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Urinary Concentrations of Phthalate Metabolites in Relation to Pregnancy Loss among Women Conceiving with Medically Assisted Reproduction

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Abstract

Background—Animal studies demonstrate that several phthalates are embryofetotoxic and are associated with increased pregnancy loss and malformations. Results from human studies on phthalates and pregnancy loss are inconsistent.

Methods—We examined pregnancy loss prospectively in relation to urinary phthalate metabolite concentrations among women undergoing medically assisted reproduction. We used data from 256 women conceiving 303 pregnancies recruited between 2004 and 2012 from the Massachusetts General Hospital Fertility Center. We quantified eleven phthalate metabolite concentrations and calculated the molar sum of four di(2-ethylhexyl) phthalate (DEHP) metabolites (ΣDEHP). We estimated risk ratios (RRs) and 95% confidence intervals (CIs) for biochemical loss and total

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pregnancy loss (<20 weeks' gestation) across quartiles using repeated measures log-binomial models, adjusted for age, body mass index, smoking and infertility diagnosis.

Results—Of the 303 pregnancies, 83 (27%) ended in loss less than 20 weeks' gestation and among these, 31 (10%) ended in biochemical loss. Although imprecise, the RRs for biochemical loss increased across quartiles of ∑DEHP and three individual DEHP metabolites. For ∑DEHP, the RRs (CIs) were: 2.3 (0.63, 8.5), 2.0 (0.58, 7.2), and 3.4 (0.97, 11.7) for quartiles two, three and four, compared to one, respectively (p-trend=0.04). RRs for total pregnancy loss were elevated in the highest quartiles of ΣDEHP and three DEHP metabolites. The remaining seven phthalate metabolite concentrations evaluated were not associated with either outcome.

Conclusions—We found a suggestive pattern of association between conception cycle-specific urinary concentrations of DEHP metabolites and biochemical and total pregnancy loss among women undergoing medically assisted reproduction.

INTRODUCTION

Healthy reproduction requires complex hormonal processes to work in synchrony. Endocrine-disrupting chemicals that interfere with this delicate balance may alter critical pathways required to achieve conception, maintain pregnancy, and deliver healthy offspring. Mounting epidemiologic evidence associates such chemicals with various adverse reproductive and developmental outcomes,[1–13] including, more recently, pregnancy loss.[7,14] The ubiquitous nature of several classes of chemicals, such as phthalates, continues to prompt considerable concern as our understanding of their role in human fertility and reproduction is still in its infancy.[15]

Phthalates are widely used to impart flexibility and durability to plastics including polyvinyl chloride. Phthalates are used in a wide variety of products ranging from vinyl tiles and flooring, adhesives, detergents, lubricants, medical devices, pharmaceuticals (in the coating of certain oral medications), clothing, food packing, and toys, and are also used as solubilizing agents in the preparation of cosmetics and personal care products.[16] Widespread consumer use of such products has led to near-universal human exposure.[5,16] Once ingested, inhaled or absorbed, phthalates have a short half-life, undergoing rapid hydrolysis into bio-active monoesters, some of which may then be further metabolized by oxidation or phase II conjugation. Metabolites are excreted mainly in urine.[17] More than 95% of US and Canadian populations have detectable urinary concentrations of one or more phthalate metabolites.[18,19] Studies suggest that the developing embryo and fetus are most sensitive to potential adverse effects, and biomonitoring studies report the highest concentration of many urinary phthalate metabolites in women and children.[5,16,17,20,21]

Experimental studies have demonstrated embryofetotoxic and teratogenic effects of di-n-butyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP) among breeding mice and rats,[22–27] with dose, timing, and route of exposure strongly mediating deleterious effects.[28] Oral administration of DBP to pregnant or pseudopregnant rats was associated with increases in preimplantation and postimplantation losses at high and moderate doses, respectively;[22] such losses may be mediated by impairment in uterine function.[22] Tomita et al. (1986) showed that timing of exposure resulted in different fetotoxic endpoints, with

mono(2-ethylhexyl) phthalate (MEHP), a metabolite of DEHP, given to mice on gestation day 7 increasing early fetal deaths, compared to dosing on day 8 increasing late fetal deaths.[28] Other studies show that dietary or orally dosed DEHP and DBP in breeding rats or mice resulted in fewer litters, fewer live pups per litter, and a decrease in the proportion of pups born alive, in a dose-dependent manner.[25,29]

While substantial experimental evidence linking phthalates to teratogenicity and fetal demise exists, little is known about its impact on embryo development and pregnancy maintenance in humans, especially in relation to exposure in the very early stages of conception. Three recent studies have examined the effect of various phthalates on pregnancy loss in couples conceiving naturally with conflicting results.[7,13,30] Others have investigated the effect of phthalates on gestational length[31–33] and preterm birth[34,35] with varying methods and conclusions.

Pregnancy loss is the most frequent unintended pregnancy outcome, affecting 31% of all conceptions.[36] Among subfertile women undergoing medically assisted reproduction, pregnancy loss is a costly and emotional outcome, and, although predictors of its occurrence are not well established, environmental causes may play a role.[37–41] Our primary objective was to examine the prospective association between eleven urinary phthalate metabolites and pregnancy loss among women conceiving through medically assisted reproduction. We examined both biochemical pregnancy loss and total pregnancy loss of less than 20 weeks' gestation.

METHODS

Participants

The Environment and Reproductive Health Study (EARTH) is a prospective cohort of couples seeking infertility investigation and treatment at the Massachusetts General Hospital Fertility Center; EARTH is designed to evaluate the effects of diet and environmental exposures on fertility and pregnancy outcomes. Details of the cohort have been described previously.[9] The EARTH study has been ongoing since 2004 and has recruited approximately 700 women and 400 men to date. Women between the ages of 18 and 46 were eligible to participate and were followed from time of entry, throughout their infertility care and eventual pregnancy. The present study included women enrolled in EARTH between November 2004 and October 2014 with two or more positive serum beta Human Chorionic Gonadotropin (β-hCG) measurements (N=600).[41] *A priori* we excluded: any natural conceptions (i.e., conceived without assisted reproduction) as we had missing early β-hCG measurements for almost 26% of all such cycles (n=127); conceptions through the use of egg donors (n=23); and conceptions with unknown cycle outcomes (n=4), leaving 446 eligible conceptions before merging with our phthalate database which extends only to April 2012. The final study cohort consisted of 303 conceptions after either fresh or frozen in vitro fertilization (IVF), or ovarian stimulation with or without intrauterine insemination, from 256 women with conception cycle-specific urinary concentrations of phthalate metabolites. The study was approved by the Institutional Review Boards of MGH, Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC). Prior to

signing informed consent, subjects spoke with a trained research nurse who explained all procedures and answered questions.

Exposure ascertainment

Study participants provided a spot urine sample at study entry, and up to two spot urine samples per fertility treatment cycle: the first specimen (not necessarily a fasting sample) corresponding to days 3 to 9 of the monitoring phase of the cycle, and the second at the time of oocyte retrieval or intrauterine insemination. Both conception cycle-specific urine samples collected prior to the index conception were included in the analysis. Urine samples were collected using a sterile phthalate-free polypropylene cup. Each sample was analyzed for specific gravity with a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA), divided into aliquots, and frozen for long-term storage at −80 °C. Samples were shipped on dry ice overnight to the CDC (Atlanta, GA, USA) for quantification of urinary phthalate metabolite concentrations using solid phase extraction coupled with high performance liquid chromatography-isotope dilution tandem mass spectrometry.[42] The eleven phthalate metabolites were: MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono(3-carboxypropyl) phthalate (MCPP), monocarboxyisooctyl phthalate (MCOP), monocarboxyisononyl phthalate (MCNP), monobenzyl phthalate (MBzP), monoethyl phthalate (MEP), mono-isobutyl phthalate (MiBP), and mono-n-butyl phthalate (MBP). The limits of detection were 0.5–1.2 μg/L (MEHP), 0.2–0.7 μg/L (MEHHP, MEOHP), 0.2–0.6 μg/L (MECPP), 0.1–0.2 μg/L (MCPP), 0.2–0.7 μg/L (MCOP), 0.2–0.6 μg/L (MCNP), 0.2–0.3 μg/L (MBzP), 0.4–0.8 μg/L (MEP), 0.2–0.3 μg/L (MiBP), and 0.4–0.6 μg/L (MBP). We calculated the molar sum of DEHP metabolites (∑DEHP) by dividing each metabolite concentration by its molecular weight and then summing: [(MEHP*(1/278.34)) + (MEHHP*(1/294.34)) + (MEOHP*(1/292.33)) + (MECPP*(1/308.33))]. Values below the limit of detection were assigned the limit of detection divided by the square root of two.[43] As analyses were based on quartiles, the method for assigning concentrations below the limit of detection had no impact on associations.

Outcome ascertainment

Routine follow-up of medically assisted reproduction at the Massachusetts General Hospital includes a quantitative serum β-hCG typically measured on day 17 (range 15–20) following oocyte retrieval and/or intrauterine insemination, and a transvaginal ultrasound at approximately 6 weeks gestation for those achieving a positive β-hCG. Pregnancy was defined as two or more β-hCG levels ≥ 6 mIU/mL, as detection of β-hCG production would indicate implantation and syncytiotrophoblastic invasion into the decidua.[40,44] This definition is also consistent with the hospital's laboratory reference threshold of ≥ 6 mIU/ml to indicate a positive pregnancy test. Biochemical pregnancy loss was defined as the demise of a β-hCG confirmed pregnancy that was never visualized on ultrasound.[41] Total pregnancy loss was defined as *any* loss of a pregnancy <20 weeks' gestational age (≤139 days), including biochemical losses. We followed committee practice guidelines from the American College of Obstetricians and Gynecologists to estimate gestational age following medically assisted reproduction.[45] For IVF based conceptions, we calculated gestational age

as: outcome date − date of transfer + 14 + cycle day of transfer.[45,46] For ovarian stimulation with or without intrauterine insemination, we used early ultrasound based gestational age estimates, and for the fraction (29/303, ~10%) whereby ultrasound and IVF data were not available, we used outcome date minus cycle start date.[45] The treating infertility physician diagnosed infertility using the Society for Assisted Reproductive Technology definitions. Other pertinent demographics such as age and race were obtained from a baseline questionnaire, and clinical information such as infertility treatment received during cycle, β-hCG levels, ultrasound data including measurements of embryo, and embryo transfer date and day were abstracted from the patients' electronic medical records by trained study staff. Age of participant was collected at time of study enrollment. Height and weight were measured at enrollment by the study nurse. Body Mass Index (BMI) measured at study entry was calculated as weight (kilograms) divided by height (meters) squared.

Statistical Analysis

Urinary phthalate metabolite concentrations were adjusted for urinary dilution by multiplying the metabolite concentration by [(1.015−1)/(SG−1)], where SG is the specific gravity of the participant's sample and 1.015 is the mean SG for all included study samples.[47,48] The specific gravity adjusted phthalate metabolite concentrations were natural log-transformed to normalize distribution and were used to estimate the geometric mean from two spot urine samples collected during each cycle. The geometric mean value was the cycle-specific summary estimate of exposure used to form quartiles. For cycles with only one urine sample (~7% of all samples), the phthalate concentration for that single sample was used as the cycle-specific concentration.

We examined the clinical and demographic characteristics, reported as means (± SD) or number of women (%), of study participants in the total cohort and by quartiles of ∑DEHP concentration. We fit generalized estimating equation (GEE) models to evaluate the association between quartiles of urinary phthalate metabolites and pregnancy loss, accounting for correlation within women contributing more than one pregnancy. GEE models were fit using a log link function and binomial distribution to yield estimated risk ratios (RRs) and 95% confidence intervals (CIs) for biochemical pregnancy loss and total pregnancy loss, with the lowest quartile as the reference category. We fit a separate model for each of the eleven individual phthalate metabolites as well as the DEHP metabolite summary measure. We conducted statistical tests for trend across quartiles using the urinary phthalate metabolite concentration as an ordinal level indicator variable of each quartile in the regression models, adjusted for covariates. Candidate covariates were selected *a priori* based on the literature and included maternal age (≤32, 33–35, 36–38, ≥39), BMI (continuous), smoking status (never smoked vs. ever smoked, defined as a current or former smoker), and infertility diagnosis (female, male [reference category], or unexplained) in adjusted models.[37,44,49–51] We performed statistical analyses with SAS (version 9.4; SAS Institute Inc., Cary, USA).

RESULTS

The study cohort comprised 256 women, predominantly Caucasian (88%) and never-smokers (74%), with an average age of 34.9 (±3.8) years at time of enrollment (Table 1). Most women were nulliparous (86%), had college or graduate degrees (92%), and about 34% had a female factor as the primary cause of infertility (Table 1). Demographics and patient characteristics did not differ by quartiles of ∑DEHP; however, the proportion of biochemical and total pregnancy loss (<20 weeks) was markedly higher in the fourth quartile compared to the first (Table 1). The distribution of the specific gravity adjusted urinary phthalate metabolite concentrations from 564 samples provided by 303 pregnancies is shown in Table 2. The percentage of urine samples with detectable concentrations of phthalate metabolites ranged from 74% (MEHP) to 100% (MEP).

In the repeated measures log-binomial regression models adjusted for age, BMI, smoking status, and infertility diagnosis, the RRs (95% CIs) for biochemical pregnancy loss increased across quartiles of ∑DEHP and across three individual DEHP metabolites (MEHP, MEHHP, MEOHP) (Table 3). For ∑DEHP, the RRs (95% CIs) were: 2.3 (0.63, 8.5), 2.0 (0.58, 7.2), and 3.4 (0.97, 11.7) in quartiles two, three, and four, compared to one, respectively (p-test for trend=0.04). The RRs were imprecise as evidenced by the width of the confidence interval. The remaining seven phthalate metabolite concentrations were not associated with biochemical pregnancy loss (Table 3).

Total pregnancy loss of <20 weeks' gestation showed modest increases in RRs across quartiles two and three of ∑DEHP and DEHP metabolites, however positive associations were observed in the highest quartiles of MEHHP and MEOHP, and borderline significant trend tests for ∑DEHP and MEHP (Table 4). For MEOHP, the RRs (95% CIs) were: 1.6 (0.90, 2.9); 1.5 (0.84, 2.9) and 2.0 (1.1, 3.5) in quartiles two, three and four, compared to one, respectively (p-test for trend=0.03). No notable associations were observed among the other phthalate metabolites examined (data not shown).

DISCUSSION

In this study of subfertile couples conceiving through medically assisted reproduction, we found that increased conception cycle-specific urinary concentrations of ∑DEHP and individual DEHP metabolites were associated with biochemical pregnancy loss. Associations were most robust for the upper two quartiles of MEHHP and MEOHP. We furthermore observed that RRs for total pregnancy loss of less than 20 gestational weeks increased in the highest compared to the lowest quartiles, with similarly stronger findings for MEHHP and MEOHP. While some results for both outcomes had significant trend tests, several effect estimates were imprecise based on the width of the corresponding confidence interval. The remaining seven phthalate metabolite concentrations examined (MEP, MBP, MiBP, MBzP, MCPP, MCOP, and MCNP) were not associated with either outcome.

To the best of our knowledge, this is the first study to examine biochemical pregnancy loss within a subfertile cohort conceiving through medically assisted reproduction. The unique nature of our study design permitted an examination of biochemical pregnancies that were

detected very early post-implantation through serum β-hCG measurement on day 17 after embryo transfer or intrauterine implantation. With about a third of all pregnancies ending before viability[36] and a limited understanding of environmental causes of human pregnancy loss, the fertility treatment setting in this study offered a glimpse into the so-called 'black box' of events in the post-implantation period.[51] Our results suggest that ∑DEHP metabolites and the specific metabolites MEHP, MEHHP, and MEOHP may be associated with one or more adverse pregnancy outcomes involving early stages of implantation, decidualization, placentation or embryogenesis through possibly uterine-embryo hormonal signaling.[52] Pregnancy loss of up to 20 weeks' gestation was also elevated at the highest concentrations of DEHP metabolites. It is possible however, that assessment of exposure at alternate time points, for example during pregnancy itself, may have produced different (possibly stronger) results especially in light of the short half-life and episodic nature of phthalate exposure. Urinary levels of metabolites in the follicular phase of a cycle are only a proxy of exposure in the first 20 weeks of pregnancy and the most sensitive time point of exposure may differ for different pregnancy loss endpoints.

Despite associations of urinary DEHP metabolites with pregnancy loss, the overall frequency of loss in our study population was not elevated compared to what we would expect clinically in a fertile population.[36] This is consistent with a large study that compared early pregnancy loss among women conceiving with IVF (fresh and frozen) with fertile women conceiving naturally.[53] Furthermore, we would not expect the overall frequency of pregnancy loss to be higher in our cohort because our urinary concentrations of DEHP metabolites were comparable to NHANES (geometric mean of MEHP 2.72 μg/L and median of 2.10 μg/L for years 2005–2006).[54]

In a recent prior study from our cohort, we reported that urinary metabolites of DEHP and the metabolite MCNP were associated with decreased oocyte yield and number of mature oocytes at retrieval, as well as reduced fertilization rates for the metabolites MCOP and MCPP.[1] Urinary DEHP metabolites were also associated with reduced clinical pregnancy rates and live birth rates among initiated IVF cycles,[1] suggesting that there is a degree of loss along the continuum of clinical pregnancy to live birth. The difference between clinical pregnancy rates and live birth rates could be interpreted as representing clinical losses. Our current findings directly show that even after fertilization and implantation, among women achieving a β-hCG confirmed pregnancy (by two or more positive serum results), exposure to DEHP may continue to adversely impact early embryo development or uterine receptivity. It is possible that embryos that survived transfer were potentially already destined to fail through earlier adverse processes involving exposures to phthalates. Or, perhaps, phthalate metabolites may alter hormonal signaling and secretion of key endogenous hormones such estrogen and progesterone[55] resulting in a less favorable uterine milieu toward implantation and placentation, even for viable and healthy embryos.

While our study was not designed to elucidate the mechanism through which exposure to phthalates may adversely impact embryo development and pregnancy maintenance, our results are consistent with animal studies suggesting that DEHP affects early reproductive endpoints and is embryofetotoxic in mice and rat models.[22,24,25,27,28] Suppression of decidualization causing impairment in uterine function through dysregulation of

progesterone has been proposed as one possible mechanism by Ema and colleagues (2000).[22] MEHP has also been detected in the fetuses of mice, likely due to transplacental crossing.[28] Unlike several experimental studies that show DBP to also be fetotoxic,[22,24] we observed no evidence of an association between MBP, the main DBP metabolite, with pregnancy loss in our cohort.

Three recent epidemiologic studies examined comparable endpoints of pregnancy loss in relation to urinary phthalate metabolites in women planning or attempting pregnancy.[7,13,30] Our results are consistent with those of a Danish study by Toft and colleagues, who examined pregnancy loss, defined as subclinical embryonal losses and clinical losses combined.[7] The authors enrolled couples planning their first pregnancy after discontinuation of birth control, and followed them prospectively until a clinically recognized pregnancy occurred or for six menstrual cycles. Their analysis - like ours and that by Jukic - included only women who achieved a pregnancy during the study period (N=128), excluding those not at risk for the outcome. Also similar to our analyses, Toft and colleagues analyzed conception specific urinary phthalate metabolites from day 10 after the last day of the menstrual cycle before pregnancy. They reported an elevated odds ratio (OR) of pregnancy loss in the upper tertile of conception specific to MEHP concentrations [adjusted OR: 2.87 (95% CI: 1.09, 7.57)]. We obtained similar RRs for biochemical pregnancy loss in the upper quartile of MEHP after additionally adjusting for infertility diagnosis (Table 3, model 2) [adjusted RR: 2.8 (95% CI: 0.99, 8.1), despite our substantially lower reported concentrations (Table 2) compared to the Danish women. Unlike our study, however, Toft and colleagues reported no significant associations for the two other DEHP metabolites examined (MEHHP, MEOHP) despite substantially higher urinary concentrations in their cohort. One important distinction, however is that our population is a subfertile group of women undergoing medically assisted reproduction and a potentially more sensitive, or high-risk group, for the early endpoints of biochemical pregnancy loss. They also had a higher reported incidence of total pregnancy loss (37.5%, ascertained by interview after one year) compared to ours (27%). Non-differential misclassification of outcome could dilute associations, leading to a false null conclusion, but it would seem unlikely that this would be chemical specific.

A case-control study of women without a history of infertility was conducted in China by Mu et al. (2015). The cases included clinically identified hospital-based pregnancy losses while the controls were pregnant women recruited from the same hospital confirmed to have a viable fetus with cardiac activity. The study was relatively small (132 cases and 172 controls) and the timing of collection of urine samples for measurement of phthalate metabolites relative to the pregnancy loss was 4 days after ascertainment of pregnancy status via transvaginal ultrasound. In contrast to our study, they found an elevated adjusted odds ratio (OR) of clinical pregnancy loss associated with urinary concentrations of MEP, MiBP, and MBP, which was consistent with some experimental animal studies.[22,24] However, they did not find associations of pregnancy loss with urinary DEHP metabolites.

The study by Jukic and colleagues (2015) is comparable to ours in that they reported an overall loss of approximately 32% if early (<6 weeks) and up to 25 gestational weeks losses were combined - our total pregnancy loss (biochemical losses and those up to 20 weeks

gestation) occurred in 27% of pregnancies.[13] The authors, however, found an inverse association between urinary DEHP metabolites and early loss: higher urinary DEHP metabolite concentrations were associated with reduced early loss. One possible explanation is that timing of exposure measurement may be a critical factor in detecting a risk of early losses. Jukic and colleagues pooled three different urine samples, one of which came from the luteal phase of the menstrual cycle. This pooling may have resulted in a different exposure profile that may have been less relevant to the endpoint under study. Our study and the Danish study included only follicular phase urine, with our analysis using the geometric mean concentration of two different time points (day 3 to 9 and again at time of oocyte retrieval) as the summary estimate of exposure.

Our study provides preliminary evidence that early pregnancy may be adversely affected by DEHP exposure. The prospective nature of this design, relying upon an infertile study population from a large academic fertility setting, permitted a careful examination of the direction of the relationship between phthalate metabolite concentrations and post-implantation pregnancy failure. The urinary concentrations of the phthalate metabolites measured are within the ranges reported for the US general population.[54] However, these findings may not be generalizable to women from the general population without fertility concerns, co-exposures to other select chemicals were also not accounted for, and exposure to phthalates may be reflective of other unknown lifestyle or fertility factors that might be associated with pregnancy loss. However, we attempted to control for these factors by adjusting for age, infertility diagnosis, BMI, and smoking. We also evaluated multiple phthalate metabolites at the same time to account for multiple co-exposures, and all samples were collected in one clinical location and processed under one protocol by the CDC. Furthermore, phthalates are short-lived chemicals and exposures are likely episodic, making the assessment of long-term exposure difficult. We attempted to partially account for the variability in phthalate metabolite concentrations by using the average concentration of two urine samples provided at two time points in the follicular phase of the conception cycle. These time points correspond most proximally to levels at the time of implantation and decidualization, making biochemical pregnancy loss a sensitive endpoint relevant to the exposure window we assessed.

CONCLUSIONS

We found a positive association between conception cycle specific urinary concentrations of DEHP metabolites and both biochemical pregnancy loss and total pregnancy loss of <20 gestational weeks. Our findings were consistent with one of two previous studies that examined similar endpoints in relation to phthalate metabolites. Our findings are unique, however, in that this is the first study to examine and demonstrate an association with biochemical pregnancy losses among women conceiving through medically assisted reproduction, suggesting that subfertile women may be potentially more sensitive to early adverse reproductive outcomes. Our findings, however, should be interpreted cautiously in light of the inherent limitations and additional studies are needed to confirm our results.

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Table 1

Characteristics and outcomes in the total cohort and in quartiles of urinary ∑DEHP concentrations (μmol/L) among 256 women with 303 β-hCG confirmed pregnancies enrolled in the Environment and Reproductive Health Study (EARTH) between 2004 and 2012.

Characteristic	Total Cohort (N=256)	∑DEHP			
		Q1 (n=63)	Q2 (n=61)	Q3 (n=67)	Q4 (n=65)
Age at study entry (years)					
Mean (SD)	34.9 (3.8)	35.1 (3.8)	34.6 (3.6)	35.0 (3.8)	34.9 (4.0)
Min-Max	24–44	27–42	27–42	24–44	26–43
Age > 35, No. (%)	111 (43%)	26 (23%)	24 (22%)	33 (30%)	28 (25%)
BMI (kg/m^2)					
Mean (SD)	24.1 (4.5)	23.2 (3.9)	24.6 (3.6)	24.2 (3.7)	24.6 (5.3)
Min-Max	16.1–42.3	16.5–39.4	16.1–42.3	18.0–36.3	27.5–41.1
Underweight/normal (< 25), No. (%)	178 (70%)	50 (28%)	40 (22%)	45 (25%)	43 (24%)
Overweight/obese (>= 25), No. (%)	78 (30%)	13 (17%)	21 (27%)	22 (28%)	22 (28%)
Education[a]					
< College graduate, No. (%)	18 (8%)	5 (28%)	2 (11%)	7 (39%)	4 (22%)
College graduate, No. (%)	82 (34%)	18 (22%)	20 (24%)	22 (27%)	22 (27%)
Graduate degree, No. (%)	140 (58%)	37 (26%)	33 (24%)	33 (24%)	37 (26%)
Smoking, No. (%)					
Never smoked	189 (74%)	47 (25%)	42 (22%)	29 (26%)	51 (27%)
Ever smoked					
Current smoker	7 (3%)	2 (29%)	3 (43%)	2 (29%)	0 (0)
Former smoker	60 (23%)	14 (23%)	16 (27%)	16 (27%)	14 (23%)
Race, No. (%)					
Caucasian	224(88%)	53 (24%)	54 (24%)	57 (25%)	60 (27%)
Black/African American	4 (2%)	1 (25%)	1 (25%)	1 (25%)	1 (25%)
Asian	18 (7%)	6 (33%)	4 (22%)	5 (28%)	3 (17%)
Other	10 (4%)	3 (30%)	2 (20%)	4 (40%)	1 (10%)
Nulligravida, No. (%)	159 (62%)	39 (25%)	36 (23%)	43 (27%)	41 (26%)
Nullipara, No. (%)	220 (86%)	53 (24%)	54 (25%)	58 (26%)	55 (25%)
Primary SART diagnosis, No. (%)					

Characteristic	Total Cohort (N=256)	∑DEHP Q1 (n=63)	Q2 (n=61)	Q3 (n=67)	Q4 (n=65)
Female factor	87 (34%)	18 (21%)	23 (26%)	23 (26%)	23 (26%)
Diminished ovarian reserve	14 (5.5%)	3 (21%)	4 (29%)	3 (21%)	4 (29%)
Ovulation disorders	35 (14%)	4 (11%)	11 (31%)	6 (17%)	15 (40%)
Endometriosis	14 (5.5%)	2 (14%)	4 (29%)	4 (29%)	4 (29%)
Uterine disorders	1 (0.4%)	0	0	1 (100%)	0
Tubal factor	13 (5%)	4 (31%)	2 (15%)	6 (46%)	1 (8%)
Male factor	87 (34%)	18 (21%)	23 (26%)	23 (26%)	23 (26%)
Unexplained	92 (36%)	32 (35%)	17 (18%)	24 (26%)	19 (21%)
FSH Day 3 in IU/L					
Mean (SD)	7.05 (2.2)	6.9 (2.1)	6.9 (2.2)	7.1 (2.6)	7.2 (2.1)
Min-Max	0.2–15.5	0.2–14.0	1.0–13.5	2.6–15.5	2.0–14.2
Type of Treatment Cycle, No. (%)					
IVF	222 (73%)[b]	56 (25%)	54 (24%)	61 (27%)	51 (23%)
IUI	81 (27%)[b]	19 (25%)	(22 (29%)	15 (20%)	25 (33%)
Pregnancy Loss Outcomes, No. (%)[c,d]					
Biochemical Loss	31 (10%)	3 (4%)	8 (10%)	7 (9%)	13 (17%)
Loss <20 weeks	82 (27%)	17 (23%)	18 (24%)	17 (22%)	30 (39%)

Abbreviations: Quartile (Q); Body Mass Index (BMI); Standard Deviation (SD); Minimum (Min); Maximum (Max); Follicle Stimulating Hormone (FSH, measured in serum on day 3); Society for Assisted Reproductive Technology (SART) primary diagnosis at study entry; In-vitro Fertilization (IVF); Intrauterine Insemination (IUI).

[a]Missing education values: n=16.

[b]Proportion of treatment type in total number of pregnancies (n/303).

[c]Pregnancy was defined as two or more serum β-hCG levels >= 6 mIU/mL. Biochemical pregnancy loss was defined as the demise of a non-visualized β-hCG confirmed pregnancy. Pregnancy loss <20 weeks' gestation was defined as the loss of any pregnancy (including biochemical losses) of less than 20 weeks gestation (<=139 days).

[d]Proportion of pregnancy loss outcomes in total number of pregnancies (n/303) and across quartiles Q1 (n=75); Q2 (n=76); Q3 (n=76); Q4 (n=76).

Table 2

Distribution of urinary phthalate metabolite concentrations (metabolite or molar sum) measured from 303 β-hCG confirmed pregnancies providing 564 cycle-specific urine samples, among 256 women enrolled in the Environment and Reproductive Health (EARTH) Study between 2004 and 2012.

Metabolite	Sample Size N	LOD (μg/L)	% Detect[a]	SG-Adjusted GM (GSD)[b]	SG-Adjusted Median (μg/L)	IQR (25^{th}, 75^{th}) (μg/L)
MEP	564	0.4–0.8	100	58.5 (4.4)	50.6	21.4, 133.2
MBP	564	0.4–0.6	97	11.8 (0.68)	12.6	6.8, 21.4
MiBP	564	0.2–0.3	96	6.3 (0.33)	7.2	3.8, 12.0
MBzP	564	0.2–0.3	92	3.3 (0.19)	3.2	1.7, 6.1
MEHP	564	0.5–1.2	74	2.9 (0.20)	2.6	1.5, 6.4
MEHHP	564	0.2–0.7	99	16.8 (1.1)	14.2	7.8, 35.4
MEOHP	564	0.2–0.7	99	11.4 (0.73)	10.2	5.5, 24.4
MECPP	564	0.2–0.6	95	29.2 (1.7)	25.2	14.3, 57.2
∑*DEHP*[c]	--	--	--	0.21 (0.01)	0.18	0.10, 0.40
MCPP	564	0.1–0.2	95	3.6 (0.22)	3.4	1.7, 6.7
MCOP	502	0.2–0.7	98	21.7 (1.7)	21.9	8.6, 56.2
MCNP	502	0.2–0.6	93	4.6 (0.30)	4.2	2.4, 7.2

Abbreviations: number of urinary samples (N); Limit of Detection (LOD); specific gravity (SG); geometric mean (GM); geometric standard deviation (GSD); interquartile range (IQR); 25^{th} percentile (25^{th}); 75^{th} percentile (75^{th}) MEP: monoethyl phthalate; MBP: mono-n-butyl phthalate; MiBP: mono-isobutyl phthalate; MBzP: monobenzyl phthalate; MEHP: mono(2-ethylhexyl) phthalate; MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP: mono(2-ethyl-5-oxohexyl) phthalate; MECPP: mono(2-ethyl-5-carboxypentyl) phthalate; DEHP: di(2-ethylhexyl) phthalate; MCPP: mono(3-carboxypropyl) phthalate; MCOP: monocarboxyisooctyl phthalate; MCNP: monocarboxyisononyl phthalate.

[a]Percentage of phthalate metabolite concentrations above the limit of detection (μg/L). All values below the LOD (<LOD) were assigned a value equal to the LOD divided by √2.

[b]Geometric mean of cycle-specific urinary SG-adjusted phthalate metabolite concentrations per pregnancy (N=303) expressed in μg/L. For MCOP and MCNP, N=270 pregnancies.

[c]∑DEHP: Molar sum of DEHP metabolites (MEHP, MEHHP, MEOHP and MECPP) expressed in μmol/L.

Table 3

Risk Ratios (RR) and 95% Confidence Intervals (CIs) for biochemical pregnancy loss across quartiles of urinary ∑DEHP and 11 individual phthalate metabolite concentrations using 564 cycle-specific samples from 303 pregnancies in the Environment and Reproductive Health (EARTH) Study.

Phthalate Metabolite Quartile (μg/L)	RR (95% CI) Biochemical Loss Unadjusted[a] Model	RR (95%CI) Biochemical Loss Adjusted[b] Model 1	RR (95% CI) Biochemical Loss Adjusted[c] Model 2
∑DEHP Metabolites (in μmol/L)			
Q1 (0.02, 0.10)	Ref	Ref	Ref
Q2 (0.10, 0.18)	2.6 (0.7, 9.5)	2.6 (0.73, 9.0)	2.3 (0.63, 8.5)
Q3 (0.18, 0.40)	2.3 (0.6, 8.5)	2.3 (0.63, 8.1)	2.0 (0.58, 7.2)
Q4 (0.40, 4.3)	4.3 (1.3, 14.2)	3.9 (1.2, 13.3)	3.4 (0.97, 11.7)
p-trend[d]	0.01	0.02	0.04
MEHP			
Q1 (<LOD, 1.3)	Ref	Ref	Ref
Q2 (1.3, 2.6)	1.7 (0.54, 5.5)	1.7 (0.54, 5.7)	1.6 (0.48, 5.1)
Q3 (2.6, 6.3)	1.5 (0.45, 4.9)	1.5 (0.46, 4.8)	1.5 (0.47, 4.6)
Q4 (6.3, 78.0)	3.4 (1.2, 9.8)	3.3 (1.1, 9.7)	2.8 (0.99, 8.1)
p-trend[d]	0.02	0.03	0.03
MEHHP			
Q1 (0.93, 7.7)	Ref	Ref	Ref
Q2 (7.7, 14.0)	2.0 (0.51, 7.7)	2.0 (0.51, 7.5)	1.8 (0.45, 7.0)
Q3 (14.0, 35.0)	3.3 (0.96, 11.3)	3.2 (0.97, 10.7)	2.8 (0.84, 9.1)
Q4 (35.0, 378)	3.9 (1.2, 13.2)	3.7 (1.1, 12.3)	3.1 (0.91, 10.5)
p-trend[d]	0.007	0.01	0.03
MEOHP			
Q1 (0.90, 5.3)	Ref	Ref	Ref
Q2 (5.3, 10.2)	3.4 (0.76, 15.7)	3.2 (0.73, 14.5)	3.1 (0.68, 13.8)
Q3 (10.2, 24.0)	4.4 (1.0, 19.3)	4.4 (1.0, 18.7)	4.2 (0.99, 17.5)
Q4 (24.4, 266)	6.4 (1.5, 26.8)	6.0 (1.4, 25.0)	5.2 (1.2, 21.9)
p-trend[d]	0.002	0.003	0.006
MECPP			
Q1 (2.4, 14.3)	Ref	Ref	Ref
Q2 (14.3, 25.1)	1.7 (0.53, 5.7)	1.7 (0.53, 5.3)	1.4 (0.42, 4.7)
Q3 (25.2, 57.2)	1.7 (0.53, 5.6)	1.7 (0.53, 5.3)	1.5 (0.46, 4.7)
Q4 (57.2, 613)	3.2 (1.1, 9.3)	3.0 (1.0, 8.9)	2.4 (0.78, 7.6)
p-trend[d]	0.02	0.04	0.07
MEP			
Q1 (2.40, 21.4)	Ref	Ref	Ref
Q2 (21.4, 50.3)	1.10 (0.50, 2.69)	1.01 (0.40, 2.56)	0.96 (0.41, 2.30)
Q3 (50.3, 133)	0.74 (0.25, 2.17)	0.72 (0.24, 2.21)	0.65 (0.23, 1.87)

Phthalate Metabolite Quartile (μg/L)	RR (95% CI) Biochemical Loss Unadjusted[a] Model	RR (95%CI) Biochemical Loss Adjusted[b] Model 1	RR (95% CI) Biochemical Loss Adjusted[c] Model 2
Q4 (133, 3879)	0.99 (0.38, 2.59)	0.88 (0.31, 2.46)	0.86 (0.34, 2.16)
p-trend[d]	0.79	0.71	0.63
MBP			
Q1 (0.84, 6.7)	Ref	Ref	Ref[e]
Q2 (6.8, 12.6)	0.86 (0.31, 2.4)	0.70 (0.23, 2.1)	0.66 (0.22, 1.9)
Q3 (12.6, 21.3)	1.1 (0.46, 2.7)	0.85 (0.32, 2.3)	0.78 (0.28, 2.2)
Q4 (21.3, 4406)	0.86 (0.33, 2.23)	0.69 (0.24, 2.0)	0.58 (0.19, 1.7)
p-trend[d]	0.91	0.61	0.43
MCPP			
Q1 (<LOD, 1.7)	Ref	Ref	Ref
Q2 (1.7, 3.4)	0.99 (0.39, 2.5)	0.88 (0.35, 2.2)	0.84 (0.35, 2.0)
Q3 (3.4, 6.7)	0.98 (0.41, 2.4)	0.86 (0.36, 2.0)	0.92 (0.40, 2.1)
Q4 (6.7, 222)	0.86 (0.31, 2.4)	0.76 (0.29, 2.0)	0.75 (0.29, 1.9)
p-trend[d]	0.79	0.56	0.60
MiBP			
Q1 (<LOD, 3.7)	Ref	Ref	Ref
Q2 (3.7, 7.1)	0.62 (0.21, 1.8)	0.49 (0.16, 1.5)	0.53 (0.17, 1.6)
Q3 (7.1, 12.0)	1.48 (0.68, 3.2)	1.22 (0.57, 2.6)	1.2 (0.59, 2.5)
Q4 (12.0, 55.6)	0.74 (0.28, 2.0)	0.74 (0.27, 2.0)	0.71 (0.26, 1.9)
p-trend[d]	0.95	0.97	0.90
MBzP			
Q1 (<LOD, 1.7)	Ref	Ref	Ref
Q2 (1.7, 3.1)	1.3 (0.49, 3.6)	1.2 (0.45, 3.37)	1.2 (0.46, 3.3)
Q3 (3.2, 6.1)	1.5 (0.57, 3.8)	1.2 (0.45, 3.16)	1.2 (0.42, 3.2)
Q4 (6.1, 71.0)	1.3 (0.46, 3.7)	1.3 (0.47, 3.70)	1.1 (0.39, 3.1)
p-trend[d]	0.57	0.68	0.97
MCOP			
Q1 (0.62, 8.6)	Ref	Ref	Ref
Q2 (8.6, 21.6)	1.8 (0.64, 5.1)	1.9 (0.68, 5.5)	2.08 (0.75, 5.7)
Q3 (21.6, 56.2)	1.6 (0.54, 4.6)	1.4 (0.53, 4.0)	1.73 (0.66, 4.5)
Q4 (56.2, 384)	1.0 (0.30, 3.4)	0.92 (0.27, 3.1)	0.92 (0.29, 2.9)
p-trend[d]	0.92	0.70	0.85
MCNP			
Q1 (0.5, 2.4)	Ref	Ref	Ref
Q2 (2.4, 4.2)	1.2 (0.41, 3.3)	1.0 (0.36, 2.8)	1.1 (0.38, 2.9)
Q3 (4.2, 7.2)	1.1 (0.41, 3.2)	0.90 (0.31, 2.6)	0.84 (0.28, 2.5)
Q4 (7.2, 566)	1.2 (0.40, 3.4)	1.0 (0.35, 2.8)	1.0 (0.39, 2.7)
p-trend[d]	0.80	0.96	0.93

Abbreviations: DEHP: di(2-ethylhexyl) phthalate; MEHP: mono(2-ethylhexyl) phthalate; MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP: mono(2-ethyl-5-oxohexyl) phthalate; MECPP: mono(2-ethyl-5-carboxypentyl) phthalate; MEP: monoethyl phthalate; MBP: mono-n-butyl phthalate; MCPP: mono(3-carboxypropyl) phthalate; MiBP: mono-isobutyl phthalate; MBzP: monobenzyl phthalate; MCOP: monocarboxyisooctyl phthalate; MCNP: monocarboxyisononyl phthalate.

[a] Unadjusted and adjusted models estimated risk ratios and 95% CIs with repeated measures log-binomial regression.

[b] Model 1 estimated RRs and 95% CIs adjusting for age (categorical), BMI (continuous), smoking status (never/ever).

[c] Model 2 estimated RRs and 95% CIs adjusting for age (categorical), BMI (continuous), smoking status (never/ever), and infertility diagnosis (male factor [reference category], female factor, and unexplained).

[d] Tests for trend were performed using the urinary phthalate metabolite concentration quartile as an ordinal level indicator variable in the regression model, adjusted for covariates.

[e] Log-binomial model did not converge: final presented model obtained using logistic regression.

Table 4

Risk Ratios (RR) and 95% Confidence Intervals (CIs) for total pregnancy loss (<20 weeks' gestation) across quartiles of urinary ∑DEHP and 4 individual DEHP metabolite concentrations using 564 cycle-specific samples from 303 pregnancies in the Environment and Reproductive Health (EARTH) Study.

Phthalate Metabolite Quartile (μg/L)	RR (95% CI) Pregnancy Loss Unadjusted[a] Model	RR (95%CI) Pregnancy Loss Adjusted[b] Model
∑DEHP Metabolites (in μmol/L)		
Q1 (0.02, 0.10)	Ref	Ref
Q2 (0.10, 0.18)	1.0 (0.57, 1.9)	1.1 (0.61, 2.0)
Q3 (0.18, 0.40)	1.00 (0.54, 1.8)	1.00 (0.56, 1.8)
Q4 (0.40, 4.3)	1.7 (1.0, 2.9)	1.6 (0.96, 2.7)
p-trend[c]	0.03	0.06
MEHP		
Q1 (<LOD 1.3)	Ref	Ref
Q2 (1.3, 2.6)	1.1 (0.60, 2.0)	1.1 (0.64, 2.0)
Q3 (2.6, 6.3)	0.93 (0.50, 1.7)	0.94 (0.53, 1.7)
Q4 (6.3, 78.0)	1.7 (1.0, 2.9)	1.6 (0.99, 2.7)
p-trend[c]	0.04	0.06
MEHHP		
Q1 (0.93, 7.7)	Ref	Ref
Q2 (7.7, 14.0)	1.2 (0.65, 2.2)	1.2 (0.67, 2.1)
Q3 (14.0, 35.0)	1.4 (0.76, 2.5)	1.3 (0.76, 2.4)
Q4 (35.0, 378)	1.8 (1.1, 3.2)	1.7 (1.0, 2.9)
p-trend[c]	0.02	0.03
MEOHP		
Q1 (0.90, 5.3)	Ref	Ref
Q2 (5.3, 10.2)	1.6 (0.86, 2.9)	1.6 (0.90, 2.9)
Q3 (10.2, 24.0)	1.5 (0.81, 2.9)	1.5 (0.84, 2.9)
Q4 (24.4, 266)	2.1 (1.2, 3.8)	2.0 (1.1, 3.5)
p-trend[c]	0.01	0.03
MECPP		
Q1 (2.4, 14.3)	Ref	Ref
Q2 (14.3, 25.1)	0.88 (0.47, 1.6)	0.87 (0.47, 1.5)
Q3 (25.2, 57.2)	1.1 (0.63, 1.9)	1.1 (0.62, 1.8)
Q4 (57.2, 613)	1.5 (0.92, 2.5)	1.4 (0.85, 2.4)
p-trend[c]	0.05	0.09

Abbreviations: DEHP: di(2-ethylhexyl) phthalate; MEHP: mono(2-ethylhexyl) phthalate; MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP: mono(2-ethyl-5-oxohexyl) phthalate; MECPP: mono(2-ethyl-5-carboxypentyl) phthalate.

[a] Unadjusted and adjusted models estimated risk ratios and 95% CIs with repeated measures log-binomial regression.

[b] Adjusted model estimated RRs and 95% CIs adjusting for age (categorical), BMI (continuous), smoking status (never/ever), and infertility diagnosis (male factor [reference category], female factor, and unexplained).

[c] Tests for trend were performed using the urinary phthalate metabolite concentration quartile as an ordinal level indicator variable in the regression model, adjusted for covariates.