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Associations between paternal urinary phthalate metabolite concentrations and reproductive outcomes among couples seeking fertility treatment

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Abstract

INTRODUCTION—Limited evidence suggests that male exposure to ubiquitous environmental phthalates may result in poor reproductive outcomes among female partners.

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AUTHORS' ROLES

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METHODS—This analysis included male-female couples undergoing in vitro fertilization (IVF) and/or intrauterine insemination (IUI). We evaluated associations between the geometric mean of paternal specific gravity-adjusted urinary phthalate concentrations prior to the female partners' cycle and fertilization, embryo quality, implantation, and live birth using generalized linear mixed models.

RESULTS—Two-hundred eighteen couples underwent 211 IVF and 195 IUI cycles. Trends were observed between paternal urinary mono-3-carboxypropyl phthalate (MCPP; P=0.01) and mono(carboxyoctyl) phthalate (MCOP; P=0.01) and decreased odds of implantation. MCPP and MCOP were also associated with decreased odds of live birth following IVF (P=0.01 and P=0.04, respectively), and monobutyl phthalate above the first quartile was significantly associated with decreased odds of live birth following IUI (P=0.04). However, most urinary phthalate metabolites were not associated with these reproductive outcomes.

CONCLUSION—Selected phthalates were associated with decreased odds of implantation and live birth.

Keywords

Phthalates; Fertility; Male Reproduction; Assisted Reproduction

INTRODUCTION

Human exposure to phthalates, a group of industrial synthetic plasticizers and solvents used in a broad variety of consumer products is widespread [1]. The National Health and Nutrition Examination Survey (NHANES), which samples a nationally-representative population, has estimated that the majority of the U.S. population is exposed to phthalates [2]. High molecular-weight phthalates, such as di(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), and benzylbutyl phthalate (BBzP), are primarily used as plasticizers of flexible vinyl used in flooring, food packaging, or medical devices [3], and human exposure occurs largely through ingestion [4]. Low molecular-weight phthalates, such as diethyl phthalate (DEP), di-n-butyl pththalate (DBP), and diisobutyl phthalate (DiBP), are primarily used as solvents in personal care products [3] and in the coatings of some medications [5]; for these phthalates, dermal absorption and inhalation of dust in indoor air are also relevant routes of exposure [4].

Several important findings regarding the mechanism of reproductive toxicity of phthalates have emerged from animal studies. These include disruption into adulthood of the pituitary-gonadal axis in both male and female mice offspring that were exposed to DEHP in the prenatal and perinatal periods [6] and dose-dependent alterations in the expression of genes involved in cholesterol transport and steroidogenesis as well as testosterone production in the testis of male rats exposed prenatally [7]. Some phthalates, especially DEHP, inhibit synthesis of testosterone by Leydig cells in male rats [8,9]. Reduced prenatal testosterone can cause reduced anogenital distance, impaired testicular descent, and morphological changes in primary and secondary reproductive organs, including reduced genital size [10]. In addition, mono(2-ethylhexyl) phthalate (MEHP), a metabolite of DEHP, has been shown

to increase apoptosis *in vitro* in the germ cells of cultured testes from human male fetuses [11]. DiNP, which is increasingly substituting DEHP because of concerns about DEHP adverse reproductive effects, has also been shown to exhibit anti-androgenic effects in rats [12].

Limited research has been conducted on couple-level outcomes following paternal exposure to environmental chemicals, though the Longitudinal Investigation of Fertility and Environment (LIFE) Study has made important recent contributions [13–15]. The LIFE Study enrolled 501 couples who were planning pregnancy and evaluated them for exposures including phthalates, heavy metals, bisphenol A, and persistent environmental chemicals such as polychlorinated biphenyls and polybrominated diphenyl ethers. Couples were followed for pregnancy for up to one year, and those who became pregnant were followed until delivery. A recent publication described a negative association of fecundability with higher concentrations of urinary metabolites of DBP and BBzP in the male partner [13]. These associations were found among couples that conceived without medical assistance, and thus the study was unable to identify the impact of paternal exposure on very early developmental outcomes such as fertilization, implantation, or very early post-implantation pregnancy loss. Examining couples undergoing assisted reproduction, however, may allow for the investigation of whether there are paternal effects on very early development, which are outcomes that are typically unobservable in couples conceiving naturally.

In the past, the male contribution to a healthy pregnancy may have been underestimated, as in addition to their genome, sperm are responsible for making contributions important for successful fertilization and embryo development [16,17]. Sperm contribute spermatozoal RNAs, which include functional mRNAs and miRNAs that are delivered to the oocyte via the sperm during fertilization [18–21]. Spermatozoal RNAs may act as clinical markers of male fertility, and they may have direct effects on fertility in men, which could occur through epigenetic modifications during early embryonic development [22–25].

Given the compelling experimental studies on the impact of phthalates on male reproductive health and the limited human studies recently published, we conducted the present study to examine associations of paternal urinary concentrations of phthalate metabolites with fertilization, embryo quality, implantation, and live birth among couples from a fertility clinic.

MATERIALS AND METHODS

Study Setting

The present study is a sub-analysis within the Environment and Reproductive Health (EARTH) Study. The EARTH Study is a prospective cohort study aimed at identifying environmental and nutritional determinants of fertility among couples undergoing fertility treatment at the Massachusetts General Hospital (MGH) Fertility Center. Since 2004, men age 18–51 and women age 18–45 have enrolled either as individuals or as couples and have been followed from study entry until a live birth or until discontinuing treatment at the MGH Fertility Center. This analysis includes all male-female couples from the EARTH Study whose male partner had urinary concentrations of phthalate metabolites measured during his

female partner's cycle(s) in 2004–2012. Because few couples underwent more than three intrauterine insemination (IUI) or IVF cycles, this analysis includes up to three IUI and/or three fresh non-donor IVF cycles. The cycles included in the analysis are not necessarily consecutive because a small proportion of cycles without male phthalate measurements (4.4%) were excluded; for example, a man whose female partner underwent three cycles where the second cycle was missing male phthalate measurements would contribute cycles 1 and 3 to the analysis. We used overall cycle number, which counted all IVF and IUI cycles prior to cycles being excluded for any reason, to account for the total number of overall treatment cycles. Cycles using donor sperm or donor oocytes were excluded from the analysis, as were cryothaw cycles and cycles that were converted from IUI to IVF or vice versa.

Information on demographic characteristics, medical history, lifestyle, and occupation were collected by a research nurse at the time of recruitment and in subsequent self-administered questionnaires. Electronic medical records were used to obtain clinical information, and infertility diagnoses were classified according to definitions of the Society for Assisted Reproductive Technology (SART).

Ethical Approval

This study was approved by the institutional review boards at the MGH Fertility Center, the Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC).

Clinical Protocols

Briefly, couples underwent an infertility evaluation, and the female partner then underwent IUI or IVF. It was common for women to first undergo IUI and then proceed to IVF when IUI was unsuccessful. For IUI cycles, clomiphene citrate or gonadotropins were started on cycle day 3 or 5 according to physician preference. Ovulation was induced with recombinant human chorionic gonadotropin (hCG), and insemination was performed approximately 36 hours later. One of the following three treatments was used for women undergoing IVF cycles: 1) luteal phase GnRH-agonist protocol using low, regular, and high-dose leuprolide (Lupron), where pituitary desensitization was initiated in the luteal phase; 2) follicular phase GnRH-agonist/Flare protocol, where Lupron was begun in the follicular phase on day 2 of menses; or 3) GnRH-antagonist protocol, where GnRH antagonist was initiated when the lead follicle reached 14 mm in size. Exogenous gonadotropins were initiated on day 3 of menses, and ovulation was induced with hCG when at least three dominant follicles ≥16 mm were noted and peak estradiol was >600 pg/ml. Oocytes were retrieved approximately 36 hours after ovulation induction, and the retrieved oocytes were fertilized either using insemination or intracytoplasmic sperm injection (ICSI). At the MGH Fertility Center, ICSI is used for couples with severe male factor infertility, and it is used very rarely for couples who experienced failed fertilization in a prior IVF cycle. At the MGH Fertility Center, the fertilization rate ranges from 65–70%, and 5–10% of oocytes that undergo ICSI do not survive the physical manipulation (unpublished data). An embryologist evaluated the resulting embryos and made selections for transfer on day 2, 3, or 5 of embryo maturation in culture.

Outcome and Exposure Measurements

Both men and women provided one spot urine sample in a clean polypropylene specimen cup at the time of oocyte retrieval for those whose female partner was undergoing IVF or at the time of insemination for those whose female partner was undergoing IUI. Female partners provided one additional spot urine sample in the monitoring phase of both IUI and IVF cycles. Specific gravity (SG) was measured at room temperature using a handheld refractometer (National Instrument Co. Inc., Baltimore, MD), which was calibrated before each measurement using deionized water. Urine samples were divided into aliquots and frozen and stored at −80°C before they were shipped overnight on dry ice to the CDC, where they were then stored at ≤ −40°C until blinded analysis.

We measured monoethyl phthalate (MEP), mono-n-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), mono(carboxyoctyl) phthalate (MCOP), mono(carboxynonyl) phthalate (MCNP), MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP). The urinary concentrations of phthalate metabolites were obtained after enzymatic deconjugation of the metabolites from their glucuronidated form, solid-phase extraction, separation with high-performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry [26]. Isotopically-labeled internal standards and conjugated internal standards were used to increase precision of the measurements. In addition to the study samples, each analytical run included calibration standards, reagent blanks, and quality control materials of high and low concentration to monitor for accuracy and precision. The parent compounds of these metabolites and the limits of detection (LODs) of the metabolites are shown in Supplemental Table 1.

The proportion of normally fertilized oocytes, henceforth referred to as the fertilization rate, was defined as the number of oocytes with two pronuclei divided by the total number of mature metaphase II oocytes. Embryo quality was defined as high-quality embryos, which were those with four cells on day 2 of culture, eight cells on day 3 of culture, and institution-specific quality scores of either one or two on both culture days 2 and 3, which are based on even size and appearance of blastomeres and absence of fragmentation, and non-high-quality embryos. Implantation was defined as a positive pregnancy test (βhCG ≥6 mIU/ml), which was administered 17 days after the embryo transfer. Chemical pregnancy was defined as implantation that did not develop into a clinical pregnancy, which was defined by the presence of fetal sacs and/or heartbeats on ultrasound. Live birth was defined as the delivery of one or more live born infants. The full cohort (n=218 couples undergoing 406 cycles) was used to evaluate the outcome of live birth, while the sub-cohort of men whose female partner underwent IVF (n=152 couples undergoing 211 cycles) was used to evaluate fertilization rate, embryo quality, and implantation. Due to their indication of poor treatment response, IVF cycles with a culture day 2 embryo transfer were excluded from the models for implantation and live birth.

Statistical Analysis

In addition to exploring associations with individual phthalate metabolites, we also calculated the molar sum (in μmol/L) of the four DEHP metabolites (∑DEHP = MEHP + MEHHP + MEOHP +MECPP). Phthalate metabolite concentrations were log-transformed and metabolite concentrations below the LOD were assigned a value of the LOD divided by the square root of two for analysis [27]. Multivariable generalized linear mixed models were used to evaluate the associations of approximate quartiles of SG-adjusted paternal urinary phthalate metabolite concentrations with the outcomes of fertilization rate, embryo quality, implantation, and live birth. Embryo quality was modeled as a proportion of high-quality embryos among all embryos within a single IVF cycle. Estimates of the rate ratio (RR) for fertilization rate and embryo quality were obtained for each quartile compared to the lowest quartile using a binomial distribution with a logit link. Estimates of the odds ratio (OR) for implantation and live birth were obtained for each quartile compared to the lowest quartile using a binary distribution with a logit link. Log-binomial models for the relative risk were attempted but did not all converge given the limited sample size, and thus the ORs are presented; of those that did converge, the conclusions were similar. Repeated measures analysis was used to account for multiple cycles per couple. For all analyses, the man or the couple was treated as the unit of observation.

Potential confounders were chosen based on prior literature and consisted of overall cycle number, paternal and maternal age, paternal and maternal smoking (ever vs. never), and paternal and maternal body mass index (BMI; normal weight vs. overweight/obese, overweight vs. normal weight/obese, and obese vs. normal weight/overweight) [28,29]. Underweight was defined as having a BMI of <18.5 kg/m^2, normal weight was defined as having a BMI of 18.5–25 kg/m^2, overweight was defined as having a BMI of 25 –<30 kg/m^2, and obese was defined as having a BMI of ≥30 kg/m^2 [30]. Variables that were considered to be potential mediators, e.g., on the causal pathway of paternal urinary phthalate metabolite concentration and the outcome of interest, were not adjusted for in the models because this can lead to overadjustment bias [31]. These variables included any diagnosis of male factor infertility, the IVF treatment protocol (flare/antagonist vs. luteal phase) used, the number of oocytes retrieved, the use of ICSI, the number of embryos transferred, and the day of embryo transfer.

Potential confounders were evaluated in maternal age-adjusted models; maternal age was forced into these models due to its biological relevance. Potential confounders that changed the estimate for the male phthalate metabolite log concentration by >10% on the log-odds scale and that had a P-value of <0.2 were included in the adjusted models. When all confounders were included in the model, those with a P-value of ≥0.15 were removed. After adjusted models were specified, quartile of maternal phthalate concentration was additionally adjusted for in a subsequent model. Models are presented as unadjusted, adjusted for confounders, and adjusted for confounders and quartile of maternal phthalate concentration. The median urinary phthalate metabolite concentration within each quartile was modeled as a continuous measure to obtain a test of linear trend. All analyses were conducted with SAS 9.3 (SAS Institute, Cary, North Carolina), and two-sided P-values <0.05 were considered to be statistically significant.

RESULTS

BASELINE CHARACTERISTICS

Two hundred eighteen eligible couples were included in the analysis; these couples underwent a total of 406 cycles (195 IUI and 211 IVF cycles). The number of couples who underwent one, two, and three IUI cycles was 102, 66, and 30 respectively; these cycles occurred approximately four weeks apart. The number of couples who underwent one, two, and three IVF cycles was 124, 45, and 30, respectively; these cycles occurred approximately 16 weeks apart. Fifty-three percent of couples underwent IVF only, 30% underwent IUI only, and 17% underwent IUI before undergoing IVF. At the time of enrollment, the mean age of participants was 36.7 (male) and 35.0 (female) years. Most (83%) participants were white, and the majority (69%) had never smoked. The primary SART diagnoses among the 218 couples was roughly evenly split between female factor infertility (32%), male factor infertility (33%), and unexplained infertility (35%). Thirty-nine percent of couples had male factor infertility as a primary or secondary diagnosis. Two-thirds of women (67%) had a BMI <25 kg/m^2; five of them (2.3%) had BMI <18.5 kg/m^2. Although being underweight is an established risk factor for adverse reproductive outcomes [32,33], given their small numbers, these women were combined with women of normal weight. Thirty percent of men were of normal weight, and none were underweight. The characteristics of the men included in the analysis were similar to those of the cohort as a whole. Participant characteristics are shown in Table 1.

The 406 total cycles were nearly evenly split between IVF (52%) and IUI (48%). For women undergoing IVF, the majority (70%) underwent luteal phase stimulation, and approximately half (56%) underwent ICSI. Almost all embryos were transferred on day 3 (61%) or day 5 (33%); 15% of cycles used single embryo transfer, and the majority (64%) underwent double embryo transfer. Among all IVF cycles, 43% resulted in a live birth, while among IUI cycles, 12% resulted in live birth. Cycle characteristics and pregnancy outcomes are shown in Table 2.

The median geometric means of paternal unadjusted phthalate metabolite concentrations are shown in Table 3, along with comparisons to the concentrations reported in NHANES. The medians of the paternal SG-adjusted geometric mean phthalate metabolite concentrations were similar between all IVF (Supplemental Table 2) and IUI (Supplemental Table 3) cycles, and the single measurements taken at each cycle were similar to the cycle-specific median geometric means.

COVARIATES ASSOCIATED WITH IUI AND IVF OUTCOMES

Among the covariates considered for adjustment (with $p<0.20$ in univariate models), increasing maternal age, paternal age, and cycle numbers all suggested decreased odds of live birth after IVF. Unexpectedly, both maternal and paternal normal weight (vs. overweight and obese) suggested a decrease in the proportion of high-quality embryos. Embryo transfer on culture day 5 compared to transfer on culture day 3 suggested increased odds of achieving a life birth following IVF.

ASSOCIATIONS WITH REPRODUCTIVE OUTCOMES

Among IVF cycles, after adjusting for maternal age, paternal urinary phthalate metabolite concentrations were not associated with fertilization rate in any of the models (Table 4).

Aside from the second quartile of paternal urinary MCOP concentrations (aOR: 0.56, 95% CI: 0.32–0.99), no associations were found with embryo quality (Supplemental Table 4). Additionally adjusting for quartile of maternal phthalate metabolite concentration did not result in any substantial changes to these results. In adjusted models, dose-response relationships were observed between decreased odds of implantation and paternal urinary concentrations of MCPP and MCOP (Table 5).

Paternal urinary concentrations of MCOP in the third and fourth quartiles were associated with decreased odds of implantation (aOR: 0.31, 95% CI: 0.11–0.89 and aOR: 0.30, 95% CI: 0.10–0.88, respectively) after controlling for maternal age. Paternal urinary concentrations of MiBP in the third quartile also showed an association with decreased odds of implantation (aOR: 0.22, 95% CI: 0.08–0.63) after adjusting for maternal age. Further adjustment for quartile of maternal phthalate metabolite concentration did not result in any changes to the conclusions regarding associations with implantation.

Among IVF cycles, paternal urinary concentrations of MCPP and MCOP were associated with overall dose-response trends towards reduced odds of live birth (P=0.01 and P=0.04, respectively) after controlling for maternal age (Table 6).

The associations with live birth did not change substantially after additional adjustment for quartile of maternal phthalate metabolite concentration. Among IUI cycles, paternal urinary concentrations of MBP above the first quartile was associated with 78–88% decreased odds of live birth (P-trend=0.04) after adjusting for maternal age and maternal smoking, though the fourth quartile and trend were no longer significant after additionally adjusting for quartile of maternal MBP concentration (Supplemental Table 5). No other phthalate metabolites showed significant dose-response relationships with live birth following IUI. Apart from MBP, the only individual quartile associated with live birth following IUI was paternal concentrations of MEP in the second quartile (aOR: 0.20; 95% CI: 0.05–0.88), which remained significant after adjusting for quartile of maternal MEP concentration.

DISCUSSION

These findings suggest that paternal urinary concentrations of select phthalate metabolites (MBP, MCOP, and MCPP) were associated with adverse reproductive outcomes in female partners undergoing fertility treatments. This was shown by significant trends of increasing concentrations of paternal urinary MCOP and MCPP with decreased implantation and live birth following IVF; additionally, there was an association between higher urinary MBP concentrations and reduced odds of live birth following IUI. MCOP is a metabolite of DiNP, MBP is the major metabolite of DBP, and MCPP is a non-specific metabolite of several high molecular weight phthalates (e.g., DnOP, DiNP, and DiDP) and a minor metabolite of DBP.

In the LIFE study, they reported a negative association between fecundability and urinary concentrations of MBP and MBzP in the male partner [13]. Decreased fecundability could result from a variety of unobservable intermediate endpoints, such as failed fertilization, poor embryo quality, or implantation failure. However, because the LIFE Study is conducted among couples conceiving naturally, the investigators were unable to assess these early outcomes. In our cohort, urinary concentrations of MBzP and MBP were inversely associated with fertilization rate and odds of live birth following IUI, respectively; these findings are consistent with the reduced fecundity reported among LIFE study participants. That MBP was not associated with reduced odds of live birth following IVF may indicate that the more intense clinical interventions inherent in IVF are able to overcome the potential biologic vulnerabilities that cannot be overcome in natural or IUI conceptions. Our study and the LIFE Study assessed many of the same phthalate metabolites. However, in the LIFE Study, the authors presented the urinary concentrations without correction for urinary dilution, although the models adjusted for creatinine as a covariate, whereas the urinary concentrations in our study were adjusted using specific gravity; thus, the concentrations reported in these two studies may not be directly comparable. We chose to adjust for specific gravity as opposed to creatinine because creatinine is affected by age, muscle mass, diet, and sex [34,35]. However, by comparing results between the natural conceptions occurring in the LIFE Study and the assisted conceptions occurring in the EARTH Study, these studies offer insights into potential effects of paternal exposure to phthalates.

In our earlier publication on the same clinic population reported here, male urinary MEHP concentrations were inversely associated with circulating serum testosterone and estradiol levels, consistent with the anti-androgenic effects of phthalates as endocrine disruptors [36]. These altered hormone levels may have adverse implications for spermatogenesis; as a driver of spermatogenesis, reduced testosterone can result in decreased sperm count [37], and as estradiol can promote the survival of male germ cells [38,39], lowered levels could also reduce the efficiency of spermatogenesis. The anti-androgenic effects of phthalates may also result in poorer semen quality. In our earlier publication using the same clinic population as the current study, we found sperm concentration to be inversely associated with concentrations of MBP [40], which in our current study was inversely associated with odds of live birth following IUI. In this context, the decreased odds of live birth following IUI could be a result of low sperm concentration due to the anti-androgenic effects of phthalate metabolites such as MBP. Low sperm counts reduce the probability of pregnancy, but it is also possible that phthalates may have effects on other developmental endpoints. For instance, we found that concentrations of MBzP were inversely associated with fertilization rate. Additionally, MCOP concentrations above the first quartile showed point estimates well below 1, though only the third and fourth quartiles were statistically significant, and this is consistent with findings that concentrations in the second quartile were associated with a lower proportion of high-quality embryos among couples undergoing IVF. The lack of more consistent dose-response relationships could be due to a lack of power from small samples and/or the existence of nonlinear relationships between urinary phthalate concentrations and reproductive outcomes. Additionally, the findings that some phthalate metabolites were associated with particular outcomes while others were not may indicate differing mechanisms of action among the phthalates.

Strengths and Limitations

This study provides some of the first evidence regarding the reproductive effects of paternal exposure to various phthalate metabolites. Strengths of this study include its ability to observe the intermediate outcomes of fertilization, embryo quality and implantation, as well as the standardized assessments of the outcomes. In addition, IUI cycles were also examined, which allows for additional insight into the relevant biological mechanisms associated with phthalate exposure and reproductive outcomes. A limitation of our study is that there may be exposure misclassification due to the short half-life of phthalates (measured in hours) [41–43]. The short half-lives and the likely episodic nature of the exposures can result in considerable within-individual variability in urinary concentrations of the phthalate metabolites. Previous work in our cohort has shown the intraclass correlation coefficients (ICC) to be 0.49 (MEP), 0.24 (MCOP), 0.14 (MCPP), and 0.23 (MCNP), while the ICCs for the DEHP metabolites have been shown to range from 0.13 to 0.39 [44]. We attempted to minimize misclassification by using the geometric mean concentration of all samples collected prior to insemination or embryo transfer, as opposed to the concurrent measure. If misclassification remained, it was expected to be non-differential with respect to the outcome, which would bias these results towards the null. Small sample size is a concern, and it especially limited our power to detect associations between live birth following IUI and paternal urinary concentrations of phthalate metabolites due to the few live births occurring after IUI. It also prevented us from stratifying the analyses by ICSI, which is important in the setting of male exposures. Given this limited power, borderline significant associations may be meaningful and should be examined in a larger cohort. Additionally, it may be possible that for later pregnancy outcomes such as live birth rates, the toxicological burden on the fetus in utero may be more important than the paternal exposure. Phthalate metabolites cross the placenta and have an extended half-life compared to the maternal circulation [45,46]. While it was not possible in this study to measure concentrations of phthalate metabolites in amniotic fluid or fetal serum, the combination of fetal exposure combined with preconception paternal and maternal exposure is important for future investigations [47]. Future studies should also consider adjusting for maternal urinary concentrations during pregnancy. Finally, although multiple comparisons may be a concern, given the need to better understand the potential effect of paternal exposures on reproduction, we wanted to explore the associations between all the measured outcomes and urinary phthalate metabolite concentrations.

Additionally, NHANES data suggest that race and ethnicity are determinants of urinary phthalate concentrations [1]. Thus, the phthalate metabolite concentrations reported in our analysis, from a population that was 83% Caucasian, may not be representative of population concentrations among other racial/ethnic groups. Finally, our study population of couples undergoing IVF and IUI differs from the general population in that our participants have subfertility. Couples undergoing assisted reproduction also tend to be older and have higher socioeconomic status than couples from the general population [48]. While these factors have important implications for generalizability, this population of couples who are already having difficulty achieving a live birth may represent a subpopulation particularly sensitive to phthalate exposures should these adversely affect human reproduction, which is advantageous for this particular investigation.

Conclusion

Our study results suggest that male exposure to certain high molecular weight phthalates may adversely affect reproductive outcomes in couples undergoing IVF and IUI, though most phthalates were not associated with the outcomes. However, these associations would benefit from confirmation within larger and more diverse cohorts. Additionally, it will be important to investigate exposure to mixtures of environmental chemicals, both within individuals and within couples, to gain a more complete understanding of the effects these chemicals may have on reproduction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Centers for Disease Control and Prevention. Department of Health and Human Services, Centers for Disease Control and Prevention; Jan 9. 2013 Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables. www.cdc.gov/exposurereport/pdf/fourthreport_updatedtables_sep2013.pdf
2. Silva MJ, Barr DB, Reidy JA, et al. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999–2000. Environ Health Perspect. 2004; 112:331–338. [PubMed: 14998749]
3. Hauser R, Calafat AM. Phthalates and human health. Occup Environ Med. 2005; 62:806–818. [PubMed: 16234408]
4. Koch HM, Lorber M, Christensen KL, Palmke C, Koslitz S, Bruning T. Identifying sources of phthalate exposure with human biomonitoring: Results of a 48h fasting study with urine collection and personal activity patterns. Int J Hyg Environ Health. 2013; 216:672–681. [PubMed: 23333758]
5. Kelley KE, Hernandez-Diaz S, Chaplin EL, Hauser R, Mitchell AA. Identification of phthalates in medications and dietary supplement formulations in the United States and Canada. Environ Health Perspect. 2012; 120:379–384. [PubMed: 22169271]
6. Pocar P, Fiandanese N, Secchi C, et al. Exposure to di(2-ethyl-hexyl) phthalate (DEHP) in utero and during lactation causes long-term pituitary-gonadal axis disruption in male and female mouse offspring. Endocrinology. 2012; 153:937–948. [PubMed: 22147016]
7. Saillenfait AM, Sabate JP, Robert A, et al. Dose-dependent alterations in gene expression and testosterone production in fetal rat testis after exposure to di-n-hexyl phthalate. J Appl Toxicol. 2013; 33:1027–1035. [PubMed: 23754470]
8. Welsh M, Saunders PT, Fisken M, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J Clin Invest. 2008; 118:1479–1490. [PubMed: 18340380]
9. 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]
10. Foster PM. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int J Androl. 2006; 29:140–147. [PubMed: 16102138]

11. Lambrot R, Muczynski V, Lecureuil C, et al. Phthalates impair germ cell development in the human fetal testis in vitro without change in testosterone production. Environ Health Perspect. 2009; 117:32–37. [PubMed: 19165384]
12. Boberg J, Christiansen S, Axelstad M, et al. Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reprod Toxicol. 2011; 31:200–209. [PubMed: 21075200]
13. 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
14. Buck Louis GM, Sundaram R, Schisterman EF, et al. Heavy metals and couple fecundity, the LIFE Study. Chemosphere. 2012; 87:1201–1207. [PubMed: 22309709]
15. Buck Louis GM, Sundaram R, Schisterman EF, et al. Persistent environmental pollutants and couple fecundity: the LIFE study. Environ Health Perspect. 2013; 121:231–236. [PubMed: 23151773]
16. Cummins JM. Cytoplasmic inheritance and its implications for animal biotechnology. Theriogenology. 2001; 55:1381–1399. [PubMed: 11327691]
17. Sutovsky P, Schatten G. Paternal contributions to the mammalian zygote: fertilization after sperm-egg fusion. Int Rev Cytol. 2000; 195:1–65. [PubMed: 10603574]
18. Ostermeier GC, Dix DJ, Miller D, Khatri P, Krawetz SA. Spermatozoal RNA profiles of normal fertile men. Lancet. 2002; 360:772–777. [PubMed: 12241836]
19. Ostermeier GC, Miller D, Huntriss JD, Diamond MP, Krawetz SA. Reproductive biology: delivering spermatozoan RNA to the oocyte. Nature. 2004; 429:154. [PubMed: 15141202]
20. Ostermeier GC, Goodrich RJ, Moldenhauer JS, Diamond MP, Krawetz SA. A suite of novel human spermatozoal RNAs. J Androl. 2005; 26:70–74. [PubMed: 15611569]
21. Lalancette C, Miller D, Li Y, Krawetz SA. Paternal contributions: new functional insights for spermatozoal RNA. J Cell Biochem. 2008; 104:1570–1579. [PubMed: 18393352]
22. Platts AE, Dix DJ, Chemes HE, et al. Success and failure in human spermatogenesis as revealed by teratozoospermic RNAs. Hum Mol Genet. 2007; 16:763–773. [PubMed: 17327269]
23. Yatsenko AN, Roy A, Chen R, et al. Non-invasive genetic diagnosis of male infertility using spermatozoal RNA: KLHL10 mutations in oligozoospermic patients impair homodimerization. Hum Mol Genet. 2006; 15:3411–3419. [PubMed: 17047026]
24. Zhao C, Huo R, Wang FQ, Lin M, Zhou ZM, Sha JH. Identification of several proteins involved in regulation of sperm motility by proteomic analysis. Fertil Steril. 2007; 87:436–438. [PubMed: 17074334]
25. Hamatani T. Human spermatozoal RNAs. Fertil Steril. 2012; 97:275–281. [PubMed: 22289287]
26. Silva MJ, Samandar E, Preau JL Jr, Reidy JA, Needham LL, Calafat AM. Quantification of 22 phthalate metabolites in human urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2007; 860:106–112.
27. Hornung R, Reed L. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990; 5:46–51.
28. Sharma R, Biedenharn KR, Fedor JM, Agarwal A. Lifestyle factors and reproductive health: taking control of your fertility. Reprod Biol Endocrinol. 2013; 11:66. [PubMed: 23870423]
29. Rooney KL, Domar AD. The impact of lifestyle behaviors on infertility treatment outcome. Curr Opin Obstet Gynecol. 2014; 26:181–185. [PubMed: 24752004]
30. World Health Organization. Global Database of Body Mass Index: BMI Classification. 2013
31. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009; 20:488–495. [PubMed: 19525685]
32. Ehrenberg HM, Dierker L, Milluzzi C, Mercer BM. Low maternal weight, failure to thrive in pregnancy, and adverse pregnancy outcomes. Am J Obstet Gynecol. 2003; 189:1726–1730. [PubMed: 14710105]
33. Schieve LA, Cogswell ME, Scanlon KS, et al. Prepregnancy body mass index and pregnancy weight gain: associations with preterm delivery. The NMIHS Collaborative Study Group. Obstet Gynecol. 2000; 96:194–200. [PubMed: 10908762]

34. Boeniger MF, Lowry LK, Rosenberg J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. Am Ind Hyg Assoc J. 1993; 54:615–627. [PubMed: 8237794]
35. Duty SM, Silva MJ, Barr DB, et al. Phthalate exposure and human semen parameters. Epidemiology. 2003; 14:269–277. [PubMed: 12859026]
36. Meeker 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; 30:287–297. [PubMed: 19059903]
37. Smith LB, Walker WH. The regulation of spermatogenesis by androgens. Semin Cell Dev Biol. 2014 Jun.:2–13. [PubMed: 24598768]
38. Hess RA, Bunick D, Lee KH, et al. A role for oestrogens in the male reproductive system. Nature. 1997; 390:509–512. [PubMed: 9393999]
39. Pentikainen V, Erkkila K, Suomalainen L, Parvinen M, Dunkel L. Estradiol acts as a germ cell survival factor in the human testis in vitro. J Clin Endocrinol Metab. 2000; 85:2057–2067. [PubMed: 10843196]
40. Hauser R, Meeker JD, Duty S, Silva MJ, Calafat AM. Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiology. 2006; 17:682–691. [PubMed: 17003688]
41. Liss GM, Albro PW, Hartle RW, Stringer WT. Urine phthalate determinations as an index of occupational exposure to phthalic anhydride and di(2-ethylhexyl)phthalate. Scand J Work Environ Health. 1985; 11:381–387. [PubMed: 4071004]
42. Dirven HA, van den Broek PH, Arends AM, et al. Metabolites of the plasticizer di(2-ethylhexyl)phthalate in urine samples of workers in polyvinylchloride processing industries. Int Arch Occup Environ Health. 1993; 64:549–554. [PubMed: 8314612]
43. Albro PW. Absorption, metabolism, and excretion of di(2-ethylhexyl) phthalate by rats and mice. Environ Health Perspect. 1986; 65:293–298. [PubMed: 3086077]
44. Meeker JD, Calafat AM, Hauser R. Urinary phthalate metabolites and their biotransformation products: predictors and temporal variability among men and women. J Expo Sci Environ Epidemiol. 2012; 22:376–385. [PubMed: 22354176]
45. Frederiksen H, Skakkebaek NE, Andersson AM. Metabolism of phthalates in humans. Mol Nutr Food Res. 2007; 51:899–911. [PubMed: 17604388]
46. Latini G, De FC, Presta G, et al. In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. Environ Health Perspect. 2003; 111:1783–1785. [PubMed: 14594632]
47. Grandjean P, Barouki R, Bellinger DC, et al. Life-Long Implications of Developmental Exposure to Environmental Stressors: New Perspectives. Endocrinology. 2015:EN20151350.
48. Martin JA, Hamilton BE, Ventura SJ, et al. Births: final data for 2009. Natl Vital Stat Rep. 2011; 60:1–70. [PubMed: 22670489]

HIGHLIGHTS

- We assessed reproductive outcomes among couples undergoing assisted reproduction
- Specific gravity-adjusted paternal urinary phthalate concentrations were quantified
- Some phthalates were associated with decreased odds of implantation and live birth

Table 1

Participant demographics and infertility diagnoses among 218 male-female couples at enrollment in the Environment and Reproductive Health (EARTH) Study

Individual characteristic	Men N (%)	Women N (%)
Age (years)—mean ± SD	36.7 ± 5.1	35.0 ± 4.0
<37	118 (54.1)	150 (68.8)
≥37	100 (45.9)	68 (31.2)
Body mass index (kg/m^2)—mean ± SD	27.3 ± 4.1*	24.2 ± 4.5
<25	65 (30.0)	146 (67.0)
25 – <30	103 (47.5)	46 (21.1)
≥30	49 (22.6)	26 (11.9)
Cycle day 3 FSH—mean ± SD	–	6.7 ± 2.2
Race		
White	183 (83.9)	178 (81.7)
Black/African American	5 (2.3)	3 (1.4)
Asian	16 (7.3)	20 (9.2)
Native American/Alaska Native	6 (2.8)	3 (1.4)
Other	7 (3.2)	14 (6.4)
Unknown	1 (0.5)	0 (0.0)
Smoking status		
Never	145 (66.5)	156 (71.6)
Ever	73 (33.5)	62 (28.4)
Former	60	55
Current	13	7

Couple characteristic	Couples N (%)
Primary SART diagnosis	
Male factor	71 (32.6)
Ovulatory dysfunction/DOR	41 (18.8)
Other female factor	29 (13.3)
Unexplained	77 (35.3)
Any diagnosis of male factor	85 (39.0)
Year of recruitment	
2004–2006	41 (18.8)
2007–2009	102 (46.8)
2010–2012 (through April)	75 (34.4)

*One value is missing

SD, standard deviation; kg, kilogram; m, meter; SART, Society for Assisted Reproductive Technology; DOR, diminished ovarian reserve

Table 2

Cycle characteristics and pregnancy outcomes overall and by cycle* among 218 couples at enrollment in the Environment and Reproductive Health (EARTH) Study

IVF cycle characteristic	All cycles N=211	Cycle 1 N=152	Cycle 2 N=40	Cycle 3 N=19
IVF protocol				
Luteal phase	148 (70.1)	123 (80.9)	19 (47.5)	6 (31.6)
Flare	39 (18.5)	16 (10.5)	14 (35.0)	9 (47.4)
Antagonist	24 (11.4)	13 (8.6)	7 (17.5)	4 (21.1)
Intracytoplasmic sperm injection	119 (56.4)	80 (52.6)	26 (65.0)	13 (68.4)
Oocytes retrieved, mean ± SD	11.0 ± 5.2	11.3 ± 5.3	10.6 ± 5.2	9.0 ± 3.7
MII oocytes retrieved, mean ± SD	9.3 ± 4.6	9.6 ± 4.5	9.1 ± 5.0	7.6 ± 3.7
Day of embryo transfer†‡				
2	11 (5.5)	9 (6.2)	2 (5.3)	0 (0.0)
3	124 (61.4)	81 (55.9)	25 (65.8)	18 (94.7)
5	67 (33.2)	55 (37.9)	11 (29.0)	1 (5.3)
# embryos transferred†				
1	30 (14.8)	24 (16.4)	6 (13.2)	1 (5.3)
2	130 (64.0)	97 (66.4)	23 (60.5)	10 (52.6)
≥3	43 (21.2)	25 (17.1)	10 (26.3)	8 (42.1)
Pregnancy outcome**				
No transfer	8 (3.8)	6 (4.0)	2 (5.0)	0 (0.0)
No oocytes retrieved	3	2	1	–
Fertilization failure	5	4	1	–
Implantation failure†	78 (38.4)	47 (32.2)	20 (52.6)	11 (57.9)
Chemical pregnancy†	13 (6.4)	9 (6.2)	3 (7.8)	1 (5.3)
Ectopic pregnancy†	3 (1.4)	3 (2.0)	0 (0.0)	0 (0.0)
Spontaneous abortion†	19 (9.0)	17 (11.2)	1 (2.5)	1 (5.3)
Therapeutic abortion†	1 (0.5)	1 (0.7)	0 (0.0)	0 (0.0)
Stillbirth†	1 (0.5)	0 (0.0)	1 (2.5)	0 (0.0)
Live birth†	88 (43.4)	69 (47.3)	13 (34.2)	6 (31.6)
IUI cycle characteristic	**All cycles N=195**	**Cycle 1 N=102**	**Cycle 2 N=66**	**Cycle 3 N=27**
Pregnancy outcome***				
Not pregnant	153 (78.5)	79 (77.5)	51 (77.3)	24 (85.7)
Chemical pregnancy	5 (2.6)	4 (3.9)	0 (0.0)	1 (3.7)
Ectopic pregnancy	1 (0.5)	1 (1.0)	0 (0.0)	0 (0.0)
Spontaneous abortion	10 (5.1)	6 (5.9)	2 (3.0)	2 (7.4)
Live birth	24 (12.2)	11 (10.8)	12 (18.2)	1 (3.7)
Unknown	2 (1.0)	1 (1.0)	1 (1.5)	0 (0.0)

Data are presented as n (%) or mean ± standard deviation (SD)

IVF, in vitro fertilization; IUI, intrauterine insemination

* IVF cycle was defined as the consecutive IVF cycle number among all IVF cycles included in the analysis; IUI cycle was defined as the consecutive IUI cycle number among all IUI cycles included in the analysis

† One value is missing

‡ Calculated among cycles with an embryo transfer

** Implantation was defined as a positive pregnancy test (βhCG ≥6 mIU/ml) 17 days following embryo transfer; chemical pregnancy was defined as implantation with no subsequent clinical pregnancy; ectopic pregnancy was defined as a pregnancy outside the uterus; spontaneous abortion was defined as spontaneous loss of a clinical pregnancy; therapeutic abortion was defined as an induced abortion of a clinical pregnancy; still birth was defined as the delivery of a dead infant; live birth was defined as delivery of a live infant

*** Not pregnant was defined as a negative pregnancy test (βhCG <6 mIU/ml) 17 days following intrauterine insemination; chemical pregnancy was defined as a positive pregnancy test (βhCG ≥6 mIU/ml) 17 days following insemination; ectopic pregnancy was defined as a pregnancy outside the uterus; spontaneous abortion was defined as spontaneous loss of a clinical pregnancy; live birth was defined as delivery of a live infant

Table 3

Median geometric means of paternal unadjusted phthalate metabolite concentrations in the present study population and unadjusted geometric mean concentrations reported among males in NHANES

Phthalate metabolite	EARTH Study 2004 – 2012	NHANES 2007 – 2008	NHANES 2009 – 2010
MEP	57.8 (23.3 – 132)	92.8 (80.1 – 107)	61.0 (54.2 – 68.6)
MBP	14.7 (7.0 – 26.2)	18.4 (16.8 – 20.3)	14.5 (13.0 – 16.3)
MiBP	8.1 (4.0 – 15.1)	7.5 (6.8 – 8.3)	7.8 (7.0 – 8.7)
MBzP	4.9 (1.7 – 10.1)	7.8 (7.0 – 8.7)	6.9 (6.1 – 7.9)
MCPP	5.0 (2.3 – 8.8)	3.0 (2.7 – 3.4)	3.4 (2.9 – 4.0)
MCOP	27.7 (9.9 – 70.4)	7.1 (6.1 – 8.4)	14.0 (11.6 – 16.8)
MCNP	4.1 (2.4 – 8.5)	2.8 (2.5 – 3.0)	3.1 (2.8 – 3.5)

NHANES, National Health and Nutrition Examination Survey

Data are presented as the median geometric mean and interquartile range (in ng/ml) for the EARTH Study and as the geometric mean and 95% confidence interval for concentrations for NHANES

Concentrations of DEHP metabolites are reported separately in NHANES and not as a sum

Table 4

Associations between fertilization rate* among initiated cycles and quartiles of geometric means of specific gravity-adjusted urinary phthalate metabolite concentrations among men whose partner underwent IVF

Phthalate metabolite quartiles	N	Unadjusted OR (95% CI)	Confounder-adjusted† OR (95% CI)	Confounder- and female phthalate-adjusted‡ OR (95% CI)
MEP (ng/ml)	211			
1 (≤26.01)	52	1.00	1.00	1.00
2 (26.02–57.07)	53	0.85 (0.57 – 1.29)	0.85 (0.56 – 1.29)	0.92 (0.60 – 1.39)
3 (57.08–140)	53	1.02 (0.67 – 1.55)	1.02 (0.67 – 1.55)	1.16 (0.75 – 1.79)
4 (141–15,634)	53	1.10 (0.72 – 1.68)	1.10 (0.72 – 1.68)	1.29 (0.83 – 2.00)
P-trend		0.42	0.42	0.15
MBP (ng/ml)	199			
1 (≤7.42)	49	1.00	1.00	1.00
2 (7.43–13.74)	50	0.73 (0.48 – 1.13)	0.73 (0.47 – 1.13)	0.66 (0.42 – 1.04)
3 (13.75–23.13)	51	0.81 (0.52 – 1.27)	0.81 (0.52 – 1.27)	0.66 (0.40 – 1.09)
4 (23.14–4,756)	49	0.85 (0.54 – 1.34)	0.85 (0.54 – 1.34)	0.69 (0.42 – 1.14)
P-trend		0.75	0.75	0.33
MiBP (ng/ml)	211			
1 (≤4.33)	51	1.00	1.00	1.00
2 (4.34–7.54)	53	1.24 (0.82 – 1.88)	1.25 (0.82 – 1.89)	1.21 (0.78 – 1.87)
3 (7.55–12.46)	54	0.71 (0.47 – 1.07)	0.71 (0.47 – 1.07)	0.67 (0.43 – 1.06)
4 (12.47–191)	53	1.06 (0.70 – 1.61)	1.06 (0.69 – 1.61)	0.95 (0.58 – 1.54)
P-trend		0.96	0.96	0.70
MBzP (ng/ml)	211			
1 (≤2.10)	52	1.00	1.00	1.00
2 (2.11–4.06)	53	0.70 (0.45 – 1.07)	0.70 (0.45 – 1.07)	0.70 (0.45 – 1.08)
3 (4.07–7.02)	53	0.73 (0.48 – 1.12)	0.73 (0.48 – 1.12)	0.78 (0.50 – 1.22)
4 (7.03–176)	53	0.66 (0.44 – 1.01)	0.67 (0.44 – 1.01)	0.69 (0.43 – 1.11)
P-trend		0.15	0.15	0.27
MCPP (ng/ml)	211			
1 (≤2.31)	53	1.00	1.00	1.00
2 (2.32–4.11)	52	1.42 (0.98 – 2.05)	1.43 (0.99 – 2.07)	1.38 (0.94 – 2.01)
3 (4.12–8.50)	53	1.41 (0.94 – 2.13)	1.42 (0.94 – 2.15)	1.37 (0.88 – 2.12)
4 (8.51–56.34)	53	1.28 (0.84 – 1.96)	1.29 (0.84 – 1.98)	1.30 (0.82 – 2.07)
P-trend		0.56	0.55	0.58
ΣDEHP (μmol/L)	211			
1 (≤0.119)	51	1.00	1.00	1.00
2 (0.120–0.219)	55	0.89 (0.60 – 1.34)	0.89 (0.60 – 1.34)	0.85 (0.55 – 1.30)
3 (0.220–0.620)	52	0.90 (0.60 – 1.36)	0.90 (0.60 – 1.36)	0.82 (0.53 – 1.28)
4 (0.630–20.51)	53	1.23 (0.80 – 1.89)	1.23 (0.80 – 1.90)	1.09 (0.67 – 1.78)
P-trend		0.14	0.14	0.31
MCOP (ng/ml)	190			

Phthalate metabolite quartiles	N	Unadjusted OR (95% CI)	Confounder-adjusted† OR (95% CI)	Confounder- and female phthalate-adjusted‡ OR (95% CI)
1 (≤9.72)	46	1.00	1.00	1.00
2 (9.73–24.43)	49	0.85 (0.55 – 1.30)	0.84 (0.55 – 1.30)	0.86 (0.52 – 1.41)
3 (24.44–71.10)	48	0.94 (0.60 – 1.47)	0.94 (0.60 – 1.47)	1.07 (0.62 – 1.83)
4 (71.11–813)	47	0.87 (0.56 – 1.37)	0.88 (0.56 – 1.38)	1.05 (0.59 – 1.87)
P-trend		0.73	0.74	0.71
MCNP (ng/ml)	190			
1 (≤2.75)	47	1.00	1.00	1.00
2 (2.76–4.54)	49	1.17 (0.77 – 1.77)	1.17 (0.77 – 1.78)	1.31 (0.83 – 2.08)
3 (4.56–8.60)	47	1.03 (0.67 – 1.58)	1.03 (0.67 – 1.59)	1.24 (0.77 – 1.99)
4 (8.61–130)	47	0.99 (0.64 – 1.53)	0.99 (0.64 – 1.54)	1.32 (0.79 – 2.20)
P-trend		0.69	0.69	0.74

*Fertilization rate was defined as the number of fertilized oocytes with two pronuclei divided by the number of mature metaphase II oocytes

†All models were adjusted for maternal age

‡These models were adjusted for maternal age and the female partner's quartile of the phthalate of interest

Table 5

Associations between implantation* among transfer cycles and quartiles of geometric means of specific gravity-adjusted urinary phthalate metabolite concentrations among men whose partner underwent IVF

Phthalate metabolite quartiles	N	Unadjusted OR (95% CI)	Confounder- adjusted† OR (95% CI)	Confounder- and female phthalate-adjusted‡ OR (95% CI)
MEP (ng/ml)	203			
1 (≤26.01)	50	1.00	1.00	1.00
2 (26.02–57.07)	50	0.78 (0.29 – 2.08)	0.75 (0.26 – 2.15)	0.82 (0.27 – 2.50)
3 (57.08–140)	53	0.79 (0.30 – 2.07)	1.07 (0.36 – 3.14)	1.14 (0.35 – 3.69)
4 (141–15,634)	50	0.52 (0.20 – 1.34)	0.45 (0.17 – 1.24)	0.48 (0.16 – 1.46)
P-trend		0.17	0.11	0.13
MBP (ng/ml)	192			
1 (≤7.42)	47	1.00	1.00	1.00
2 (7.43–13.74)	47	1.50 (0.54 – 4.15)	1.50 (0.54 – 4.17)	1.58 (0.53 – 4.74)
3 (13.75–23.13)	50	0.80 (0.31 – 2.11)	0.80 (0.30 – 2.13)	0.97 (0.32 – 2.99)
4 (23.14–4,756)	48	0.58 (0.22 – 1.51)	0.58 (0.22 – 1.52)	0.71 (0.23 – 2.17)
P-trend		0.11	0.11	0.27
MiBP (ng/ml)	203			
1 (≤4.33)	49	1.00	1.00	1.00
2 (4.34–7.54)	52	0.55 (0.19 – 1.56)	0.55 (0.19 – 1.58)	0.66 (0.22 – 1.96)
3 (7.55–12.46)	51	0.23 (0.08 – 0.63)	0.22 (0.08 – 0.63)	0.30 (0.10 – 0.88)
4 (12.47–191)	51	0.40 (0.14 – 1.11)	0.40 (0.14 – 1.12)	0.50 (0.15 – 1.65)
P-trend		0.14	0.14	0.37
MBzP (ng/ml)	203			
1 (≤2.10)	51	1.00	1.00	1.00
2 (2.11–4.06)	49	0.80 (0.30 – 2.15)	0.92 (0.33 – 2.57)	0.99 (0.34 – 2.90)
3 (4.07–7.02)	51	0.65 (0.25 – 1.66)	0.69 (0.26 – 1.86)	0.82 (0.28 – 2.37)
4 (7.03–176)	52	0.54 (0.21 – 1.40)	0.61 (0.23 – 1.62)	0.71 (0.23 – 2.20)
P-trend		0.21	0.28	0.51
MCPP (ng/ml)	203			
1 (≤2.31)	49	1.00	1.00	1.00
2 (2.32–4.11)	52	1.33 (0.50 – 3.51)	1.32 (0.49 – 3.58)	1.30 (0.46 – 3.66)
3 (4.12–8.16)	49	0.92 (0.35 – 2.46)	0.97 (0.35 – 2.69)	0.99 (0.33 – 2.93)
4 (8.17–56.34)	53	0.39 (0.15 – 1.00)	0.39 (0.15 – 1.03)	0.38 (0.13 – 1.12)
P-trend		0.01	0.01	0.02
ΣDEHP (μmol/L)	203			
1 (≤0.119)	48	1.00	1.00	1.00
2 (0.120–0.219)	54	1.61 (0.61 – 4.22)	1.62 (0.59 – 4.41)	1.76 (0.55 – 5.58)
3 (0.220–0.620)	49	0.79 (0.31 – 2.03)	0.93 (0.35 – 2.50)	0.79 (0.24 – 2.54)
4 (0.630–20.51)	52	0.67 (0.26 – 1.74)	0.56 (0.21 – 1.52)	0.48 (0.14 – 1.67)
P-trend		0.17	0.07	0.05
MCOP (ng/ml)	183			

Phthalate metabolite quartiles	N	Unadjusted OR (95% CI)	Confounder- adjusted† OR (95% CI)	Confounder- and female phthalate-adjusted‡ OR (95% CI)
1 (≤9.72)	44	1.00	1.00	1.00
2 (9.73–24.43)	47	0.55 (0.19 – 1.58)	0.55 (0.19 – 1.59)	0.38 (0.11 – 1.32)
3 (24.44–71.10)	47	0.31 (0.11 – 0.89)	0.31 (0.11 – 0.89)	0.19 (0.05 – 0.69)
4 (71.11–813)	45	0.30 (0.10 – 0.87)	0.30 (0.10 – 0.88)	0.17 (0.04 – 0.66)
P-trend		0.06	0.06	0.05
MCNP (ng/ml)	183			
1 (≤2.75)	43	1.00	1.00	1.00
2 (2.76–4.54)	48	0.60 (0.22 – 1.64)	0.48 (0.16 – 1.44)	0.59 (0.18 – 1.90)
3 (4.56–8.60)	46	0.56 (0.20 – 1.55)	0.75 (0.25 – 2.30)	0.81 (0.24 – 2.68)
4 (8.61–130)	46	0.63 (0.22 – 1.76)	0.46 (0.14 – 1.44)	0.58 (0.16 – 2.11)
P-trend		0.71	0.35	0.57

*Implantation was defined as a positive pregnancy test (βhCG ≥6 mIU/ml) 17 days after the embryo transfer

†All models were adjusted for maternal age; overall cycle number is additionally adjusted for in the models for MEP, MCNP, and ΣDEHP; paternal age is additionally adjusted for in the models for MBzP, MCPP, MEP, and MCNP; maternal smoking (ever vs. never) was additionally adjusted for in the models for MEP and MCNP; paternal smoking (ever vs. never) was additionally adjusted for in the model for ΣDEHP; maternal obesity (vs. normal weight/overweight) was additionally adjusted for in the models for MCNP

‡These models were additionally adjusted for maternal urinary phthalate concentration

Table 6

Associations between live births among transfer cycles and quartiles of geometric means of specific gravity-adjusted urinary phthalate metabolite concentrations among men whose partner underwent IVF

Phthalate metabolite quartiles	N	Unadjusted OR (95% CI)	Confounder- adjusted* OR (95% CI)	Confounder- and female phthalate-adjusted† OR (95% CI)
MEP (ng/ml)	88/211			
1 (≤26.01)	24/52	1.00	1.00	1.00
2 (26.02–57.07)	22/53	0.99 (0.41 – 2.41)	1.05 (0.43 – 2.55)	1.11 (0.44 – 2.83)
3 (57.08–140)	23/53	0.83 (0.35 – 1.97)	1.11 (0.45 – 2.71)	1.09 (0.42 – 2.82)
4 (141–15,634)	19/53	0.61 (0.25 – 1.45)	0.58 (0.24 – 1.39)	0.67 (0.26 – 1.73)
P-trend		0.20	0.13	0.25
MBP (ng/ml)	84/199			
1 (≤7.42)	18/49	1.00	1.00	1.00
2 (7.43–13.74)	27/50	2.37 (0.94 – 5.96)	2.52 (1.00 – 6.36)	2.83 (1.04 – 7.70)
3 (13.75–23.13)	23/51	1.42 (0.57 – 3.53)	1.47 (0.59 – 3.66)	1.98 (0.69 – 5.70)
4 (23.14–4,756)	16/49	0.80 (0.32 – 2.01)	0.79 (0.31 – 2.00)	1.07 (0.37 – 3.14)
P-trend		0.25	0.23	0.47
MiBP (ng/ml)	88/211			
1 (≤4.33)	21/51	1.00	1.00	1.00
2 (4.34–7.54)	27/53	1.61 (0.66 – 3.93)	1.68 (0.69 – 4.12)	1.97 (0.77 – 5.05)
3 (7.55–12.46)	19/54	0.73 (0.29 – 1.81)	0.69 (0.28 – 1.71)	0.87 (0.33 – 2.33)
4 (12.47–191)	21/53	0.92 (0.37 – 2.25)	0.90 (0.37 – 2.20)	1.15 (0.40 – 3.31)
P-trend		0.48	0.42	0.71
MBzP (ng/ml)	88/211			
1 (≤2.10)	24/52	1.00	1.00	1.00
2 (2.11–4.06)	18/53	0.72 (0.30 – 1.77)	0.77 (0.32 – 1.88)	0.88 (0.34 – 2.26)
3 (4.07–7.02)	23/53	0.82 (0.35 – 1.93)	0.88 (0.37 – 2.08)	1.11 (0.44 – 2.85)
4 (7.03–176)	23/53	0.91 (0.39 – 2.16)	0.89 (0.38 – 2.11)	1.10 (0.41 – 2.93)
P-trend		0.93	0.96	0.76
MCPP (ng/ml)	88/211			
1 (≤2.31)	23/53	1.00	1.00	1.00
2 (2.32–4.11)	30/52	1.77 (0.74 – 4.24)	1.90 (0.79 – 4.58)	2.00 (0.80 – 4.99)
3 (4.12–8.16)	21/53	0.85 (0.35 – 2.07)	0.91 (0.37 – 2.23)	0.97 (0.37 – 2.55)
4 (8.17–56.34)	14/53	0.42 (0.17 – 1.07)	0.45 (0.18 – 1.14)	0.49 (0.18 – 1.35)
P-trend		0.01	0.01	0.02
ΣDEHP (μmol/L)	88/211			
1 (≤0.118)	23/51	1.00	1.00	1.00
2 (0.119–0.219)	24/55	0.86 (0.36 – 2.04)	0.91 (0.38 – 2.16)	0.84 (0.32 – 2.20)
3 (0.220–0.620)	23/52	0.97 (0.40 – 2.35)	0.96 (0.40 – 2.31)	0.86 (0.31 – 2.36)
4 (0.630–20.51)	18/53	0.56 (0.23 – 1.38)	0.59 (0.24 – 1.47)	0.50 (0.17 – 1.50)
P-trend		0.17	0.21	0.17
MCOP (ng/ml)	80/190			

Phthalate metabolite quartiles	N	Unadjusted OR (95% CI)	Confounder- adjusted* OR (95% CI)	Confounder- and female phthalate-adjusted† OR (95% CI)
1 (≤9.72)	24/46	1.00	1.00	1.00
2 (9.73–24.43)	23/49	0.78 (0.31 – 1.94)	0.78 (0.31 – 1.95)	0.70 (0.25 – 1.98)
3 (24.44–71.10)	20/48	0.57 (0.23 – 1.45)	0.55 (0.22 – 1.40)	0.46 (0.15 – 1.39)
4 (71.11–813)	13/47	0.36 (0.14 – 0.95)	0.36 (0.14 – 0.96)	0.28 (0.08 – 0.94)
P-trend		0.04	0.04	0.049
MCNP (ng/ml)	80/190			
1 (≤2.75)	22/47	1.00	1.00	1.00
2 (2.76–4.54)	20/49	0.54 (0.21 – 1.37)	0.51 (0.20 – 1.30)	0.54 (0.19 – 1.52)
3 (4.56–8.60)	20/47	0.67 (0.26 – 1.73)	0.76 (0.29 – 1.98)	0.76 (0.26 – 2.26)
4 (8.61–130)	18/47	0.58 (0.22 – 1.51)	0.55 (0.21 – 1.45)	0.67 (0.22 – 2.07)
P-trend		0.53	0.48	0.84

*All models were adjusted for maternal age; overall cycle number and maternal smoking (ever vs. never) were additionally adjusted for in the models for MEP and MCNP

†These models were additionally adjusted for maternal urinary phthalate metabolite concentration