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Organophosphate flame retardant metabolite concentrations and pregnancy loss among women conceiving with assisted reproductive technology

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Abstract

Objective: To evaluate whether urinary concentrations of organophosphate flame retardant (PFR) metabolites are associated with pregnancy loss among women conceiving with assisted reproductive technology (ART).

Design: Prospective preconception cohort of subfertile women.

Setting: Academic hospital fertility center in Boston, Massachusetts.

Patients: 155 women conceiving 179 pregnancies with ART.

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Intervention: None. We estimated mean exposure to each of five PFR metabolites by averaging the specific-gravity adjusted natural log concentrations from two urine samples collected during the ART cycle of conception.

Main Outcome Measures: We estimated adjusted risk ratios (RRs) and 95% confidence intervals (CIs) for biochemical and total pregnancy loss (all losses <20 weeks gestation) by quartiles of PFR metabolite concentrations using a repeated measures log-binomial model, accounting for multiple pregnancies per woman.

Results: Of the 179 pregnancies, 31% ended in pregnancy loss (12% in biochemical loss). Among the three metabolites with high detection frequency [bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), and isopropylphenyl phenyl phosphate (ip-PPP)], we observed an increased risk of biochemical loss for women with DPHP concentrations in the 4th vs. 1st quartile (RR, 1.64; 95% CI: 0.61, 4.39). We also found an elevated risk of biochemical pregnancy loss among women in the highest quartile of the molar sum of urinary PFR metabolites compared to the lowest (RR, 1.89; 95% CI: 0.64, 5.58). Urinary concentrations of ip-PPP and BDCIPP were not associated with either outcome.

Conclusions: Among subfertile women, urinary DPHP metabolite concentrations measured during the ART cycle of conception may be associated with early pregnancy loss. While our study is uniquely designed to investigate early markers of pregnancy success and maintenance, our small sample size likely contributed to imprecision. Given their increasing use as replacement chemicals to traditional flame retardants, exposure to PFRs may increase, and more studies will be needed to investigate their potential to impact pregnancy and reproduction.

Capsule:

Urinary concentrations of organophosphate flame retardant metabolites measured in women undergoing ART increased the risk of early pregnancy loss.

Keywords

organophosphate flame retardants; pregnancy loss; assisted reproductive technology; infertility; environment

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Introduction

Pregnancy loss is the most frequent unintended perinatal outcome, affecting 31% of all conceptions (1). The spontaneous loss of a biochemical or clinical pregnancy contributes to reduced fecundity – the cycle probability of live birth. While some women may be able to achieve pregnancy, they may not be able to maintain that pregnancy throughout gestation and deliver a live-born infant. Thus, such women may be considered subfecund, but not technically infertile. Among subfertile women undergoing assisted reproductive technology (ART), pregnancy loss is a costly and emotional outcome. Predictors of pregnancy loss occurrence are not well established, however, environmental causes are thought to play a role (2–6).

Endocrine disrupting chemicals (EDCs) have been associated with infertility, including a longer time to pregnancy (i.e., reduced fecundability) as well as pregnancy loss (7, 8). Over the last decade, there has been growing concern over a large class of synthetic chemicals known as organophosphate flame retardants (PFRs). PFRs have been widely used in the polyurethane foam of upholstered furniture, in electronics, and as a plasticizer (e.g. in nail polish), replacing traditional polybrominated diphenyl ethers (PBDEs) after they were phased out in 2005 (9, 10). PFRs are added to household and consumer goods in order to meet flammability requirements. However, these compounds are not covalently bound to materials used in products and therefore slowly leach out into the environment (11). As such, human PFR exposure is ubiquitous in the general population, with North Americans reporting the highest body burdens of exposure (12, 13). One or more PFR metabolites has been detected in 90% of adult urine samples (14–19). While exposure to PFRs is widespread, there is limited human data about their potential endocrine disrupting effect on pregnancy or reproduction.

There is a scant but growing body of literature pointing to the endocrine disrupting effects of PFRs in experimental and human studies (20–24). PFRs have been shown to alter steroidogenesis and estrogen metabolism in chickens (20). There is also evidence that PFRs can disrupt thyroid pathways and alter embryo development in zebrafish (23, 24). Recently, concentrations of some PFR metabolites have been associated with reduced fertilization, implantation, and clinical pregnancy and live birth rates among subfertile couples (25, 26). In this study, we aimed to prospectively examine whether urinary concentrations of PFRs are associated with biochemical and total pregnancy loss (< 20 weeks gestation) among women conceiving with ART.

Methods

Study Cohort.

The Environment and Reproductive Health (EARTH) Study is an ongoing prospective cohort of couples recruited from the Massachusetts General Hospital Fertility Center. The study was designed to evaluate the effects of diet and environmental exposures on fertility and pregnancy outcomes. Women between the ages of 18 to 46 years are eligible to enroll and are followed from study entry, throughout their fertility care, eventual pregnancy, and birth. Details of the cohort have been described elsewhere (27). The present study included women who enrolled in the EARTH Study with a male partner between November 2004 and October 2015 and for whom we had at least one urine sample analyzed for the measurement of flame retardant metabolite concentrations during an in vitro-fertilization (IVF) cycle (fresh or cryo/frozen). The analysis included only those women who achieved positive pregnancy (defined as two or more positive serum beta Human Chorionic Gonadotropin (β-hCG) measurements) (6) (see Supplemental Figure 1 for Participant Flow Chart). The study was approved by the Institutional Review Boards of the Massachusetts General Hospital, Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC). Prior to signing informed consent, subjects met with trained study staff who explained all procedures and answered questions.

Urinary PFR Metabolite Assessment.

Participants provided one or two spot urine samples per ART treatment cycle. The first specimen was obtained between days 3 to 9 of the follicular phase of the woman's cycle and the second was obtained on the day of oocyte retrieval. Both urine samples collected during the ART treatment cycle of conception of the index pregnancy were included in the analysis. Urine samples among women conceiving a pregnancy following a cryo-thawed cycle (n= 15/179 pregnancies) were obtained during the cycle of oocyte retrieval and not embryo transfer. Urine samples were collected using a sterile polypropylene cup, analyzed for specific-gravity with a handheld refractometer (National Instrument Company, Inc.), and divided into aliquots and frozen at −80°C. Samples were shipped on dry ice overnight to the Stapleton laboratory at Duke University (Durham, NC, USA) for the quantification of PFR metabolites.

We measured five urinary PFR metabolites: bis(1-chloro-2-propyl) phosphate (BCIPP); bis(1,3-dichloro-2-propyl) phosphate (BDCIPP); diphenyl phosphate (DPHP); tert-butylphenyl phenyl phosphate (tb-PPP); and isopropylphenyl phenyl phosphate (ip-PPP). Extraction and analysis methods for BCIPP, BDCIPP, DPHP, tb-PPP, and ip-PPP followed methods previously developed by the Stapleton laboratory (14). Briefly, urine samples were thawed and an aliquot (2.5 to 5 ml) was transferred to a clean glass test tube. The urine was spiked with mass-labeled internal standards (d_{10}-BDCIPP = 80 ng, d_{10}-DPHP = 60 ng). Samples were diluted 1:1 with water and concentrated and cleaned using solid-phase extraction techniques (SPE) after acidifying to pH <6.5. The SPE eluent was blown to dryness under a gentle nitrogen stream, reconstituted in 500 µl of 1:1 H_2O:MeOH and spiked with the recovery standard ($^{13}C_2$-DPHP = 81.5 ng). Following methods as describe by Butt et al. 2014, extracts were analyzed by negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS). The mobile phases were methanol and water (modified with 0.8 mM ammonium acetate), with a flow rate of 300 µl/min, an injection volume of 5 µl and the column oven was set at 45°C. Chromatography was achieved under gradient conditions using a Luna C18(2) column (50 × 2.0 mm, 2.5 µm particle size, Phenomenex, Torrance, CA) preceded by a SecurityGuard Polar-RP (4 × 2.0 mm) guard cartridge. Data were acquired under multiple reaction monitoring conditions using optimized parameters. Analyte responses were normalized to internal standard responses. DPHP, ip-PPP and tb-PPP were normalized using d_{10}-DPHP, and BCIPP and BDCIPP were normalized using d_{10}-BDCIPP. Urinary specific gravity ranged from 1.002 to 1.028 with a mean of 1.016.

Samples were analyzed by LC-MS/MS in 10 separate batches. Unique method detection limits (MDLs) were calculated for each analysis batch. In the urine samples, the mean recovery of the mass-labeled standards was 152% (standard error = 2.2%) for d_{10}-BDCIPP and 119% (standard error = 0.75%) for d_{10}-DPHP. One laboratory blank (5 ml Milli-Q water only) sample was extracted with every batch (n=95). An in-house standard reference material (SRM) was prepared from pooled urine that was collected during previous studies. SRM samples were periodically analyzed during the extraction batches (n=18) and were within 15% for BDCIPP, 20% for ip-PPP, and 10% for DPHP. Two of the individual sub-samples were analyzed in duplicate to assess method precision and were generally within

35% for BDCIPP, 15% for DPHP, and 25% for ip-PPP. Very low levels of DPHP (mean = 0.58 ng) and ip-PPP (mean = 0.21 ng) were commonly detected in the laboratory blanks and analyte values were blank-corrected using the mean laboratory blank values. MDLs were calculated as three times the standard deviation of laboratory blanks normalized to the volume extracted (5 ml). MDLs ranged (n=10) from 31–300 pg/ml for BDCIPP, 68–180 pg/ml for BCIPP, 23–120 pg/ml for ip-PPP, 10–150 pg/ml for tb-PPP, 25–130 pg/ml for DPHP, respectively.

Pregnancy Loss Assessment.

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 a transvaginal ultrasound at approximately 6 weeks gestation for women with 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 (5, 28). 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 (6). 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 ART (29). We calculated gestational age as: outcome date - date of transfer + 14 + cycle day of transfer (30).

Covariates.

Age, race, and smoking status were obtained by self-reported questionnaire administered by research staff at study entry. Height and weight were measured at enrollment by the study staff. Body Mass Index (BMI) measured at study entry was calculated as weight (kilograms) divided by height (meters) squared. Clinical information such as type of fertility treatment and protocol received, 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. Levels of follicle stimulating hormone (FSH) were measured in serum obtained on third day of the menstrual cycle. The treating infertility physician diagnosed the underlying cause of infertility using the Society for Assisted Reproductive Technology definitions (31).

Statistical Analysis.

Unquantified concentrations <MDL were substituted with a value equal to the MDL/√2 (Hornung and Reed 1990). We calculated the molar sum of the 3 PFR metabolites with high detection frequencies by dividing each metabolite concentration by its molar weight and then summing: $\Sigma PFR= [(DPHP \times (1/250.04)) + (BDCIPP \times (1/319.91)) + (ip\text{-}PPP \times (1/292.09))]$. Urinary PFR metabolite concentrations were adjusted for urinary dilution by multiplying the metabolite concentration by $[(1.016 – 1)/(SG – 1)]$, where SG is the specific gravity of the participant's sample and 1.016 is the mean SG for all study urine samples (32). The specific gravity adjusted PFR metabolite concentrations were natural log-transformed to normalize the distribution and used to estimate the geometric mean from the

two urine samples obtained during the index ART cycle. For cycles where only one urine sample was available (11% of women), the concentration of the single sample was used as the estimate of PFR exposure. We calculated Spearman's correlation coefficients for natural log urinary PFR metabolite concentrations and estimated the variability within a cycle and within a woman by calculating the intraclass correlation coefficient (ICC).

We examined clinical and demographic characteristics and reported the mean (SD) or number of women (%). We fit generalized estimating equation models to evaluate the association of quartiles of urinary PFR metabolite concentrations and pregnancy loss, accounting for correlation within women contributing more than one pregnancy. Generalized estimating equation models were fit using a log link function and binomial distribution to yield estimated risk ratios (RRs) and 95% confidence intervals (CIs) for biochemical and total pregnancy loss with the lower quartile as the reference category. We fit a separate model for each of the individual PFRs metabolite concentrations and for the molar sum of PFR metabolites (ΣPFR). Statistical tests for trend were conducted across quartiles using the PFR metabolite concentration as an ordinal level indicator variable of each quartile in the regression models, adjusted for covariates. Covariates were selected *a priori* as potential confounders based on the literature and included maternal age (categorical), BMI (continuous), and smoking status (never smoked vs. ever smoked, defined as current or former smoker). In order to assess potential effect modification by age, we stratified our cohort by women <35 and ≥35 years of age, and tested for interaction by including a cross-product term (ΣPFR concentration*age) in our models. Given our limited sample size, we considered a p-value for the interaction term of <0.20 as evidence of potential interaction. We performed statistical analyses with SAS (version 9.4; SAS Institute Inc., Cary, USA).

Results

Study Cohort.

The final study cohort consisted of 155 women, who conceived 179 pregnancies with ART. Women were on average 34.9 years of age and had a BMI of 23.6 kg/m^2 at time of enrollment. Participants were mostly Caucasian (87%), never-smokers (77%), and nulliparous (87%), and 28% had a female cause as their primary infertility diagnosis (Table 1).

Urinary PFR metabolite concentrations.

In our cohort of 155 women, we obtained 339 cycle-specific urine samples. Most women provided two urine samples during the ART treatment cycle of the index conception (89%). Detection frequencies were high for DPHP (98%), BDCIPP (94%), and ip-PPP (87%) (Table 2). Low detection frequencies for tb-PPP (23%) (tb-PPP) and BCIPP (0%) precluded us from performing further analyses with these metabolites. Urinary PFRs were weakly correlated across all metabolites with measurable detection frequencies: Spearman correlation coefficients ranged from 0.05 (tb-PPP and DPHP) to 0.20 (BDCIPP and DPHP) (Table 3). ICCs indicated moderate between cycle variability (BDCIPP=0.36; DPHP=0.30; ip-PPP=0.26; ΣPFR=0.26), with higher reproducibility within the two urine samples obtained from an individual cycle (BDCIPP=0.50; DPHP=0.50; ip-PPP=0.50; ΣPFR=0.50) (data not shown).

PFR and Pregnancy Loss.

Of the 179 pregnancies, 31% ended in pregnancy loss (12% were a biochemical loss). Among the three metabolites with high detection frequency (BDCIPP, DPHP, ip-PPP), we observed a possible increased risk of biochemical loss for women with DPHP concentrations in the 4^{th} vs. 1^{st} quartile (RR, 1.64; 95% CI: 0.61, 4.39) (Table 4). Although also imprecise, we found an elevated risk of biochemical pregnancy loss for women in higher quartiles of the sum of PFR metabolite concentrations (ΣPFR) compared to those in the lowest quartile: Q2, 1.61 (95% CI: 0.49, 5.23); Q3, 1.05 (95% CI: 0.30, 3.84); Q4, 1.89 (95% CI: 0.64, 5.58) (Table 4). There was also a suggestive increased risk of pregnancy loss of up to 20 weeks gestation in relation higher quartiles of the sum of PFR metabolite concentrations (Table 4). BDCIPP and ip-PPP concentrations were not associated with either biochemical or total pregnancy loss (Table 4).

In our age-stratified sensitivity analysis, we observed an elevated risk of both biochemical and total pregnancy loss among younger (<35 years) compared to older women (≥35 years) in the highest quartile of the sum of PFR metabolite concentrations. Evidence of statistically significant interaction was significant for biochemical pregnancy loss (p-value for interaction by age=0.16) but limited for total pregnancy loss (p-value for interaction by age=0.51) (Supplemental Table 1).

Discussion

In this study of subfertile women conceiving with ART, we found suggestive evidence of an association between cycle-specific urinary concentrations of the sum of PFR metabolites and biochemical pregnancy loss. Although imprecise and non-linear, elevated risk of biochemical loss was most apparent in the highest quartile compared to the lowest. We also observed an elevated risk of biochemical pregnancy loss for DPHP metabolite concentrations among women in the highest exposure group. Total pregnancy loss was also elevated among women higher quartiles of sum of the urinary PFR metabolite concentrations. Overall, associations appeared more pronounced among younger compared to older women. However, our limited sample size precluded us from making more firm conclusions, and results from this study should be interpreted cautiously.

To the best of our knowledge, this is the first study to examine biochemical pregnancy loss within a subfertile cohort of women conceiving with ART in relation to organophosphate flame retardant exposure. In a recent prior study from our cohort, we reported that the sum of urinary PFRs were associated with reduced probability of fertilization, implantation, clinical pregnancy and live birth (25). The unique nature of our study design permitted us to further examine biochemical pregnancies that were detected very early post-implantation, by measuring serum β-hCG on day 17 (range: 15–20) after embryo transfer and confirming the pregnancy with a second positive β-hCG serum measurement. The current study further adds to our earlier work, suggesting that associations with reduced clinical pregnancy and live births (25) are partially due to impaired fertilization and implantation, however a portion of the association is likely also due a higher risk of pregnancy loss, especially very early, biochemical losses. With about a third of all pregnancies ending before viability (1) 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 (33).

Our results suggest a potential association between PFRs and pregnancy loss, potentially involving early stages of implantation, decidualization, placentation or embryogenesis and possibly through uterine-embryo hormonal signaling (34). Although not designed to elucidate mechanisms, our findings are consistent with animal studies suggesting that PFRs affect early reproductive endpoints through disruption of regulatory pathways mediated by the hypothalamus-pituitary-gonadal axis. Studies in zebrafish report decreased hatching and survival, and increased plasma estradiol (E2), testosterone and thyroid hormone levels (22–24). Similarly, studies in chicken embryos have shown delayed hatching and endocrine disruption, including reduced thyroxine and cholesterol (20, 21).

Our study provides preliminary evidence that flame retardant exposure may adversely impact very early reproductive processes resulting in pregnancy failure. Early pregnancy failure is a significant and costly outcome in the fertility clinical setting, and while our results are only suggestive they may have important clinical and public health implication on a population level. The strengths of this study include the prospective preconception design that permitted a careful examination of the direction of the relationship between PFRs metabolite concentrations and pregnancy loss. We also used cutting-edge measurement of PFR exposure biomarkers collected in one clinical location and processed under one protocol by the Duke University Stapleton laboratory. PFRs are short-lived chemicals; parent compounds in blood are rapidly metabolized to diesters and other metabolites in urine. Half-lives are short, in the order of hours (19). Exposure is therefore episodic, making assessment of long-term exposure difficult. However, we partially accounted for this variability by averaging concentrations of two urine samples provided at two time points in the follicular phase of the ART cycle of conception in the index pregnancy. These time points correspond most proximally to concentrations at the time of implantation and decidualization, making biochemical pregnancy loss a sensitive endpoint relevant to the exposure window we assessed, except in the case of 15 cryo-thawed IVF conceived pregnancies where exposure was measured at time of oocyte retrieval and not embryo transfer. However, these findings may not be generalizable to women from the general population without fertility concerns, co-exposures to other select chemicals such as phthalates and phenols were also not accounted for, and exposure to PFRs 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, BMI, and smoking. While we have not tested the equipment for the presence of DPHP, urine samples were quantified for PFR metabolites and not the parent compounds. Metabolites would not be present in the petri dishes, urine sample containers, or other equipment, and therefore likely not a source of contamination in our urine sample measurements. Nevertheless, any potential source of theoretical contamination to the parent compounds in equipment would likely be equal among all embryo processed. Furthermore, in the present study, we have not considered the effect of paternal PFR exposure on pregnancy loss outcomes via DNA methylation or other epigenetic modifications of imprinted genes in male gametes (35). Lastly, our study was based on a limited sample size, and findings should be confirmed or refuted in future larger cohorts.

Conclusions

To our knowledge, this is the first study to examine PFRs in relation to pregnancy loss. Among subfertile women, urinary PFR metabolite concentrations measured during ART may be associated with pregnancy loss. While the study setting is uniquely designed to investigate early markers of pregnancy success and maintenance, our small sample size likely contributed to imprecision. As exposure to PFRs continues to grow given their increasing use as replacement chemicals to traditional flame retardants, more studies will be needed to investigate their potential to impact pregnancy and reproduction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Baseline characteristics and outcomes among 155 women with 179 β-hCG confirmed pregnancies enrolled in the Environment and Reproductive Health Study (EARTH) between 2004 and 2015.

Characteristic	Total Cohort (N=155) N (%)
Age at study entry (years)	
Mean (SD)	34.9 (3.6)
Min-Max	27–42
Age > 35	64 (41%)
BMI (kg/m^2)	
Mean (SD)	23.6 (4.0)
Min-Max	16–37
Overweight or obese (≥25)	46 (30%)
Education[a]	
< College graduate	8 (5%)
College graduate	52 (34%)
Graduate degree	85 (55%)
Smoking	
Never smoked	120 (77%)
Ever smoked	35 (23%)
Race	
Caucasian	135(87%)
Black/African American	2 (1%)
Asian	14 (9%)
Other	4 (3%)
Nulligravida	100 (65%)
Nullipara	134 (87%)
Primary SART diagnosis	
Female factor	44 (28%)
Diminished ovarian reserve	7 (4%)
Ovulation disorders	17 (11%)
Endometriosis	8 (5%)
Uterine disorders	2 (1%)
Tubal factor	10 (6%)
Male factor	60 (39%)
Unexplained	51 (33%)
FSH Day 3 in IU/L	
Mean (SD)	6.9 (2.1)
Pregnancy Loss Outcomes[b,c,d]	
Biochemical Loss	21 (12%)
Loss <20 weeks	56 (31%)

Abbreviations: 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.

[a] Unknown/missing education values: n=10.

[b] 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).

[c] Proportion of pregnancy loss outcomes in total number of pregnancies (n/179).

[d] Biochemical pregnancy losses by quartile of the sum of organophosphate flame retardant concentrations (ΣPFR): 4/44 (9%); 6/45 (13%); 4/45 (9%); 7/45 (16%); Total losses by quartile of ΣPFR: 12/44 (27%); 17/45 (38%); 13/45 (29%); 14/45 (31%).

Table 2.

Distribution of urinary organophosphate flame retardant metabolites (ug/L) measured among 155 women with 339 cycle-specific urine samples in the EARTH Study.

Specific Gravity Adjusted[a]	N > MDL[b] (%)	GM (95% CI)	Min	10th Pctl	25th Pctl	50th Pctl	75th Pctl	90th Pctl	95th Pctl	Max
BCIPP	0 (0)	N/A	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
BDCIPP	329 (94)	0.69 (0.59, 0.80)	<MDL	0.19	0.30	0.65	1.46	2.72	4.03	6.74
DPHP	335 (98)	0.80 (0.69, 0.92)	<MDL	0.30	0.47	0.73	1.19	2.34	3.52	657
ip-PPP	315 (87)	0.09 (0.07, 0.11)	<MDL	0.02	0.05	0.24	0.39	0.32	0.55	76.1
tb-DPP	201 (23)	N/A	<MDL	<MDL	0.06	0.08	0.20	0.36	374	374
ΣPFR[c]	-	7.89 (7.12, 8.73)	1.5	3.7	4.54	7.25	1.16	1.96	2.55	2.89

[a]Adjusted to specific gravity, range (1.002–1.028).

Abbreviations: < MDL, method detection limit; Max, maximum; Pctl: percentile; Min, minimum; [bis(1,3-dichloro-2-propyl) phosphate (BDCIPP); diphenyl phosphate (DPHP); isopropylphenyl phenyl phosphate (ip-PPP); tert-butylphenyl phenyl phosphate (tb-PPP); bis(1-chloro-2-propyl) phosphate (BCIPP)].

[b]All values below MDL were assigned a value equal to the MDL divided by √2.

[c]ΣPFR is the molar sum of the three organophosphate flame retardant metabolite concentrations with high detection frequencies: (BDCIPP*(1/319.91) + (DPHP*(1/250.04) + ipPPP*(1/292.09). Concentrations shown in mol/ml X10–9.

Table 3.

Spearman's rank correlation coefficients (*r*) of urinary organophosphate flame retardant concentrations measured in 339 urine samples from 155 women in the EARTH Study.

PFR Metabolites	BDCIPP	DPHP	ip-PPP	tb-DPP
BDCIPP	1.0	0.20	0.15	−0.07
p-value	-	<0.0001	0.001	0.07
DPHP	0.20	1.0	0.16	0.05
p-value	<0.0001	-	<0.001	0.17
ip-PPP	0.15	0.16	1.00	0.10
p-value	0.001	<0.001	-	0.008
tb-DPP	−0.07	0.05	0.10	1.0
p-value	0.07	0.17	0.008	-

Metabolite Abbreviations: [bis(1,3-dichloro-2-propyl) phosphate (BDCIPP); diphenyl phosphate (DPHP); isopropylphenyl phenyl phosphate (ip-PPP); tert-butylphenyl phenyl phosphate (tb-PPP).

Table 4.

Risk Ratios (RR) and 95% Confidence Intervals (CIs) for biochemical pregnancy loss and total pregnancy loss across quartiles of urinary organophosphate flame retardant metabolite concentrations using 339 cycle-specific samples from 179 pregnancies in the Environment and Reproductive Health (EARTH) Study.

		Biochemical Loss[a]		Total Pregnancy Loss[b]	
PFR Metabolite Quartile (ng/ml)	N	RR (95% CI) Unadjusted[c] Model	RR (95%CI) Adjusted[d] Model	RR (95% CI) Unadjusted Model	RR (95% CI) Adjusted[d] Model
BDCIPP					
Q1 (0.21)	45	REF	REF	REF	REF
Q2 (0.46)	44	0.73 (0.25, 2.10)	0.70 (0.25, 2.00)	0.83 (0.45, 1.55)	0.87 (0.50, 1.54)
Q3 (0.94)	45	0.71 (0.25, 2.07)	0.71 (0.24, 2.06)	0.81 (0.45, 1.47)	0.83 (0.47, 1.49)
Q4 (2.49)	45	0.57 (0.18, 1.79)	0.52 (0.17, 1.58)	0.75 (0.40, 1.39)	0.70 (0.39, 1.26)
p-trend[e]		0.34	0.23	0.37	0.27
DPHP					
Q1 (0.34)	44	REF	REF	REF	REF
Q2 (0.58)	45	0.78 (0.23, 2.69)	0.81 (0.23, 2.77)	0.73 (0.39, 1.40)	0.71 (0.37, 1.35)
Q3 (0.92)	45	0.98 (0.33, 2.94)	1.06 (0.36, 3.10)	0.67 (0.35, 1.30)	0.84 (0.44, 1.46)
Q4 (1.88)	45	1.37 (0.49, 3.84)	1.64 (0.61, 4.39)	0.92 (0.53, 1.59)	1.15 (0.68, 1.93)
p-trend[e]		0.49	0.30	0.94	0.67
ipPPP					
Q1 (0.028)	44	REF	REF	REF	REF
Q2 (0.068)	45	0.81 (0.28, 2.42)	0.86 (0.28, 2.68)	0.75 (0.37, 1.51)	0.79 (0.40, 1.57)
Q3 (0.12)	45	0.81 (0.27, 2.45)	0.91 (0.29, 2.88)	1.28 (0.71, 2.30)	0.91 (0.82, 2.40)
Q4 (0.28)	45	0.82 (0.29, 2.32)	0.81 (0.29, 2.27)	1.05 (0.56, 1.96)	0.81 (0.56, 1.88)
p-trend[e]		0.71	0.76	0.71	0.76
ΣPFR (mol/ml × 10–9)[f]					
Q1 (0.0038)	44	REF	REF	REF	REF
Q2 (0.0060)	45	1.47 (0.45, 4.76)	1.61 (0.49, 5.23)	1.39 (0.72, 2.64)	1.41 (0.75, 2.65)
Q3 (0.0087)	45	0.98 (0.27, 3.50)	1.05 (0.30, 3.84)	1.06 (0.54, 2.09)	1.10 (0.58, 2.10)
Q4 (0.018)	45	1.71 (0.56, 5.24)	1.89 (0.64, 5.58)	1.14 (0.60, 2.18)	1.19 (0.63, 2.24)
p-trend[e]		0.48	0.65	0.82	0.70

Metabolite Abbreviations: BDCIPP: [bis(1,3-dichloro-2-propyl) phosphate; DPHP: diphenyl phosphate; ip-PPP: isopropylphenyl phenyl phosphate

[a]Biochemical pregnancy loss was defined as the demise of a non-visualized β-hCG confirmed pregnancy.

[b]Pregnancy loss <20 weeks' gestation was defined as the loss of any pregnancy (including biochemical losses) of less than 20 weeks gestation (<=139 days).

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

[d]Adjusted models included age (categorical), BMI (continuous), smoking status (never/ever).

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

[f] ΣPFR is the molar sum of the three organophosphate flame retardant metabolite concentrations with high detection frequencies: (BDCIPP*(1/319.91) + (DPHP*(1/250.04) + ipPPP*(1/292.09). Concentrations shown in mol/ml X10–9.

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