubMed: 14760016]
19. Huang L-P, Lee C-C, Hsu P-C, Shih T-S. The association between semen quality in workers and the concentration of di(2-ethylhexyl) phthalate in polyvinyl chloride pellet plant air. Fertil Steril. 2011; 96(1):90–94. [PubMed: 21621774]
20. Jönsson BA, Richthoff JE, Rylander L, Giwercman A, Hagmar L. Urinary phthalates metabolites and biomarkers of reproductive function in young men. Epidemiol. 2005; 16:487–93.
21. 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(10):1397–1403. [PubMed: 22832070]
22. 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 Letters. 2012; 213:15–20.
23. Toft G, Jönsson BA, Lindh CH, Jensen TK, Hjollund NH, Vested A, et al. Association between pregnancy loss and urinary phthalate levels around the time of conception. Environ Health Perspect. 2012; 120(3):458–63. [PubMed: 22113848]
24. Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Iiagan A, Voight RC, et al. Bisphenol A exposure causes meiotic aneuploidy in the female mouse. Curr Biol. 2003; 13:546–53. [PubMed: 12676084]
25. Kim EJ, Kim JW, Lee SK. Inhibition of oocyte development in Japanese medaka (Oryzias latipes) exposed to di-2-ethylhexyl phthalate. Environ Int. 2002; 28:359–65. [PubMed: 12437285]

26. Davis BJ, Weaver R, Gaines LJ, Heindel JJ. Mono-(2-ethylhexyl) phthalate suppresses estradiol production independent of FSH-cAMP stimulation in rat granulosa cells. Toxicol Appl Pharmacol. 1994; 128:224–8. [PubMed: 7940537]
27. Akingbemi BT, Sottas CM, Koulova AI, Kliinefelter 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. Endocrinol. 2004; 145:592–603.
28. Furuya M, Sasaki F, Hassanin AM, Kuwahara S, Tsukamoto Y. Effects of bispenhol-A on the growth of comb and testes of male chicken. Can J Vet Res. 2003; 67:68–71. [PubMed: 12528833]
29. Kato H, Ota T, Furuhashi T, Ohta Y, Iguchi T. Changes in reproductive organs of female rats treated with bisphenol A during the neonatal period. Reprod Toxicol. 2003; 17:283–8. [PubMed: 12759096]
30. Han SW, Lee H, Han SY, Lim DS, Jung KK, Kwack SJ, et al. An exposure assessment of DI-(2-ethylhexyl) phthalate (DEHP) and DI-*n*-butyl phthalate (DBP) in human semen. J Toxicol Environ Health. 2009; 72(Part A):1463–9.
31. Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum Reprod. 2002; 17(11):2839–41. [PubMed: 12407035]
32. Vandenberg LN, Chauhoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G. Urinary, circulating and tissue monitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect. 2010; 118:1055–70. [PubMed: 20338858]
33. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for di(2-ethylhexyl)phthalate update [online]. 2002. Available at URL: http://www.atsdr.cdc.gov/toxprofiles/tp9.html. 4/20/09
34. 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; 113:926–33. [PubMed: 16079060]
35. Rudel RA, Carmann DE, Spengler JD, Korn LR, Bordy JG. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environ Sci Technol. 2003; 37:4543–53. [PubMed: 14594359]
36. Buck Louis GM, Schisterman EF, Sweeney AM, Wilcosky TC, Gore-Langton R, Lynch CD, Barr DD, et al. Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development – the LIFE Study. Paediatr Perinatal Epidemiol. 2011; 25:413–24.
37. Behre HM, Kuhlage J, Gahner C, Sonntag B, Schem C, Schneider HP, et al. Prediction of ovulation by urinary hormone measurements with the home use ClearPlan® Fertility Monitor: comparison with transvaginal ultrasound scans and serum hormone measurements. Hum Reprod. 2000; 15:2478–82. [PubMed: 11098014]
38. Zhang Z, Alomirah H, Cho H-S, 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; 45:7044–50. [PubMed: 21732633]
39. 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; 45:3138–44. [PubMed: 21395215]
40. Bernert JT Jr, Turner WE, Pirkle JL, Sosnoff CS, Akins JR, Waldrep MK, et al. Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. Clin Chem. 1997; 43(12):2281–91. [PubMed: 9439445]
41. American Society for Reproductive Medicine. Age-related fertility decline: a committee opinion. Fertil Steril. 2008; 90:S154–155. [PubMed: 19007615]
42. Dunson DB, Colombo B, Baird DD. Changes with age in the level and duration of fertility in the menstrual cycle. Hum Reprod. 2002; 17:1399–1403. [PubMed: 11980771]
43. Wise LA, Palmer JR, Rosenberg L. Body size and time-to-pregnancy in black women. Hum Reprod. 2013 Aug 19. Online.

44. Ramlau-Hansen CH, Thulstrup AM, Nohr EA, Bonde JP, Sorensen TI, Olsen J. Subfecundity in overweight and obese couples. Hum Reprod. 2007; 22(6):1634–7. [PubMed: 17344224]
45. Augood C, Duckitt K, Templeton AA. Smoking and female infertility: a systematic review and meta-analysis. Hum Reprod. 1998; 13:1532–9. [PubMed: 9688387]
46. Bolumar F, Olsen J, Boldsen J. Smoking reduces fecundity: a European multicenter study on infertility and subfecundity. The European Study Group on Infertility and Subfecundity. Am J Epidemiol. 1996; 15(6):578–87. [PubMed: 8610675]
47. Cox DR. Regression Models and Life Tables (with discussion). J Royal Stat Soc Series. 1973; 20:187–220.
48. 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]
49. Schisterman EF, Whitcomb BW, Louis GM, Louis TA. Lipid adjustment in the analysis of environmental contaminants and human health risks. Environ Health Perspect. 2005; 113:853–857. [PubMed: 16002372]
50. 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]
51. Therneau, TM.; Grambsch, PM. Modeling Survival Data: Extending the Cox Model. New York: Springer Series, Statistics for Biology and Health; 2000.
52. Hassan MA, Killick SR. Negative lifestyle is associated with a significant reduction in fecundity. Fertil Steril. 2004; 81(2):384–392. [PubMed: 14967378]
53. Wozniak AL, Bulayeva NN, Watson CS. Xenoestrogens at picmolar to nanomolar concentrations trigger membrane estrogen receptor-a mediated Ca^{2+} fluxes and prolactin release in GH3/B6 pituitary tumor cells. Environ Health Perspect. 2005; 113:431–9. [PubMed: 15811834]
54. Lee HJ, Chattopadhyay S, Gong EY, Ahn RS, Lee K. Antiandrogenic effects of bisphenol A and nonylphenol on the function of the androgen receptor. Toxicol Sci. 2003; 75:40–6. [PubMed: 12805653]
55. Vogel SA. The politics of plastics: the making and unmaking of bisphenol A"safety". Am J Public Health. 2009; 99(Suppl 3):S559–S566. [PubMed: 19890158]
56. JD, Calafat AM, Hauser R. Urinary metabolites of di(2-ethylhexyl) phthalate are associated with decreased steroid hormone levels in adult men. J Androl. 2009; 30(3):287–97. [PubMed: 19059903]
57. Hauser R, Meeker JD, Duty S, Silva MJ, Calafat AM. Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiol. 2006; 17(6):682–91.
58. Lee BM, Koo HJ. Hershberger assay for antiandrogenic effects of phthalates. J Toxicol Environ Health A. 2007; 70(15–16):1365–70. [PubMed: 17654256]
59. Shen O, Du G, Sun H, Wu W, Jiang Y, Song L, et al. Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. Toxicol Lett. 2009; 191(1):9–14. [PubMed: 19643168]
60. Ye X, Wong L-Y, Bishop AM, Calafat AM. Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. Environ Health Perspect. 2011; 119(7): 984–988.
61. Yorita Christensen KL, Lorber M, Koch HM, Kolossa-Gehring M, Morgan MK. Population variability of phthalate metabolites and bisphenol A concentrations in spot urine samples *versus* 24- or 48-h collections. J Expo Sci Environ Epidemiol. 2012; 22:632–640. [PubMed: 22669498]
62. Axmon A, Rylander L, Albin M, Hagmar L. Factors affecting time to pregnancy. Hum Reprod. 2006; 21(5):1279–84. [PubMed: 16410331]

Table 1

Comparison of partners by select characteristics, LIFE Study.

Characteristic	Female Partners (n=501)	Male Partners (n=501)
	n (%)	*n (%)*
Nonhispanic white race/ethnicity	393 (78.9)	394 (79.1)
College/technical education	470 (94.6)	452 (91.1)
Health insurance	458 (92.0)	456 (91.6)
No live births[a]	263 (52.8)	259 (52.0)
	Mean (± SD)	*Mean (± SD)*
Age (years)	30.0 (±4.1)	31.8 (±4.9)
Body mass index (kg/m^2)	27.6 (±7.3)	29.8 (±5.6)
	Geometric mean (95% CI)	*Geometric Mean (95% CI)*
Urinary creatinine (mg/dl)[b]	67.13 (61.92, 72.78)	115.87 (108.34, 123.93)
Serum cotinine (ng/ml)[b]	0.06 (0.05, 0.08)	0.22 (0.16, 0.31)

[a] Includes 210 women reporting never having been pregnant, and 215 men who reported never fathering a live birth.

Table 2

Geometric mean comparison of urinary BPA and phthalate concentrations by partner and pregnancy status, LIFE Study.

Chemical (ng/mL)	Female Concentration			Male Concentration		
	% <LOQ	Pregnancy Mean (95% CI)	No Pregnancy Mean (95% CI)	% <LOQ	Pregnancy Mean (95% CI)	No Pregnancy Mean (95% CI)
BPA	2	0.63 (0.54–0.73)	0.68 (0.53–0.87)	2	0.53 (0.46–0.61)	0.49 (0.39–0.62)
mMP	65	0.89 (0.72–1.09)	0.77 (0.56–1.05)	61	**0.63 (0.51–0.78)**	**0.80 (0.60–1.06)***
mEP	2	**93.83 (79.57–110.64)**	**103.45 (76.58–139.75)***	1	82.73 (68.90–99.34)	78.47 (60.85–101.19)
mCPP	6	5.11 (4.45–5.87)	3.95 (3.19–4.91)	3	4.69 (4.03–5.46)	4.42 (3.55–5.50)
mBP	1	9.97 (8.96–11.09)	10.29 (8.62–12.30)	1	**5.94 (5.30–6.67)**	**7.09 (5.96–8.43)***
miBP	4	5.11 (4.58–5.70)	4.96 (4.09–6.01)	2	3.44 (3.09–3.83)	3.65 (3.10–4.29)
mECPP	2	21.18 (18.25–24.58)	21.21 (16.94–26.55)	<1	**17.19 (14.72–20.07)**	**19.49 (15.65–24.28)***
mCMHP	<1	15.91 (13.96–18.13)	14.57 (11.35–18.70)	<1	16.04 (13.76–18.69)	17.46 (13.94–21.87)
mEHHP	2	15.24 (13.01–17.86)	14.46 (11.52–18.14)	1	12.92 (10.76–15.51)	14.28 (11.06–18.42)
mEOHP	4	8.65 (7.40–10.10)	7.55 (5.86–9.74)	2	6.13 (5.16–7.27)	6.78 (5.33–8.62)
mCHP	96	0.02 (0.01–0.02)	0.01 (0.01–0.02)	96	0.01 (0.01–0.01)	0.01 (0.01–0.01)
mBzP	4	4.61 (4.06–5.23)	5.15 (4.29–6.18)	4	**2.79 (2.44–3.19)**	**3.84 (3.14–4.69)****
mEHP	58	4.56 (3.40–6.11)	5.60 (3.81–8.24)	48	3.39 (2.54–4.53)	4.06 (2.79–5.92)
mOP	98	0.08 (0.06–0.12)	0.05 (0.02–0.11)	96	0.07 (0.05–0.11)	0.08 (0.05–0.13)
mNP	97	0.11 (0.08–0.14)	0.07 (0.05–0.10)	95	0.07 (0.05–0.09)	0.05 (0.03–0.07)

NOTE: Pregnancy refers to all hCG-detected pregnancies observed during follow up of the cohort. Phthalates concentrations were creatinine adjusted for comparison. CI, confidence interval; LOQ, limits of quantification rounded to nearest decimal.

* $p < 0.05$

** $p < 0.01$

mCPP, mono (3-carboxypropyl) phthalate

mMP, monomethyl phthalate

mEP, monoethyl phthalate

miBP, mono (2-isobutyl phthalate)

mBP, mono-*n*-butyl phthalate

mECPP, mono (2-ethyl-5-carboxyphentyl) phthalate

mCMHP, mono-[(2-carboxymethyl) hexyl] phthalate

mEOHP, mono (2-ethyl-5-oxohexyl) phthalate

mEHHP, mono (2-ethyl-5-hydroxyhexyl) phthalate

mCHP, monocyclohexyl phthalate (mCHP)

mBzP, monobenzyl phthalate

mEHP, mono (2-ethylhexyl) phthalate

mNP, mono-isonoyl phthalate

mOP, monooctyl phthalate (mOP)

Table 3

Urinary BPA and phthalate concentrations and fecundability odds ratios by partner, LIFE Study.

Chemical (ng/mL)	Female Partners (n=454)			Male Partners (n=439)		
	Unadjusted	Adjusted[a]	Adjusted[b]	Unadjusted	Adjusted[a]	Adjusted[b]
BPA	0.93 (0.82, 1.06)	0.94 (0.82, 1.07)	0.98 (0.86, 1.13)	1.04 (0.92, 1.17)	1.00 (0.89, 1.14)	1.04 (0.91, 1.18)
mMP	0.95 (0.84, 1.07)	0.96 (0.84, 1.10)	0.93 (0.81, 1.08)	0.89 (0.79, 1.01)	**0.84 (0.73, 0.96)***	**0.80 (0.70, 0.93)****
mEP	0.95 (0.84, 1.07)	0.96 (0.83, 1.10)	0.97 (0.84, 1.12)	1.06 (0.94, 1.21)	1.02 (0.87, 1.18)	1.01 (0.86, 1.18)
mCPP	1.07 (0.95, 1.21)	**1.21 (1.01, 1.44)***	**1.20 (1.00, 1.43)***	1.07 (0.95, 1.21)	1.03 (0.90, 1.19)	0.98 (0.85, 1.13)
mBP	0.93 (0.83, 1.05)	0.92 (0.76, 1.11)	0.93 (0.77, 1.12)	0.95 (0.85, 1.07)	**0.83 (0.71, 0.98)***	**0.82 (0.70, 0.97)***
miBP	0.97 (0.86, 1.09)	1.00 (0.83, 1.19)	0.95 (0.78, 1.14)	1.00 (0.89, 1.13)	0.90 (0.76, 1.06)	0.88 (0.74, 1.04)
mECPP	0.99 (0.88, 1.11)	1.02 (0.88, 1.19)	1.05 (0.90, 1.22)	0.99 (0.87, 1.11)	0.92 (0.80, 1.06)	0.89 (0.77, 1.03)
mCMHP	0.98 (0.87, 1.10)	1.01 (0.87, 1.18)	1.02 (0.87, 1.19)	0.99 (0.88, 1.11)	0.92 (0.80, 1.06)	0.88 (0.76, 1.02)
mEHHP	0.99 (0.88, 1.12)	1.03 (0.89, 1.18)	1.06 (0.92, 1.22)	0.99 (0.88, 1.12)	0.95 (0.83, 1.09)	0.93 (0.82, 1.07)
mEOHP	1.00 (0.89, 1.13)	1.04 (0.90, 1.22)	1.06 (0.91, 1.24)	0.99 (0.88, 1.12)	0.94 (0.82, 1.08)	0.91 (0.79, 1.05)
mCHP	1.03 (0.92, 1.16)	1.04 (0.92, 1.17)	1.06 (0.95, 1.20)	1.06 (0.95, 1.18)	1.06 (0.95, 1.18)	1.07 (0.96, 1.20)
mBzP	0.93 (0.82, 1.05)	0.93 (0.77, 1.11)	0.94 (0.79, 1.13)	0.92 (0.81, 1.05)	**0.77 (0.65, 0.92)****	**0.77 (0.65, 0.92)****
mEHP	0.96 (0.86, 1.08)	0.97 (0.86, 1.10)	0.99 (0.87, 1.12)	1.01 (0.90, 1.13)	0.99 (0.88, 1.11)	0.98 (0.87, 1.10)
mOP	1.11 (0.99, 1.26)	1.12 (0.99, 1.26)	1.09 (0.96, 1.23)	0.97 (0.85, 1.09)	0.97 (0.85, 1.10)	0.99 (0.87, 1.12)
mNP	1.04 (0.92, 1.17)	1.04 (0.92, 1.18)	1.04 (0.91, 1.19)	1.03 (0.91, 1.16)	1.02 (0.91, 1.15)	1.01 (0.90, 1.14)

NOTE: Separate models were run for each chemical and partner. Chemicals were log transformed and rescaled by their standard deviations for analysis. All models accounted for left truncation or time couple was off contraception.

Model[a] adjusts for each partner's urinary creatinine (continuous).

Model[b] adjusts for each partner's urinary creatinine (continuous), and also for age (continuous), BMI (continuous), serum cotinine (continuous), and research site (Michigan/Texas).

* $p < 0.05$;

** $p < 0.01$

mCPP, mono (3-carboxypropyl) phthalate

mMP, monomethyl phthalate

mEP, monoethyl phthalate

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mCHP, monocyclohexyl phthalate (mCHP)

mBzP, monobenzyl phthalate

mEHP, mono (2-ethylhexyl) phthalate

mNP, mono-isonoyl phthalate

mOP, monooctyl phthalate (mOP)

Table 4

Couples' urinary BPA and phthalate concentrations and fecundability odds ratios, LIFE Study (n=424 couples).

Chemical (ng/mL)	Adjusted Model[a]		Adjusted Model[b]	
	Females	Males	Females	Males
BPA	0.93 (0.80,1.07)	1.01 (0.89,1.15)	0.96 (0.83,1.10)	1.05 (0.92,1.20)
mMP	1.00 (0.87,1.16)	**0.85 (0.73,0.98)***	0.99 (0.85,1.15)	**0.81 (0.70,0.94)****
mEP	0.96 (0.82,1.11)	1.03 (0.88,1.20)	0.97 (0.83,1.13)	1.00 (0.84,1.17)
mCPP	**1.21 (1.01,1.46)***	1.01 (0.88,1.17)	**1.22 (1.02,1.47)***	0.97 (0.83,1.12)
mBP	0.96 (0.79,1.16)	0.85 (0.72,1.00)	0.95 (0.78,1.16)	0.87 (0.73,1.04)
miBP	1.01 (0.84,1.22)	0.90 (0.76,1.08)	0.97 (0.80,1.18)	0.91 (0.76,1.09)
mECPP	1.04 (0.89,1.22)	0.91 (0.79,1.06)	1.06 (0.91,1.24)	0.89 (0.76,1.03)
mCMHP	1.02 (0.87,1.20)	0.91 (0.78,1.05)	1.04 (0.88,1.23)	0.86 (0.74,1.01)
mEHHP	1.03 (0.88,1.19)	0.94 (0.82,1.08)	1.06 (0.91,1.24)	0.92 (0.80,1.06)
mEOHP	1.07 (0.91,1.25)	0.92 (0.80,1.06)	1.08 (0.92,1.27)	0.90 (0.78,1.04)
mCHP	1.02 (0.91,1.15)	1.05 (0.94,1.18)	1.05 (0.93,1.18)	1.07 (0.95,1.20)
mBzP	0.98 (0.80,1.19)	**0.78 (0.65,0.93)****	0.98 (0.81,1.20)	**0.80 (0.67,0.97)***
mEHP	0.98 (0.86,1.11)	1.00 (0.89,1.13)	0.99 (0.87,1.12)	1.02 (0.91,1.15)
mOP	**1.19 (1.04,1.37)***	0.90 (0.78,1.04)	**1.18 (1.03,1.35)***	0.92 (0.80,1.06)
mNP	1.02 (0.89,1.16)	1.01 (0.89,1.15)	1.03 (0.90,1.18)	1.01 (0.89,1.14)

NOTE: Separate models were run for each chemical and partner. Chemicals were log transformed and rescaled by their standard deviations for analysis. All models accounted for left truncation or time couple was off contraception.

Model[a] adjusts for both partners' chemical concentrations (continuous) and urinary creatinine (continuous).

Model[b] adjusts for both partners' chemical concentrations (continuous) and urinary creatinine (continuous), and also for female age (continuous), difference in couples' ages (continuous), research site (Michigan/Texas), and both partners' BMIs (continuous) and serum cotinine (continuous).

* $p < 0.05$;

** $p < 0.01$

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