HHS Public Access

Author manuscript

Environ Int. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:

Environ Int. 2018 February ; 111: 232–238. doi:10.1016/j.envint.2017.12.005.

Paternal Urinary Concentrations of Organophosphate Flame Retardant Metabolites, Fertility Measures, and Pregnancy Outcomes among Couples Undergoing *in Vitro* Fertilization

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Abstract

Background—Use of organophosphate flame retardants (PFRs) has increased over the past decade following the phase out of some brominated flame retardants, leading to increased human exposure. We recently reported that increasing maternal PFR exposure is associated with poorer pregnancy outcomes among women from a fertility clinic. Because a small epidemiologic study previously reported an inverse association between male PFR exposures and sperm motility, we sought to examine associations of paternal urinary concentrations of PFR metabolites and their partner's pregnancy outcomes.

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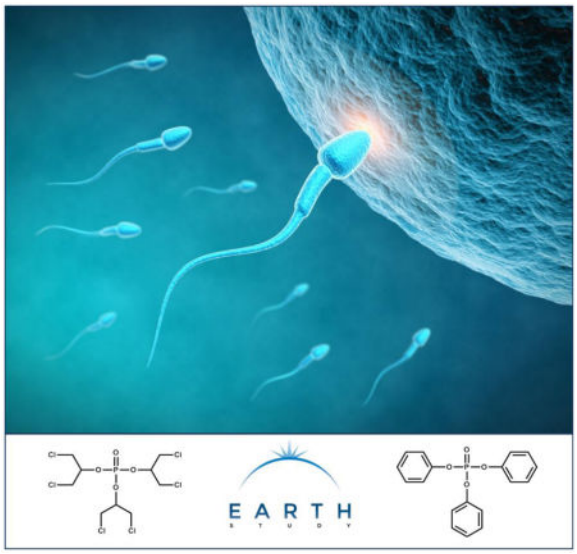
Competing financial interests: The authors declare they have no actual or potential competing financial interests.

Methods—This analysis included 201 couples enrolled in the Environment and Reproductive Health (EARTH) prospective cohort study (2005–2015) who provided one or two urine samples per IVF cycle. In both the male and female partner, we measured five urinary PFR metabolites [bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), tert-butylphenyl phenyl phosphate (tb-PPP) and bis(1-chloro-2-propyl) phosphate (BCIPP)] using negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS). The sum of the molar concentrations of the urinary PFR metabolites was calculated. We used multivariable generalized linear mixed models to evaluate the association of urinary concentrations of paternal PFR metabolites with IVF outcomes, accounting for multiple in vitro fertilization (IVF) cycles per couple. Models were adjusted for year of IVF treatment cycle and primary infertility diagnosis as well as paternal age, body mass index, and race/ethnicity.

Results—Detection rates were high for paternal urinary concentrations of BDCIPP (84%), DPHP (87%) and ip-PPP (76%) but low for tb-PPP (12%) and zero for BCIPP (0%). We observed a significant 12% decline in the proportion of fertilized oocytes from the first to second quartile of male urinary ΣPFR and a 47% decline in the number of best quality embryos from the first to third quartile of male urinary BDCIPP in our adjusted models. An 8% decline in fertilization was observed for the highest compared to lowest quartile of urinary BDCIPP concentrations (95% CI: 0.01, 0.12, p-trend=0.06).

Conclusions—Using IVF as a model to investigate human reproduction and pregnancy outcomes, we found that paternal urinary concentrations of BDCIPP were associated with reduced fertilization. In contrast to previously reported findings for the female partners, the paternal urinary PFR metabolites were not associated with the proportion of cycles resulting in successful implantation, clinical pregnancy, and live birth. These results indicate that paternal preconception exposure to TDCIPP may adversely impact successful oocyte fertilization, whereas female preconception exposure to ΣPFRs may be more relevant to adverse pregnancy outcomes.

Graphical Abstract



Keywords

Endocrine disruption; epidemiology; fertility; flame retardants; reproductive health

1. INTRODUCTION

Use of organophosphate flame retardants (PFRs) has increased over the past decade in the polyurethane foam of upholstered furniture with the phase out PentaBDE (Stapleton et al. 2009). PFRs are not chemically bonded to foam and have been shown to migrate into the air and dust of indoor environments (van der Veen and de Boer 2012). This has led to ubiquitous human exposure, with PFR urinary metabolites detected in 90 to 100% of adult urine samples (Butt et al. 2014; Butt et al. 2016; Carignan et al. 2013a; Cequier et al. 2015; Hammel et al. 2016; Hoffman et al. 2014; Meeker et al. 2013a; Van den Eede et al. 2015).

We previously reported that maternal exposure to PFRs may adversely impact female reproductive health and pregnancy outcomes, as evidenced by strong inverse associations for the sum of three PFR metabolites with decreased proportions of fertilization, implantation, clinical pregnancy and live birth among women recruited from an academic fertility clinic (Carignan et al. in press). This finding was consistent with animal studies, which have shown adverse effects on reproductive outcomes including decreased egg production, promotion of oocyte maturation, egg quality, hatching and survival among zebrafish and delayed hatching among chicken embryos (Farhat et al. 2013; Liu et al. 2013; Wang et al. 2015b; Wang et al. 2013). Experimental studies have also reported impacts of PFRs on male fecundity. These include a study of zebrafish exposed to tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) that reported decreased spermiation and a study of leydig cell tumor cells exposed to seven common PFRs that found adverse effects on leydig cell mitochondrial activity, cell survival, and superoxide production to a greater extent than the brominated flame retardants they have replaced (Schang et al. 2016; Wang et al. 2015b). In a small epidemiologic study on PFRs and male reproductive health among men recruited from a fertility clinic, there was a suggestive inverse association of sperm concentrations and motility with urinary metabolites of TDCIPP and triphenyl phosphate (TPHP) (Meeker et al. 2013b).

Although our earlier study showed associations of maternal urinary concentrations of PFRs with poorer pregnancy outcomes (Carignan et al. in press), the paternal contribution to these outcomes needs to be considered given the potential for correlated exposures among couples and the limited data showing associations with poorer semen quality. Therefore we explored associations of *paternal* urinary concentrations of PFR metabolites and pregnancy outcomes among couples in a prospective cohort study, the Environment and Reproductive Health Study (EARTH) Study, using assisted reproductive technologies (ART) as a model to early developmental endpoints and pregnancy outcomes.

2. METHODS

2.1 Participants

Study participants included male and female partners recruited into the EARTH study between 2005 and 2015 to evaluate environmental and dietary determinants of fertility among patients from Massachusetts General Hospital (MGH) Fertility Center. Age requirements for participation were 18–55 for men and 18–45 for women. The EARTH study was approved by the Human Studies Institutional Review Boards of the MGH and Harvard T.H. Chan School of Public Health. Participants signed an informed consent after the study procedures were explained by trained study staff and any questions were answered. A staff-administered questionnaire was used to collect demographic information from each participant including race/ethnicity, smoking history, education, and history of previous pregnancies. To be included in the present analysis, couples must have used their own fresh gametes and each partner must have provided at least one urine sample for the measurement of flame retardant metabolites during an in vitro fertilization (IVF) cycle. We included up to three IVF attempts per couple. Our final dataset included 201 couples with 276 IVF cycles who had complete information on the exposure and outcome variables.

2.2 Clinical Data and IVF Outcomes

At study entry, both male and female participants' date of birth was collected and their weight and height were measured by trained study staff. Body mass index (BMI) was calculated as weight (in kilograms) per height (in meters) squared. Clinical information on the IVF treatment cycle was collected or abstracted from the female partner's electronic medical record by trained study staff. Infertility diagnosis was physician determined according to the Society for Assisted Reproductive Technology (SART 2016; Mok-Lin et al. 2010). IVF treatment protocols include: (1) luteal phase gonadotropin releasing hormone (GnRH) agonist (low-, regular-, or high-dose leuprolide acetate, Lupron), (2) follicular phase GnRH-agonist/Flare stimulation, or (3) GnRH-antagonist. Fertilization was confirmed 17–20 hours after insemination by the presence of a fertilized oocyte with two pronuclei. For analysis we classified embryos as best quality if they had 4 cells on day 2, 8 cells on day 3, and a morphologic quality score of 1 or 2 on days 2 and 3 (Veeck and Zaninovic 2003). Implantation was defined as a serum β-hCG level > 6 mIU/mL approximately 17 days (range 15–20 days) after egg retrieval, clinical pregnancy as the presence of an intrauterine pregnancy confirmed by ultrasound at approximately 6 weeks gestation, and live birth as the birth of a neonate on or after 24 weeks gestation.

2.3 PFR Assessment in Urine Samples

Urine samples were provided during the IVF cycle, which for men was typically during the visit to provide a semen sample. All men included in this analysis provided a single urine sample per IVF cycle and women provided up to two urine samples per IVF cycle. Following collection of each sample, specific gravity (SG) was measured using a handheld refractometer (National Instrument Company, Inc.).

Extraction and analysis methods for BCIPP, BDCIPP, DPHP, ip-PPP and tb-PPP followed methods previously developed by Dr. Stapleton's laboratory at Duke University (Butt et al.

2014). Briefly, urine samples were thawed and a 2.5 to 5 ml aliquot was transferred to a clean glass test tube where it was spiked with mass-labeled internal standards (d_{10}-BDCIPP = 80 ng, d_{10}-DPHP = 60 ng). After acidifying to pH <6.5 with formic acid, samples were diluted 1:1 with water and concentrated and cleaned using solid-phase extraction techniques (SPE). 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). Extracts were analyzed by negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described (Butt et al. 2014). Chromatography was achieved under gradient conditions using a Luna C18(2) column (50 x 2.0 mm, 2.5 µm particle size, Phenomenex, Torrance, CA) preceded by a SecurityGuard Polar-RP (4 x 2.0 mm) guard cartridge. The mobile phases were methanol and water (modified with 0.8 mM ammonium acetate), flow rate was 300 µl/min, the injection volume was 5 µl and the column oven was 45°C. Data were acquired under multiple reaction monitoring conditions using optimized parameters. Analyte responses were normalized to internal standard responses. BCIPP and BDCIPP were quantified using d_{10}-BDCIPP, while DPHP, ip-PPP and tb-PPP were quantified using d_{10}-DPHP. Urinary SG ranged from 1.002 to 1.038 with a mean of 1.018.

Samples were analyzed by LC-MS/MS in 10 separate batches and unique method detection limits (MDLs) were calculated for each analysis batch. In the urine samples, the mean recovery of the mass-labeled standards was 119% (standard error = 0.75%) for d_{10}-DPHP and 152% (2.2%) for d_{10}-BDCIPP. 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 generally within 10% for DPHP, 15% for BDCIPP and 20% for ip-PPP. Two of the individual sub-samples were analyzed in duplicate to assess method precision and were generally within 15% for DPHP, 25% for ip-PPP and 35% for BDCIPP. 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 of water extracted (5 ml). MDLs ranged (n=10) from 68–180 pg/ml for BCIPP, 31–300 pg/ml for BDCIPP, 25–130 pg/ml for DPHP, 23–120 pg/ml for ip-PPP, 10–150 pg/ml for tb-PPP, respectively.

2.4 Statistical Analysis

Demographic and clinical characteristics were reported using median ± interquartile range (IQR) or percentages. Concentrations of urinary metabolites <MDL were substituted with a value equal to the MDL/√2 (Hornung and Reed 1990). To account for urinary dilution, we adjusted by SG as described in Pearson *et al.* (2009) and all analyses used the SG-adjusted urinary metabolite concentrations. While all men in our dataset provided only one urine sample for each IVF cycle, the women provided up to two. For these women we took the geometric mean (GM) of the two urinary metabolite concentrations to determine correlations with the male urinary metabolite concentrations. Due to the skewed distributions of urinary metabolite concentrations, these measures were log-transformed prior to analysis.

Relationships among urinary PFR metabolites were determined using Spearman's correlation. Intraclass correlation coefficients (ICC) were calculated to determine variability of urinary metabolite concentrations across cycles within men and within women. For women who contributed two urines per cycle, ICCs were calculated to estimate variability of urinary metabolite concentrations within a cycle. To evaluate associations between potential predictors and urinary metabolites, we fit multivariable generalized linear mixed models with random intercepts to account for multiple IVF cycles in the couple. Final models included race/ethnicity, BMI, season, and year.

Cycle specific concentrations for men were divided into quartiles for use in regression models, with values <MDL falling into Q1. This approach was selected because it does not constrain the dose-response relationship to be linear, given the demonstrated non-linearities in the literature (Liu et al. 2013), underlying mechanistic support for such non-linearities (Birnbaum 2012), and insufficient statistical power for using smooth spline models to evaluate such non-linearities. To evaluate associations between the urinary metabolites and IVF outcomes, we fit multivariable generalized linear mixed models with random intercepts to account for multiple IVF cycles in the couple. A Poisson distribution and log link function were specified for the number of best quality embryos. A binomial distribution and logit link function were specified for fertilization. Finally, a binary distribution with a logit link function were specified for the clinical outcomes (implantation, clinical pregnancy and live birth). Tests for trend were conducted across quartiles using the median log-transformed urinary metabolite concentration in each quartile as a linear variable in the regression models. To allow for better interpretation of the results, all results are presented as population marginal means adjusted for covariates set to their mean for continuous variables weighted according to their frequencies for categorical variables. Percent decrease was calculated as the difference in marginal means from Q1 to Q4 divided by the marginal mean from Q1. In our primary analysis of the early developmental outcomes we excluded six couples (13 IVF cycles) with unsuccessful oocyte retrieval.

We evaluated confounding using prior knowledge and descriptive statistics from our cohort. A *priori* these included paternal and maternal age (continuous), race/ethnic group (Black/Asian/Other, White/Caucasian), and BMI (continuous) as well as maternal urinary PFR concentration (continuous) and primary infertility diagnosis (female factor, male factor, and unexplained). Year was included as a covariate because it was associated with both the exposure and outcome. Season was not included as a covariate as it was not associated with the IVF outcomes. In a sensitivity analysis its inclusion did not alter effect estimates. Statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC) and two-sided significance levels less than 0.05 were considered statistically significant.

3. RESULTS

3.1 Study population

Our analysis included 201 couples and 276 IVF cycles. The first and second IVF cycles were an average of 170 (IQR: 131, 201) days apart. Male and female partners had similar demographic characteristics (Table 1). At study enrollment, male partners were on average 35.6 years of age; 88% were Caucasian, 77% had never smoked, and 30% reported having

ever made a partner pregnant. Female partners were on average 35.0 years of age; 87% were Caucasian, 77% had never smoked, and 34% reported a prior pregnancy. The initial SART diagnosis was approximately equally distributed between female factor (36%), male factor (31%) and unexplained (33%).

3.2 Urinary PFR metabolites

Among urine samples collected from men, detection frequencies were high for BDCIPP (84%), DPHP (87%) and ip-PPP (76%) but low for tb-PPP (12%) and zero for BCIPP (0%) (Table 2). Concentrations of DPHP were 7% higher than BDCIPP on average and 66% higher than ip-PPP. BDCIPP was weakly but significantly correlated with DPHP (r_s=0.24) and ip-PPP (r_s=0.19), whereas DPHP and ip-PPP were moderately correlated among the male partner urine samples (r_s=0.30). ICCs among urine samples collected from men indicated weak to moderate within-person variability across cycles (ΣPFR=0.36; BDCIPP=0.56; DPHP=0.19; ip-PPP=0.31). Spearman correlations between urinary PFR concentrations in male and female partners were higher for BDCIPP (r_s=0.48), DPHP (r_s=0.33), and ΣPFR (r_s=0.33) compared to ip-PPP (r_s=0.13) (each with p<0.0001). GM urinary metabolite concentrations were 48–82% lower among men compared to women in our study population for BDCIPP (0.52, 95% CI=0.34, 0.70), DPHP (0.33, 95% CI=0.19, 0.47), and ip-PPP (0.18, 95% CI=0.04, 0.33) (Supplemental Material Table S1).

Paternal urinary BDCIPP concentrations were significantly associated with race/ethnicity, BMI, season, and year. In multivariate analyses, concentrations of paternal urinary BDCIPP were 48% (0.53, 95% CI=0.32, 0.86) lower among Caucasian men compared to other race/ethnicities and increased by an average of 5% (1.05, 95% CI=1.01, 1.09) for every unit increase in BMI. Compared to urine samples collected during the fall, paternal urinary BDCIPP was 60% (1.60, 95% CI=1.12, 2.28) higher during the summer and 34% lower in the winter (0.66, 95% CI=0.47, 0.93). Examination of overall temporal trends indicated that each year there was an average 7% (1.07, 95% CI=1.01, 1.14) increase in concentrations of paternal urinary BDCIPP, a 5% (0.95, 0.91, 1.00) decline for ip-PPP, and a non-significant 4% (0.96, 95% CI=0.92, 1.01) decline for DPHP. No other significant associations with the demographic variables and no associations of paternal urinary PFR quartiles with the reproductive/cycle characteristics presented in Table 1 were observed.

3.3 IVF Outcomes

We observed a significant 12% decline in the proportion of fertilized oocytes from the first to second quartile of male urinary ΣPFR and the number of best quality embryos declined by 47% from the first to third quartile of male urinary BDCIPP in our fully adjusted model (Figure 1, Table 3). We also observed an 8% decline in adjusted means for the proportion of fertilized oocytes from the lowest to highest quartile of male urinary BDCIPP that approached statistical significance [95% confidence interval (CI): 0.01, 0.12, p-trend=0.06]. No other associations were observed between urinary metabolites and the other early developmental outcomes. We observed no associations of paternal urinary PFR metabolite concentrations and later clinical outcomes including the proportion of cycles resulting in successful implantation, clinical pregnancy, and live birth (Table 4). Results were similar in unadjusted models as well as in the sensitivity analysis adjusting for maternal urinary

metabolite concentration, age, race/ethnic group, and BMI (Supplemental Material Tables S2–S3).

4. DISCUSSION

As far as we are aware, this is the first epidemiologic study to explore associations between PFRs and reproductive outcomes among couples. We used the model of IVF to investigate human reproduction and early pregnancy outcomes, ranging chronologically from oocyte fertilization, embryo quality, and implantation to clinical pregnancy and live birth. Paternal urinary BDCIPP was associated with reduced probability of fertilization, whereas paternal urinary PFRs (including BDCIPP) were *not* associated with the proportion of cycles resulting in successful implantation, clinical pregnancy, and live birth. The suggestive negative association of BDCIPP with embryo quality is consistent with that for fertilization as well as previously reported negative associations of semen quality with both fertilization and embryo quality (Loutradi et al. 2006). While no studies have investigated paternal exposure to PFRs and embryo quality, a non-monotonic dose response was previously observed between male zebrafish TDCIPP exposure and testosterone as well as with the estradiol/testosterone ratio (Liu et al. 2013). We previously reported strong negative associations of maternal urinary ΣPFR with these reproductive outcomes as well as maternal urinary ip-PPP with the proportion of cycles resulting in fertilization (Carignan et al. in press). Therefore our results suggest that *paternal* exposure to TDCIPP and maternal exposure to mono-ITP exposure may adversely impact fertilization whereas maternal exposure to the sum of TDCIPP, TPHP and mono-ITP may adversely impact implantation, clinical pregnancy, and live birth.

Our finding for TDCIPP and fertilization is consistent with reports of reduced spermiation among male zebrafish (Wang et al. 2015b). Such effects may be influenced by action on the hypothalamus-pituitary-gonadal axis as TDCIPP has been found to have estrogenic and anti-androgenic activity in zebrafish (Liu et al. 2012; Liu et al. 2013). Effects on spermiation may also be influenced by action of TDCIPP on the hypothalamus-pituitary-thyroid axis as both human and animal studies have reported inverse relationships with the thyroid pro-hormone, thyroxine (T4) (Farhat et al. 2013; Kim et al. 2015; Meeker and Stapleton 2010; Wang et al. 2015a; Wang et al. 2013) and thyroid hormone may contribute to normal spermatogenesis and metabolic processes in the adult testis (Wajner et al. 2009). PFRs may adversely affect female reproduction through disruption of regulatory pathways mediated by these axes as low pregnancy levels of estradiol has been associated with increased fetal loss (Schindler 2004) and sub-clinical hypothyroidism can adversely affect female fertility (Abdel Rahman et al. 2010; Bussen et al. 2000; Scoccia et al. 2012; Velkeniers et al. 2013).

Exposure levels in our study population were similar to those reported in the literature for general population exposure with GM concentrations of the PFR metabolites similar to other adult populations in China (Feng et al. 2016), Norway (Cequier et al. 2015), and the U.S. considering that differences in the timing of sample collection and population size complicate such comparisons (Butt et al. 2014; Butt et al. 2016; Carignan et al. 2013b; Castorina et al. 2017; Cooper et al. 2011; Dodson et al. 2014; Kate Hoffman et al. 2017; Hoffman et al. 2015; Preston et al. 2017; Romano et al. 2017) (Supplemental Material Table

S4). Concentrations of BDCIPP and DPHP among men in our study population were approximately 2 fold and 90% higher, respectively, than previously measured among a population of 45 men recruited from the Vincent Memorial Andrology laboratory at Massachusetts General Hospital in 2002–2007 (Meeker et al. 2013a). For urinary BDCIPP this difference may reflect differences in the period of sample collection as we observed a positive association with sampling year. This increasing trend was also observed in a previous study (Hoffman et al. 2017) and is consistent with increased use of TDCIPP in the polyurethane foam of upholstered furniture over the past decade (Stapleton et al. 2012).

We previously reported inverse associations of paternal urinary phthalate metabolites with only implantation and live birth among this study population (Dodge et al. 2015). As DPHP is used as both a flame retardant and plasticizer we examined relationships of paternal urinary metabolites of PFRs with phenols and phthalates. Spearman correlations were weak, with the strongest observed for DPHP (r_s<0.33) and similar maximums for both phenols and phthalates (Supplemental Material Table S5). This correlation may have confounded associations with DPHP and should be considered in future studies.

Strengths of our study include the prospective study design, state-of-the-art measurement of urinary PFR metabolites in samples collected preconception (during the IVF cycle), assessment of early developmental outcomes (i.e., fertilization, implantation) that are not observable in non-IVF populations, clinical outcome data obtained from electronic medical records, and consideration of potential confounders including maternal urinary concentrations. Associations may have been attenuated by exposure misclassification as the half-life of the PFRs is estimated on the order of hours and the single paternal urine provided may not represent exposure during the full spermatogenic cycle, which has a length of approximately 90 days. For the female partner, we collected up to two samples and would thus expect less exposure misclassification. Our modest sample size limited our statistical power, and our evaluation of both multiple urinary metabolites and multiple outcomes increased the likelihood of type I error. This possibility may be decreased in future studies with increased sample sizes, which would provide greater statistical power and may have the ability to more carefully evaluate nonlinearities in dose-response relationships using techniques such as smooth spline models. Given these limitations we were unable to condition on a previous stage (i.e., successful implantation), however we did limit to cycles with successful oocyte retrieval for the early developmental outcomes. While our assessment of maternal preconception exposure was improved by use of repeated urine samples, male partners contributed only one urine sample per cycle. Our findings are generalizable to infertile couples, which has a prevalence of 15% (Thoma et al. 2013), and may be more broadly generalizable assuming that couples undergoing IVF have similar biological responses to PFR exposure as those not undergoing IVF.

In conclusion, using IVF as a model to investigate human reproduction and pregnancy outcomes we found that paternal urinary BDCIPP was associated with reduced fertilization. In contrast to previously reported findings for the female partners, the paternal urinary PFRs metabolites were not associated with the proportion of cycles resulting in implantation, clinical pregnancy, and live birth. These results indicate that paternal preconception exposure to TDCIPP may adversely impact successful oocyte fertilization, whereas female

preconception exposure to ΣPFRs may be more relevant to adverse pregnancy outcomes. This finding is important as use ofthese flame retardants has increased over the past decade.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge Spencer Pecha for sample extraction and all members of the EARTH study team, specifically the Harvard T.H. Chan School of Public Health research nurses Jennifer B. Ford and Myra G. Keller, research staff Ramace Dadd and Patricia Morey, physicians and staff at Massachusetts General Hospital fertility center and a special thanks to all the study participants.

Funding: This work was supported by the National Institutes of Environmental Health Sciences [ES009718, ES022955, ES000002, and T32ES007069].

Abbreviations

ART	assisted reproductive technologies
BCIPP	bis(1-chloro-2-propyl) phosphate
BDCIPP	bis(1,3-dichloro-2-propyl) phosphate
BMI	body mass index
CI	confidence interval
DPHP	diphenyl phosphate
EARTH	Environment and Reproductive Health Study
GM	geometric mean
GnRH	gonadotropin releasing hormone
ICC	intraclass correlation coefficients
ip-PPP	isopropylphenyl phenyl phosphate
IQR	interquartile range
IVF	in vitro fertilization
LC-MS/MS	liquid chromatography tandem mass spectrometry
MDL	method detection limits
MGH	Massachusetts General Hospital
Mono-ITP	mono-substituted isopropyl triphenyl phosphate
PFR	Organophosphate flame retardant
SG	specific gravity

SRM	standard reference material
tb-PPP	tert-butylphenyl phenyl phosphate
TDCIPP	tris(1,3-dichloro-2-propyl) phosphate
TPHP	triphenyl phosphate

References

Birnbaum LS. Environmental chemicals: evaluating low-dose effects. Environ Health Perspect. 2012; 120(4):A143–144. [PubMed: 22470049]

Butt CM, Congleton J, Hoffman K, Fang M, Stapleton HM. Metabolites of organophosphate flame retardants and 2-ethylhexyl tetrabromobenzoate in urine from paired mothers and toddlers. Environ Sci Technol. 2014; 48:10432–10438. [PubMed: 25090580]

Butt CM, Hoffman K, Chen A, Lorenzo A, Congleton J, Stapleton HM. Regional comparison of organophosphate flame retardant (PFR) urinary metabolites and tetrabromobenzoic acid (TBBA) in mother-toddler pairs from California and New Jersey. Environ Int. 2016; 94:627–634. [PubMed: 27397928]

Carignan CC, McClean MD, Cooper E, Watkins D, Fraser AJ, Heiger-Bernays W, et al. Predictors of tris(1,3-dichloro-2-propyl) phosphate metabolite in the urine of office workers. Environ Int. 2013a; 55:56–61. [PubMed: 23523854]

Carignan CC, McClean MD, Cooper EM, Watkins DJ, Fraser AJ, Heiger-Bernays W, et al. Predictors of tris(1,3-dichloro-2-propyl) phosphate metabolite in the urine of office workers. Environ Int. 2013b; 55:56–61. [PubMed: 23523854]

Carignan CC, Mínguez-Alarcón L, Butt CM, Williams PL, Meeker J, Stapleton HM, et al. Flame retardant exposure and treatment outcomes among women undergoing in vitro fertilization. Environ Health Persp. in press.

Castorina R, Butt C, Stapleton HM, Avery D, Harley KG, Holland N, et al. Flame retardants and their metabolites in the homes and urine of pregnant women residing in California (the CHAMACOS cohort). Chemosphere. 2017; 179:159–166. [PubMed: 28365501]

Cequier E, Sakhi AK, Marce RM, Becher G, Thomsen C. Human exposure pathways to organophosphate triesters - a biomonitoring study of mother-child pairs. Environ Int. 2015; 75:159–165. [PubMed: 25461425]

Cooper EM, Covaci A, van Nuijs ALN, Webster TF, Stapleton HM. Analysis of the flame retardant metabolites bis(1,3-dichloro-2-propyl) phosphate (BDCPP) and diphenyl phosphate (DPP) in urine using liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2011; 401:2123–2132. [PubMed: 21830137]

Dodge LE, Williams PL, Williams MA, Missmer SA, Souter I, Calafat AM, et al. Associations between paternal urinary phthalate metabolite concentrations and reproductive outcomes among couples seeking fertility treatment. Reproductive toxicology (Elmsford, NY). 2015; 58:184–193.

Dodson RE, Van den Eede N, Covaci A, Perovich LJ, Brody JG, Rudel RA. Urinary biomonitoring of phosphate flame retardants: levels in California adults and recommendations for future studies. Environ Sci Technol. 2014; 48:13625–13633. [PubMed: 25388620]

Farhat A, Crump D, Chiu S, Williams KL, Letcher RJ, Gauthier LT, et al. In Ovo effects of two organophosphate flame retardants--TCPP and TDCPP--on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos. Toxicol Sci. 2013; 134:92–102. [PubMed: 23629516]

Feng L, Ouyang F, Liu L, Wang X, Wang X, Li YJ, et al. Levels of Urinary Metabolites of Organophosphate Flame Retardants, TDCIPP, and TPHP, in Pregnant Women in Shanghai. Journal of environmental and public health. 2016; 2016:9416054. [PubMed: 28115951]

Hammel SC, Hoffman K, Webster TF, Anderson KA, Stapleton HM. Measuring personal exposure to organophosphate flame retardants using silicone wristbands and hand wipes. Environ Sci Technol. 2016; 50:4483–4491. [PubMed: 26975559]

Hoffman K, Daniels JL, Stapleton HM. Urinary metabolites of organophosphate flame retardants and their variability in pregnant women. Environ Int. 2014; 63:169–172. [PubMed: 24316320]

Hoffman K, Garantziotis S, Birnbaum LS, Stapleton HM. Monitoring indoor exposure to organophosphate flame retardants: hand wipes and house dust. Environ Health Perspect. 2015; 123:160–165. [PubMed: 25343780]

Hoffman K, Butt CM, Webster TF, Preston EV, Hammel SC, Makey C, et al. Temporal Trends in Exposure to Organophosphate Flame Retardants in the United States. Environmental science & technology letters. 2017

Hoffman K, Butt CM, Webster TF, Preston EV, Hammel SC, Makey CM, et al. Temporal trends in exposure to organophosphate flame retardants in the United States. Environ Sci Technol Letters. 2017 in press.

Kim S, Jung J, Lee I, Jung D, Youn H, Choi K. Thyroid disruption by triphenyl phosphate, an organophosphate flame retardant, in zebrafish (Danio rerio) embryos/larvae, and in GH3 and FRTL-5 cell lines. Aquatic toxicology (Amsterdam, Netherlands). 2015; 160:188–196.

Loutradi KE, Tarlatzis BC, Goulis DG, Zepiridis L, Pagou T, Chatziioannou E, Grimbizis GF, Papadimas I, Bontis I. The effects of sperm quality on embryo development after intracytoplasmic sperm injection. J Assist Reprod Gen. 2006; 23(2):69–74.

Liu X, Ji K, Choi K. Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. Aquatic toxicology (Amsterdam, Netherlands). 2012; 114–115:173–181.

Liu X, Ji K, Jo A, Moon HB, Choi K. Effects of TDCPP or TPP on gene transcriptions and hormones of HPG axis, and their consequences on reproduction in adult zebrafish (Danio rerio). Aquatic toxicology (Amsterdam, Netherlands). 2013; 134–135:104–111.

Meeker JD, Stapleton HM. House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environ Health Persp. 2010; 118:318–323.

Meeker JD, Cooper EM, Stapleton HM, Hauser R. Urinary metabolites of organophosphate flame retardants: temporal variability and correlations with house dust concentrations. Environ Health Perspect. 2013a; 121:580–585. [PubMed: 23461877]

Meeker JD, Cooper EM, Stapleton HM, Hauser R. Exploratory analysis of urinary metabolites of phosphorus-containing flame retardants in relation to markers of male reproductive health. Endocrine disruptors (Austin, Tex). 2013b; 1:e26306.

Mok-Lin E, Ehrlich S, Williams PL, Petrozza J, Wright DL, Calafat AM, et al. Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. Int J Androl. 2010; 33:385–393. [PubMed: 20002217]

Pearson MA, Lu CS, Schmotzer BJ, Waller LA, Riederer AM. Evaluation of physiological measures for correcting variation in urinary output: Implications for assessing environmental chemical exposure in children. Journal of Exposure Science and Environmental Epidemiology. 2009; 19:336–342. [PubMed: 18841168]

Preston EV, McClean MD, Claus Henn B, Stapleton HM, Braverman LE, Pearce EN, et al. Associations between urinary diphenyl phosphate and thyroid function. Environ Int. 2017; 101:158–164. [PubMed: 28162782]

Romano ME, Hawley NL, Eliot M, Calafat AM, Jayatilaka NK, Kelsey K, et al. Variability and predictors of urinary concentrations of organophosphate flame retardant metabolites among pregnant women in Rhode Island. Environmental health : a global access science source. 2017; 16:40. [PubMed: 28399857]

SART (Society for Assisted Reproductive Technologies). [Accessed May 27, 2016] Clinical Summary Report: All SART Member Clinics. 2013. Available: https://www.sartcorsonline.com/rptCSR_PublicMultYear.aspx?ClinicPKID=0

Schang G, Robaire B, Hales BF. Organophosphate Flame Retardants Act as Endocrine-Disrupting Chemicals in MA-10 Mouse Tumor Leydig Cells. Toxicol Sci. 2016; 150:499–509. [PubMed: 26794138]

Stapleton HM, Klosterhaus S, Eagle S, Fuh J, Meeker JD, Blum A, et al. Detection of organophosphate flame retardants in furniture foam and U.S. house dust. Environ Sci Technol. 2009; 43:7490–7495. [PubMed: 19848166]

Stapleton HM, Sharma S, Getzinger G, Ferguson PL, Gabriel M, Webster TF, et al. Novel and High Volume Use Flame Retardants in US Couches Reflective of the 2005 PentaBDE Phase Out. Environ Sci Technol. 2012; 46:13432–13439. [PubMed: 23186002]

Thoma ME, McLain AC, Louis JF, King RB, Trumble AC, Sundaram R, et al. Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. Fertility and sterility. 2013; 99:1324–1331. e1321. [PubMed: 23290741]

Van den Eede N, Heffernan AL, Aylward LL, Hobson P, Neels H, Mueller JF, et al. Age as a determinant of phosphate flame retardant exposure of the Australian population and identification of novel urinary PFR metabolites. Environ Int. 2015; 74:1–8. [PubMed: 25277340]

van der Veen I, de Boer J. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere. 2012; 88:1119–1153. [PubMed: 22537891]

Veeck, LL., Zaninovic, N. An atlas of human blastocycsts. Boca Raton, FL: Panthenon Publishing Group; 2003.

Wajner SM, Wagner MS, Maia AL. Clinical implications of altered thyroid status in male testicular function. Arq Bras Endocrinol Metab. 2009; 53:976–982.

Wang Q, Liang K, Liu J, Yang L, Guo Y, Liu C, et al. Exposure of zebrafish embryos/larvae to TDCPP alters concentrations of thyroid hormones and transcriptions of genes involved in the hypothalamic-pituitary-thyroid axis. Aquatic toxicology (Amsterdam, Netherlands). 2013; 126:207–213.

Wang Q, Lai NL, Wang X, Guo Y, Lam PK, Lam JC, et al. Bioconcentration and transfer of the organophorous flame retardant 1,3-dichloro-2-propyl phosphate causes thyroid endocrine disruption and developmental neurotoxicity in zebrafish larvae. Environ Sci Technol. 2015a; 49:5123–5132. [PubMed: 25826601]

Wang Q, Lam JC, Han J, Wang X, Guo Y, Lam PK, et al. Developmental exposure to the organophosphorus flame retardant tris(1,3-dichloro-2-propyl) phosphate: estrogenic activity, endocrine disruption and reproductive effects on zebrafish. Aquatic toxicology (Amsterdam, Netherlands). 2015b; 160:163–171.

Highlights

- Preconception cohort of couples undergoing IVF as a model of human reproduction
- Measured concentrations of urinary organophosphate flame retardant metabolites
- No associations were observed for implantation, clinical pregnancy, or live birth
- Fertilization declined with increasing paternal exposure to TDCIPP

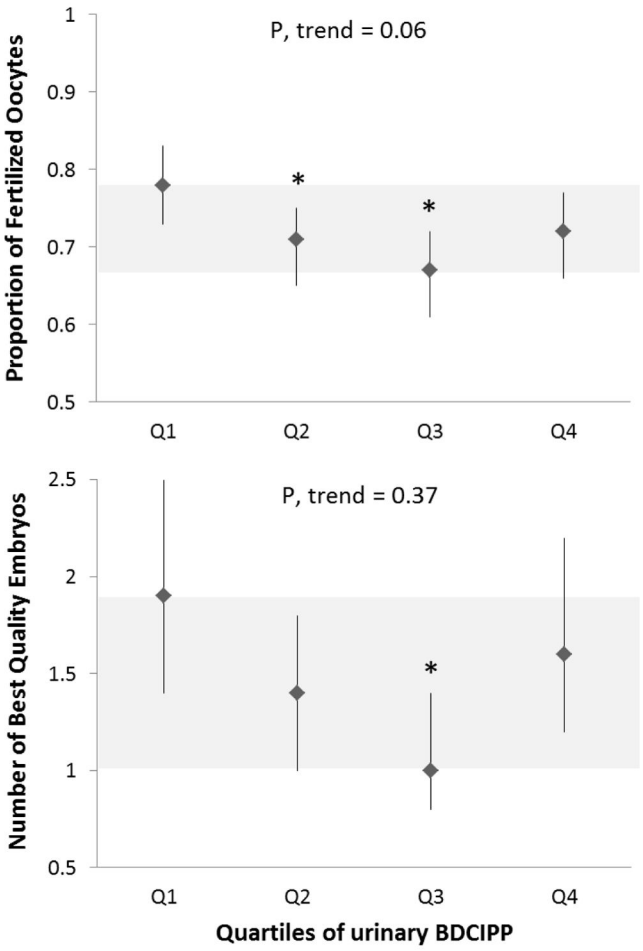


Figure 1.

Adjusted mean (95% CI) proportion of fertilized oocytes and number of best quality embryos by quartile of urinary BDCIPP concentrations among 195 men with partners undergoing 263 IVF cycles that had oocyte retrieval. Gray shading indicates change in means from the first to third quartile. Adjusted models control for maternal urinary PFR metabolite (continuous), year of IVF treatment cycle (continuous) and primary SART infertility diagnosis at study entry (female, male, unknown) as well as paternal and maternal age (continuous), body mass index (continuous) and race/ethnicity (black/Asian/other, white/Caucasian). Adjusted means are presented for the mean year of IVF treatment cycle (2010), primary SART infertility diagnosis at study entry (female=1, male=0, unexplained=0) as well as mean paternal age (36.9), body mass index (27.1), and race/ethnicity (white); and maternal age (35.1), body mass index (23.8), and race/ethnicity (white). *Significantly different from the lowest quartile (Q1) at the alpha=0.05 level.

Table 1

Demographic and reproductive characteristics of 201 couples (276 IVF cycles) in the EARTH Study, Median (IQR) or N (%)

Demographic characteristics	Male Partner	Female Partner
Age, years	35.6 (32.9, 39.2)	35.0 (32.0, 37.0)
Race/Ethnic group, n (%)		
Black/Asian/Other	24 (12)	27 (13)
White/Caucasian	177 (88)	174 (87)
Body Mass Index, kg/m^2 [a]	26.8 (24.1, 28.9)	22.8 (20.8, 25.8)
Ever smoker, n (%)	46 (23)	46 (23)
Education, n (%)[a]		
High school/some college	28 (17)	12 (7)
College graduate	54 (32)	63 (35)
Graduate degree	84 (51)	107 (59)
Reproductive/Cycle characteristics	**Couple**	
History of ever been pregnant	68 (34)	
Day 3 FSH Levels, IU/L	6.7 (5.9, 8.0)	
Initial infertility diagnosis, n (%)		
Female factor	73 (36)	
Male factor	61 (31)	
Unexplained	67 (33)	
Previous IUI, n (%)[a]	82 (41)	
Previous IVF, n (%)[a]	39 (19)	
Treatment protocol, n (%)		
Antagonist	36 (13)	
Flare[b]	48 (17)	
Luteal phase agonist[c]	192 (70)	
E2 Trigger Levels, pmol/L	1978 (1525, 2893)	
ICSI cycles, n (%)	155 (56)	
Cycle cancelled prior to transfer, n (%)	13 (5)	
Embryo Transfer Day, n (%)		
No embryos transferred	13 (5)	
Day 2	15 (5)	
Day 3	152 (55)	
Day 5	96 (35)	
Number of Embryos Transferred, n (%)		
0 embryos	13 (5)	
1 embryo	53 (19)	
2 embryos	153 (55)	
3+ embryos	57 (21)	

IQR: interquartile range, FSH: follicle stimulating hormone, IUI: intrauterine insemination, IVF: in vitro fertilization, E2: estradiol.

[a] Has missing data (1 male missing body mass index, 19 female missing education, 35 male missing education)

[b] Follicular phase GnRH-agonist/Flare protocol

[c] Luteal phase GnRH-agonist protocol

Table 2

Distribution of urinary organophosphate flame retardant metabolites (ug/L) measured among 276 urine samples collected from 201 men with partners undergoing 276 IVF cycles in the EARTH Study.

	N > MDL (%)	GM (95% CI)	Min	10th Pctl	25th Pctl	50th Pctl	75th Pctl	90th Pctl	95th Pctl	Max
Specific Gravity Adjusted[a]										
BCIPP	0 (0.0)	NA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
BDCIPP	233 (84)	0.42 (0.36, 0.48)	<MDL	<MDL	0.19	0.46	0.88	1.97	3.11	12.39
DPHP	241 (87)	0.60 (0.54, 0.66)	<MDL	<MDL	0.35	0.57	0.96	2.10	3.18	8.54
ip-PPP	186 (76)	0.20 (0.18, 0.22)	<MDL	<MDL	<MDL	0.21	0.37	0.65	0.90	3.60
tb-PPP	34 (12)	NA	<MDL	<MDL	<MDL	<MDL	<MDL	0.15	0.26	1.40
Wet weight (Unadjusted)										
BCIPP	0 (0.0)	NA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
BDCIPP	233 (84)	0.43 (0.37, 0.50)	<MDL	<MDL	0.19	0.49	1.11	2.36	3.42	10.30
DPHP	241 (87)	0.61 (0.54, 0.70)	<MDL	<MDL	0.30	0.69	1.30	2.47	4.21	10.57
ip-PPP	186 (76)	0.20 (0.18, 0.23)	<MDL	<MDL	<MDL	0.20	0.48	0.87	1.18	4.56
tb-PPP	34 (12)	NA	<MDL	<MDL	<MDL	<MDL	<MDL	0.11	0.18	2.24

MDL: method detection limit, GM: geometric mean, BCIPP: bis(1-chloro-2-propyl) phosphate, BDCIPP: bis(1,3-dichloro-2-propyl) phosphate, DPHP: diphenyl phosphate, ip-PPP: isopropylphenyl phenyl phosphate, tb-PPP: tert-butylphenyl phenyl phosphate.

[a]Adjusted to specific gravity, range (1.002–1.038)

Abbreviations: < MDL, method detection limit; Max, maximum; Pctl: percentile; Min, minimum. All values below MDL were assigned a value equal to the MDL divided by √2

Table 3

Adjusted mean (95% CI) proportion of fertilized oocytes and number of best quality embryos by quartile of urinary organophosphate flame retardant metabolite concentrations among 195 men with partners undergoing 263 IVF cycles that had oocyte retrieval.

	ΣPFR	BDCIPP	DPHP	ip-PPP
Fertilization, proportion				
Q1	0.75 (0.70, 0.79)	0.78 (0.73, 0.83)	0.73 (0.68, 0.78)	0.75 (0.71, 0.80)
Q2	0.67 (0.62, 0.72)*	0.71 (0.65, 0.75)*	0.69 (0.63, 0.73)	0.70 (0.65, 0.75)
Q3	0.71 (0.66, 0.76)	0.67 (0.61, 0.72)*	0.73 (0.68, 0.78)	0.70 (0.65, 0.75)
Q4	0.75 (0.70, 0.79)	0.72 (0.66, 0.77)	0.72 (0.67, 0.77)	0.73 (0.68, 0.77)
p-trend	0.69	0.06	0.85	0.41
Best quality embryos, count				
Q1	1.6 (1.2, 2.1)	1.9 (1.4, 2.5)	1.5 (1.1, 1.9)	1.6 (1.2, 2.1)
Q2	1.1 (0.8, 1.5)	1.4 (1.0, 1.8)	1.1 (0.8, 1.5)	1.3 (1.0, 1.8)
Q3	1.4 (1.0, 1.8)	1.0 (0.8, 1.4)*	1.5 (1.2, 2.0)	1.3 (1.0, 1.8)
Q4	1.9 (1.4, 2.4)	1.6 (1.2, 2.2)	1.8 (1.3, 2.3)	1.6 (1.2, 2.1)
p-trend	0.25	0.37	0.15	0.91

Models control for maternal urinary PFR metabolite (continuous), year of IVF treatment cycle (continuous) and primary SART infertility diagnosis at study entry (female, male, unknown) as well as paternal and maternal age (continuous), body mass index (continuous) and race/ethnicity (black/Asian/other, white/Caucasian). Adjusted means are presented for the mean year of IVF treatment cycle (2010), primary SART infertility diagnosis at study entry (female) as well as mean paternal age (36.9), body mass index (27.1), and race/ethnicity (white); and maternal age (35.1), body mass index (23.9), and race/ethnicity (white).

p-trend calculated using median of ln-transformed urinary metabolite concentrations.

*Significantly different from Q1 at the p=0.05 level

Table 4

Adjusted mean (95% CI) proportion of cycles resulting in implantation, live birth and clinical pregnancy by quartile of urinary organophosphate flame retardant metabolite concentrations among 201 men with partners undergoing 276 IVF cycles.

	ΣPFR	BDCIPP	DPHP	ip-PPP
Implantation				
Q1	0.63 (0.49, 0.75)	0.55 (0.40, 0.69)	0.65 (0.51, 0.77)	0.59 (0.45, 0.72)
Q2	0.54 (0.40, 0.67)	0.63 (0.49, 0.75)	0.54 (0.40, 0.67)	0.50 (0.37, 0.64)
Q3	0.62 (0.48, 0.74)	0.58 (0.44, 0.70)	0.65 (0.51, 0.76)	0.71 (0.58, 0.82)
Q4	0.65 (0.51, 0.76)	0.68 (0.53, 0.80)	0.60 (0.46, 0.73)	0.63 (0.49, 0.75)
p-trend	0.65	0.29	0.86	0.32
Clinical				
Pregnancy				
Q1	0.55 (0.41, 0.68)	0.47 (0.33, 0.61)	0.54 (0.41, 0.67)	0.52 (0.39, 0.65)
Q2	0.46 (0.33, 0.59)	0.52 (0.38, 0.64)	0.46 (0.34, 0.59)	0.44 (0.32, 0.57)
Q3	0.49 (0.35, 0.62)	0.53 (0.39, 0.66)	0.53 (0.40, 0.66)	0.57 (0.44, 0.69)
Q4	0.57 (0.44, 0.70)	0.56 (0.42, 0.69)	0.54 (0.40, 0.67)	0.54 (0.40, 0.66)
p-trend	0.68	0.40	0.90	0.62
Live birth				
Q1	0.47 (0.33, 0.61)	0.41 (0.28, 0.56)	0.38 (0.25, 0.52)	0.45 (0.32, 0.59)
Q2	0.33 (0.22, 0.46)	0.41 (0.29, 0.55)	0.35 (0.24, 0.49)	0.26 (0.16, 0.39) *
Q3	0.34 (0.23, 0.48)	0.35 (0.24, 0.49)	0.40 (0.28, 0.54)	0.45 (0.32, 0.59)
Q4	0.45 (0.32, 0.58)	0.41 (0.27, 0.56)	0.44 (0.31, 0.58)	0.41 (0.28, 0.55)
p-trend	0.98	0.86	0.43	0.94

Models control for maternal urinary PFR metabolite (continuous), year of IVF treatment cycle (continuous) and primary SART infertility diagnosis at study entry (female, male, unknown) as well as paternal and maternal age (continuous), body mass index (continuous) and race/ethnicity (black/Asian/other, white/Caucasian). Adjusted means are presented for the mean year of IVF treatment cycle (2010), primary SART infertility diagnosis at study entry (female) as well as mean paternal age (36.9), body mass index (27.1), and race/ethnicity (white); and maternal age (35.1), body mass index (23.8), and race/ethnicity (white).

p-trend calculated using median of ln-transformed urinary metabolite concentrations.

*Significantly different from Q1 at the p=0.05 level