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Paternal and maternal preconception urinary phthalate metabolite concentrations and child behavior

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Abstract

Background—Prenatal phthalate exposure has been associated with behavioral problems and lower performance on measures of cognitive ability in children. However, the potential effect of phthalate exposure during the sensitive preconception period is unknown.

Objectives—To estimate the association of maternal and paternal preconception urinary phthalate metabolite concentrations with child behavior and evaluate potential modification by child sex.

Methods—We used data from 166 children (111 singletons, 26 pairs of twins, and 1 set of triplets) born to 134 mothers and 100 fathers participating in a prospective preconception cohort study of subfertile couples from the Massachusetts General Hospital Fertility Center. We estimated mean maternal and paternal preconception exposures by averaging individual phthalate metabolite

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Competing interests

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

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.07.032.

concentrations in multiple urine samples collected before pregnancy. We assessed children's behavior at 2–9 years of age by parent report using the Behavior Assessment System for Children-2 (BASC-2). We estimated the covariate-adjusted association between individual phthalate metabolite concentrations and the sum of di(2-ethylhexyl) phthalate metabolites (ΣDEHP) and behavior scores, and evaluated differences in associations by child sex using linear regression with Generalized Estimating Equations. Models were further adjusted for prenatal phthalate concentrations in sensitivity analyses.

Results—Each $\log_e$-unit increase in maternal and paternal preconception concentrations of ΣDEHP was associated with a 2.0 (95% CI: − 3.2, − 0.7) and 1.8 (95% CI: − 3.1, − 0.4) point decrease in BASC-2 internalizing behavior scores among all children, respectively. We observed sex-specific associations for some phthalate biomarkers: among boys, maternal monoisobutyl phthalate (MiBP) was positively associated with externalizing behaviors, and paternal MiBP and mono-n-butyl phthalate were positively associated with internalizing behaviors.

Conclusions—In this cohort, paternal and maternal preconception concentrations of some phthalate biomarkers were associated with specific aspects of child behavior, even after adjustment for prenatal concentrations. While additional research is warranted to confirm these results, our findings suggest that the preconception period of exposure may be a critical window for offspring neurodevelopment.

1. Introduction

Neurodevelopmental disorders affect approximately 15% of children between the ages of 3 and 17 years in the United States (Boyle et al., 2011). Children with atypical neurodevelopment can experience difficulties in a wide range of areas including speech and language, fine and gross motor functioning, memory, learning, and behavior. Over the past 30 years, there has been a growing body of literature suggesting that environmental chemical exposures, including lead (Lanphear et al., 2005) and organic pollutants (Braun, 2016) during pregnancy, infancy, or childhood may increase the risk of these disorders.

Phthalate diesters are a family of high production volume chemicals that are used to soften polyvinyl chloride plastics and can be found in flooring, electronics, medical equipment, pharmaceuticals, clothing, food packing, and toys. Some phthalates are also used as solubilizing agents in cosmetics and personal care products. While phthalates have a short biological half-life and are non-persistent (Wittassek and Angerer, 2008), frequent and repeated exposure from numerous sources has resulted in the detection of urinary phthalate metabolites in more than 95% of the U.S. population (Hauser and Calafat, 2005; CDC, 2015; Zota et al., 2014).

Several phthalates, including di(2-ethylhexyl) phthalate (DEHP), have been shown to exhibit anti-androgenic effects in rodents (Borch et al., 2006), and some aspects of brain development are dependent on the action of gonadal hormones, particularly androgens (Fedotova et al., 2017). Experimental animal studies have shown that prenatal DEHP and di-n-butyl phthalate can lead to impaired spatial learning and memory, and decreased grooming behavior (Hoshi and Ohtsuka, 2009; Tanaka, 2002; Li et al., 2009). Epidemiological studies report that urinary concentrations of low molecular weight phthalate metabolites, such as

mono-n-butyl phthalate (MnBP) and monoisobutyl phthalate (MiBP), measured during the 2nd and 3rd trimesters of pregnancy were associated with neonatal behavior and reflexes (Yolton et al., 2011; Engel et al., 2009), aggression, rule breaking, and conduct problems (Engel et al., 2010; Whyatt et al., 2012; Kobrosly et al., 2014; Lien et al., 2015), autistic traits (Miodovnik et al., 2011), lower mental and psychomotor development (Whyatt et al., 2012; Balogh et al., 2011), emotional problems (Whyatt et al., 2012) and reduced IQ (Factor-Litvak et al., 2014); whereas others have not (Braun et al., 2014; Huang et al., 2015; Gascon et al., 2015).

While there has been legitimate emphasis on studying the neurotoxicity of phthalate exposures during gestation and childhood, new and emerging research suggests that the preconception period may be highly sensitive to environmental perturbations and paternal exposures may be an underappreciated determinant of offspring health, including neurodevelopment (Braun et al., 2017). As most studies on child neurodevelopment have focused on prenatal phthalate exposures in the latter two-thirds of pregnancy, we know far less about the impact of exposure during the potentially sensitive preconception period on child behavior. In this study we examined the association between maternal and paternal preconception urinary phthalate metabolite concentrations and child behavior in a prospective cohort from Boston, MA.

2. Methods

2.1. Study cohort

The Environment and Reproductive Health (EARTH) Study is a prospective preconception cohort of subfertile couples from the fertility center at the Massachusetts General Hospital (MGH). The study was designed to evaluate the effects of diet and environmental exposures on fertility and pregnancy outcomes. Details of the cohort have been described previously (Ehrlich et al., 2012). The EARTH study has been ongoing since 2004 and has recruited approximately 800 women and 500 men to date. Women 18 – 46 years and men 18 – 55 years were eligible to participate and could enroll independently or as a couple. Participants were followed from study entry throughout their fertility care, pregnancy, and birth, or until they discontinue treatment or withdraw from the study. At enrollment, participants completed a nurse-administered sociodemographic, lifestyle, and medical history questionnaire. They also completed a comprehensive questionnaire on family, medical, reproductive and occupational history, stress, product use, tobacco and drug use, and physical activity. Urine and blood samples were collected at enrollment into the study, and subsequently multiple urine and blood samples were collected during follow-up when couples underwent medically assisted reproduction, including in-vitro fertilization (IVF) or intrauterine insemination (IUI) treatments, as well as throughout pregnancy.

EARTH study participants with singletons, twins, or triplets born between 2005 and 2015 who were 2.5 years or older in 2014 were invited to participate in a neurobehavioral child follow-up study. Approximately 69% (138/201) of participants who agreed to partake in the study completed and returned questionnaires. Among the 138 parents agreeing, 134 mothers and 4 fathers participated and completed neurodevelopmental questionnaires on 166 children (4 fathers agreed to partake, but their female partners were not EARTH study participants).

Preconception urine samples and chemical analysis data were available on all 134 mothers and 96 male partners (fathers) who participated in the EARTH Study before their child was conceived, as well as paternal preconception urine samples on all 4 fathers who participated independently (see Participant Flow Chart, Supplemental Appendix Figure 1A). Trained study staff described the study protocol to participants in detail and answered questions. All participating mothers or fathers provided written informed consent. 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).

2.2. Phthalate exposure assessment

Both men and women provided a single spot urine sample at study entry. Women provided up to two additional preconception urine samples per fertility treatment cycle: the first specimen was obtained on days 3–9 of the follicular phase of the cycle, and the second at the time of oocyte retrieval or intrauterine insemination procedures. During pregnancy, women also provided one spot urine sample per trimester (at median 6, 21 and 35 weeks gestation). Men provided one additional preconception spot urine sample per treatment cycle at the time when their female partner underwent oocyte retrieval or intrauterine insemination.

Urine was collected in a polypropylene specimen cup and 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 online solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry (Silva et al., 2007). The urinary concentrations of the following nine phthalate metabolites were determined: mono(2-ethylhexyl) phthalate (MEHP); mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP); mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP); MnBP; MiBP; monobenzyl phthalate (MBzP); monoethyl phthalate (MEP); and mono(3-carboxypropyl) phthalate (MCPP). The limits of detection (LOD) were in the low parts-*per*-billion range (0.1–1.2 ng/ml). Concentrations below the LOD were assigned the LOD divided by the square root of two (Hornung and Reed, 1990). We calculated the molar sum of four DEHP metabolites by dividing each metabolite concentration by its molecular weight and then summing: ΣDEHP = [(MEHP*(1/278.34)) + (MEHHP*(1/294.34)) + (MEOHP*(1/292.33)) + (MECPP*(1/308.33))]. We then multiplied the molar sum by the molecular weight of MECPP (308.33) to convert the expression of ΣDEHP to ng/ml.

2.3. Child behavior assessment

We assessed child behavior using the second edition of the Behavior Assessment System for Children (BASC-2) (Reynolds and Kamphaus, 2002). The BASC-2 is a valid and reliable instrument that assesses overall behavioral and emotional functioning as well as specific problem and adaptive behaviors in children 2–21 years of age. We mailed questionnaires including the 134-item Parent Rating Scale for Preschool (BASC-2 PRS-P) and the 160-item Parent Rating Scale for Children (BASC-2 PRS-C) to participating parents to assess behavior in children 2–5 years and 6–11 years, respectively. These instruments rate child behavior using Likert-style responses and individual items are summed into 4 composite

scales: Behavioral Symptoms Index (BSI), Internalizing Problems (INZ), Externalizing Problems (EXT), and Adaptive Skills. While the BASC-2 generates additional clinical subscales (e.g., anxiety), we chose to focus on the BSI, externalizing, and internalizing problem scales since they provide a broader survey of children's problem behaviors related to specific clinical domains. BASC-2 scores are expressed as age- and sex-standardized T-Scores, with a mean and standard deviation of 50 and 10, respectively. For these maladaptive behavior scales, higher scores represent more problematic behavior.

2.4. Covariates

Pertinent sociodemographic features of study participants (men and women) including age, race, education and smoking were obtained from the baseline enrollment questionnaires. A study nurse measured their height and weight at study entry. Body Mass Index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Smoking status was self-reported at baseline and categorized as never smoked vs. ever smoked, defined as a current or former smoker. Clinical information about infertility treatment was abstracted from electronic medical records by trained study staff per cycle. Mode of conception for the index pregnancy was categorized as either IVF-based (fresh or frozen IVF treatment protocols including intracytoplasmic sperm injection) or non-IVF based (IUI with or without ovulation induction/stimulation, ovulation induction with timed intercourse, or not medically assisted/naturally conceived). The treating infertility physician diagnosed the underlying cause of infertility using the Society for Assisted Reproductive Technology definitions. Relevant information on the child including date of birth, child sex, and twin or triplet birth status was abstracted from the delivery records.

2.5. Statistical analysis

Urinary phthalate metabolite concentrations were adjusted for urine dilution by multiplying the metabolite concentration by $[(SG_p-1)/(SG_i-1)]$, where SG_i is the specific gravity of the participant's sample and SG_p is the mean specific gravity for all male or all female participants included in the study samples (Pearson et al., 2009). The specific gravity adjusted phthalate metabolite concentrations were $\log_e$-transformed to reduce the influence of extreme observations. We estimated mean maternal and paternal preconception phthalate exposure by calculating the geometric mean of each participant's $\log_e$-transformed phthalate metabolite concentrations obtained from study entry and at each treatment cycle up until and including the index cycle of conception of the child under study. We also estimated mean maternal prenatal phthalate exposure by averaging all three trimester-specific urinary concentrations of the phthalate biomarkers during the same index pregnancy. The $\log_e$-mean value was the summary estimate of exposure used in the analysis. When only one urine sample was available (4%, 9%, and 1% of all maternal, paternal preconception and prenatal urine samples, respectively) the phthalate concentration for that single sample was used for the corresponding window of exposure. We calculated descriptive statistics for phthalate metabolite concentrations for the exposure windows as well as the proportion below the LOD. We also calculated Pearson's correlation coefficients for $\log_e$ concentrations between couples and exposure windows.

We examined the sociodemographic and clinical characteristics in mothers and fathers, and birth characteristics of children by sex, reported as means (±SD) or number (%). We estimated associations of maternal and paternal $\log_e$-phthalate metabolite concentrations and Behavioral Symptoms Index, Internalizing, and Externalizing T-scores using linear regression with Generalized Estimating Equation (GEE) models to account for correlation within mothers/fathers with more than one child in the follow-up study (28/166 children were siblings). We fit a separate model for each individual phthalate metabolite and for the summary measure ΣDEHP. Beta coefficients and 95% confidence intervals (CI) represent the difference in T-Scores for every $\log_e$-unit increase in phthalate metabolite concentration. We evaluated potential effect measure modification by sex of child by including a cross-product term for sex and the metabolite of interest for each of the separate models ($\log_e$[phthalate]*sex). We considered a p-value for the interaction term < 0.20 as possible effect-modification by child sex on the multiplicative scale.

Covariates were selected *a priori* as potential confounders based on substantive knowledge using a directed acyclic graph (DAG) to identify covariates associated with both exposure and outcome that were not intermediates on the causal path (see Supplemental Appendix Figure 2A) (Textor et al., 2011). We applied two sets of main models: one for the maternal preconception window of exposure and the other for the paternal preconception window of exposure. Maternal preconception base models included: maternal age and BMI (continuous), maternal education (< college, college, graduate degree), smoking status (never vs. ever), and IVF treatment vs. non-IVF treatment. Paternal preconception base models included paternal and maternal age and BMI (continuous), paternal and maternal smoking (never vs. ever), maternal education (< college, college, graduate degree), and IVF treatment vs. non-IVF treatment. We performed all statistical analyses using SAS version 9.4 (SAS Institute Inc., Cary, USA).

2.6. Sensitivity analysis

We wanted to further evaluate whether maternal prenatal phthalate metabolite concentrations influenced child behavior and adjusted for $\log_e$ mean prenatal phthalate metabolite concentrations to estimate associations of preconception phthalate exposure with child behavior independent of prenatal exposure. We also accounted for potential confounding by partner's exposure and additionally adjusted for partners' preconception phthalate metabolite concentrations in sensitivity analyses. Furthermore, given that our study sample also included twins and triplets, we restricted our analysis to only singletons (excluding all 55 multiples) to rule out the possibility that our results may have been influenced by the higher proportion of preterm births among multiples, as gestational age may be on the causal pathway to neurobehavioral outcomes.

As we wanted to examine the overall effect of phthalates with similar anti-androgenic properties, we created a summary measure of metabolites MBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, and MECPP using methods developed by Varshavsky et al. (2016). The summary estimate (ΣAAPhthalate) was calculated by multiplying the specific-gravity adjusted concentration of each of these seven individual phthalate metabolites by their anti-androgenic potency and summing the weighted concentrations: ΣAAPhthalate = MnBP

+ (0.24*MiBP) + (0.26*MBzP) + (0.61*MEHP) + (0.61*MEHHP) + (0.61*MEOHP) + (0.61*MECPP). Potencies were based on benchmark doses associated with a 5% reduction as described by the National Research Council, Phthalates and Cumulative Risk Assessment (2008) (National Research Council, 2008). The $\log_e$ transformed summary values were used to estimate the mean of all maternal and paternal preconception concentrations.

3. Results

The study cohort comprised 134 mothers and 100 fathers with an average age of 35 and 36 years at time of enrollment, respectively. Participants were predominantly Caucasian (women, 92%; men, 94%), and never-smokers (women, 72%; men, 71%). Most women were nulliparous (83%) and had college or graduate degrees (98%), 29% had a BMI ≥ 25 kg/m^2, and about 28% had a female factor as the primary cause of infertility (Table 1). Among men, 70% had a BMI ≥ 25 kg/m^2 and almost a third had a male factor infertility diagnosis (Table 1). Among the 166 children, 51% were male, 69% were conceived after IVF-based treatment (76% of boys; 60% of girls) and 33% were multiples (26 sets of twins and 1 set of triplets) (Table 2). Mean BASC-2 Externalizing, Internalizing, and Behavioral Symptoms Index T-Scores were 46.6 (SD: 7.6), 47.9, (SD: 8.4), and 46.6 (SD: 7.6), respectively, with boys having slightly higher scores (reflecting worse behavioral functioning) with more variance (Table 2).

Each participant provided multiple urine samples per exposure window. On average, men provided 2.7 (25th, 75th: 1, 3) urine samples in the preconception period, and women provided 4.3 (25th, 75th: 2, 5) and 2.7 (25th, 75th: 2, 3) urine samples in the preconception and prenatal periods, respectively. The geometric mean of the specific gravity adjusted urinary phthalate metabolite concentrations from 578 maternal preconception samples ranged from 3.2 ng/ml for MBzP to 60.7 ng/ml for ΣDEHP, and within the 266 paternal preconception urine samples, concentrations ranged from 3.5 ng/ml for MBzP to 92.8 ng/ml for ΣDEHP (Supplementary Appendix Table 1A). The percentage of urine samples with detectable concentrations of phthalate metabolites ranged from 78% (maternal preconception MBzP) to 100% (maternal preconception and prenatal MEP). Log_e-transformed phthalate concentrations were moderately correlated between couples (Pearson R = 0.07 to 0.55) and maternal exposure windows (Pearson R = 0.27 to 0.50) (see Supplementary Appendix, Table 2A).

3.1. Maternal preconception exposure

In the total cohort (both sexes), we found a negative association between maternal preconception ΣDEHP concentrations and internalizing scores (β = − 2.0; 95%CI: − 3.2, − 0.7) (Table 3). We also observed significant modification of the association of maternal preconception MnBP and MiBP concentrations with internalizing behavior by sex: among boys MnBP and MiBP were associated with higher scores than for girls (p-values for interaction were < 0.06); however, the sex-specific estimates were not significant for either stratum (Table 3). In contrast, maternal preconception MEP concentrations were associated with lower internalizing scores in boys only (β = − 2.9; 95%CI: − 4.7, − 1.2, p-interaction = 0.0001). Maternal preconception phthalate metabolite concentrations were not associated

with either BSI or externalizing scores in the total cohort. However, in sex-stratified models, we observed a positive association of maternal preconception MCPP (β = 1.8; 95%CI: 0.0, 3.5), p-value for interaction = 0.01] and MiBP (β = 1.7; 95%CI: 0.3, 3.2) p-interaction = 0.12] concentrations with BSI scores among boys, but not girls. Maternal preconception MiBP concentrations were also associated with higher externalizing scores among boys (β = 2.1; 95%CI: 0.5, 3.8, p-interaction = 0.01) but not girls (β = 0.7; 95%CI: − 0.7, 2.0).

3.2. Paternal preconception exposure

Among the total study sample, we found a negative association between paternal preconception ΣDEHP concentrations and internalizing scores (β = −1.8; 95%CI: − 3.1, − 0.4), as well as negative associations of MnBP concentrations with BSI (β = − 1.7; 95%CI: − 3.0, − 0.4) and externalizing scores (β = − 1.2; 95%CI: − 2.5, 0.06) (Table 4). Furthermore, we observed sex-specific differences in paternal preconception phthalate concentrations with increased internalizing behavior among boys and decreased among girls with increasing MnBP (boys, β = 2.0; 95%CI: −1.2, 5.3; girls, β = − 4.5; 95%CI: − 8.0, − 1.0, p-interaction = 0.01; MiBP (boys, β = 2.1; 95%CI: − 0.5, 4.8; girls, β = − 2.4; 95%CI: − 5.2, 0.4, p-interaction = <0.0001); and MBzP concentrations (boys, β = 1.8; 95%CI: − 0.8, 4.3; girls, β = − 0.6; 95%CI: − 3.0, 1.9, p-interaction = 0.09) (Table 4).

3.3. Sensitivity analysis

We observed some differences in the associations of maternal preconception phthalate metabolite concentrations and behavioral scores with additional adjustment for prenatal and/or paternal preconception exposure (see Supplementary Appendix, Table 2A). Overall, adjustment for respective prenatal concentrations increased the magnitude (i.e., more positive/higher scores and away from null) of the observed associations between maternal preconception MiBP and MCPP and BSI scores, while adjustment for paternal preconception phthalates biomarkers attenuated associations. The negative association between maternal preconception ΣDEHP and internalizing behavior remained irrespective of additional adjustment. In paternal preconception models, additional adjustment for maternal preconception and/or prenatal concentrations generally attenuated the association between ΣDEHP and internalizing scores (see Supplementary Appendix, Table 2A). In sensitivity analyses restricted to singleton births (n = 111), our results remained largely consistent with our total sample, however power was more limited and confidence intervals were less precise (data not shown). However, when examining maternal preconception MEP concentrations and internalizing behavior among boys, maternal MEP concentration was associated with 2.94 (95% CI: − 4.72, − 1.17) point decrease in internalizing scores when including all births but this association was attenuated and no longer significant (β = − 1.14, 95% CI: − 2.80, 0.52) when restricting to singletons.

We observed a consistent negative association between maternal (β = − 1.91; 95%CI: − 3.26, − 0.55) and paternal preconception (β = − 1.61; 95% CI: − 3.05, − 0.17) anti-androgenic phthalate metabolite concentrations and internalizing behavior scores. While results did not significantly differ by sex, boys had modestly lower scores than girls with increasing concentrations of our anti-androgenic summary measure (Supplementary Appendix, Table 3A). The anti-androgenic summary measure was not associated with BSI or

externalizing scores. Results also remained consistent when we examined paternal preconception anti-androgenic metabolite concentrations and internalizing behavior after additional adjustment for maternal prenatal anti-androgenic metabolite concentrations (β = − 1.7; 95% CI: − 3.1, − 0.4).

4. Discussion

In this prospective cohort study of subfertile couples, we found that maternal preconception phthalate exposure appears to influence boys towards more externalizing behaviors; however, paternal preconception exposure appears to influence boys towards more internalizing behaviors. Specifically, maternal and paternal preconception urinary ΣDEHP concentrations were associated with less internalizing behaviors. In examining potential sex-specific effects, we found that maternal preconception urinary MiBP and MCPP concentrations were associated with more total behavior problems and externalizing behaviors in boys, but not girls, whereas paternal preconception urinary MnBP, MiBP, and MBzP concentrations were associated with more internalizing behaviors in boys, and less in girls.

Moreover, additional adjustment for respective maternal prenatal concentrations did not materially change the results in maternal preconception models. In paternal preconception models, additional adjustment with maternal preconception or prenatal concentrations generally attenuated results suggesting that some but not all of the observed association is driven by maternal preconception or prenatal exposure. Consistent with our ΣDEHP results, the sum of the anti-androgenic phthalate metabolite concentrations in both mothers and fathers before conception was associated with lower internalizing scores and this association was largely driven by the DEHP metabolites.

We are not aware of any other studies that have examined relations between preconception urinary phthalate metabolite concentrations and child behavior. However, several prospective cohort studies have associated urinary phthalate metabolites measured during pregnancy with neonatal behavior, behavior problems, autistic behaviors, and emotional problems (Yolton et al., 2011; Engel et al., 2009, 2010; Whyatt et al., 2012; Lien et al., 2015). While the overall epidemiological literature to date suggests that prenatal phthalate exposure may be associated with behavioral problems, there are inconsistencies and uncertainties about the specific neurobehavioral domains affected by prenatal exposure, specific metabolites responsible for any potential effects, and presence and direction of sex-specific associations (Braun, 2016). Inconsistencies across studies might be due to differences in study populations and their range of exposure, misclassification of phthalate exposure from studies using a single urine sample for assessment, and variation in the timing of when phthalates were assessed (for example, early versus late gestation) (Braun, 2016; Miodovnik et al., 2014). This latter point is critical for comparisons of this study and prior ones as previous research has estimated prenatal exposure during the second and third trimesters, whereas we were able to estimate exposure in the mother during each trimester of pregnancy, as well as during the preconception period.

During the prenatal window of exposure, phthalates may act directly on brain development via reduced androgen and/or elevated thyroid hormone action. Numerous animal studies have demonstrated that certain phthalates interfere with the synthesis, metabolism and/or action of androgen and thyroid hormones (Howdeshell et al., 2007, 2008). In experimental animal studies, *in utero* exposure to DEHP and di-isobutyl phthalate has been shown to be anti-androgenic and reduce testicular testosterone biosynthesis and decrease the expression of genes involved in steroidogenesis (Hannas et al., 2011). Some aspects of brain development have also been shown to depend on the action of gonadal hormones, particularly androgens (Fedotova et al., 2017). Furthermore, both animal and human studies show that phthalates can interfere with thyroid hormone action concentrations in pregnant women and children (Boas et al., 2012; Morgenstern et al., 2017; Yao et al., 2016). During pregnancy, higher urinary phthalate metabolite concentrations have been associated with higher gestational thyroid hormone concentrations (Johns et al., 2015). Thyroid hormone regulates early brain development processes such as the proliferation, migration and differentiation of neuronal cells (Bernal, 2005), and high-normal maternal thyroid hormone concentrations during early pregnancy are associated with lower IQ and lower gray matter volume in the child (Korevaar et al., 2016). Although little is known about the impact of phthalate exposure in the pre- or periconception periods on thyroid function, there may also be interplay of effects of preconception exposure and its influence on early pregnancy hormonal response. For example, phthalate metabolite concentrations around the time of conception may alter first trimester pregnancy thyroid function, thereby influencing early brain development via a phthalate-thyroid pathway.

Our study provides novel suggestive evidence that preconception maternal and paternal urinary phthalate metabolite concentrations were associated with altered neurobehavioral measures in preschool and school-aged children. These results are supported by the growing evidence from experimental animal studies showing that maternal and paternal preconception exposures such as psychosocial stress, diet, and chemicals such as pesticides impact offspring health (Daxinger and Whitelaw, 2012; Fernandez-Twinn et al., 2015; Rodgers et al., 2015; Yehuda, 2011; Ng et al., 2010; Rando, 2012; Soubry et al., 2014a; Huypens et al., 2016; Snijder et al., 2012a, 2012b). Although our study was not designed to examine underlying mechanisms, we hypothesize that different pathways are implicated in preconception versus pregnancy phthalate exposure on neurodevelopmental outcomes. A likely mechanism of action in the preconception window of exposure is the possibility that phthalates perturb epigenetic programming of sperm and oocytes (Wu et al., 2015; Albert and Jegou, 2014; Casas and Vavouri, 2014). For example, there is new evidence to suggest that paternal environmental and lifestyle exposures may impact sperm epigenetics and consequently the health of offspring (Rando, 2012; Kumar et al., 2013; Robinson et al., 2012; Chen et al., 2016). Sperm contribute more than paternal genetic material to the oocyte, they also transmit oocyte activation factors, centrosomes, messenger RNA, and microRNA (Krawetz, 2005; Kumar et al., 2012). The sperm and oocyte epigenome are integral to embryogenesis and to the fetal epigenome; epigenetic modifications to gametes play a critical role in fertilization potential and embryo and fetal development (Jenkins and Carrell, 2011) and may thus influence offspring phenotype (Rando, 2012; Soubry et al., 2014b; Soubry, 2015; Perera and Herbstman, 2011). While our results add to the body of literature

suggesting that the preconception period may be an important window of vulnerability for child health outcomes and that prenatal effects are likely also involved, more research is needed to elucidate such potential mechanisms, particularly for the preconception window of vulnerability.

Although our study was conducted in a large academic fertility clinic setting permitting a careful examination of preconception windows of exposure, including paternal preconception exposure – a period that has recently been shown in experimental animal studies to impact offspring health but has remained largely unexplored in humans – findings may not be generalizable to children born to couples without fertility concerns if the effect of phthalate exposure is modified by fertility or other correlated factors. However, infertile and subfertile couples represent a vulnerable population of public health importance especially because the number of children born after any medically assisted reproduction continues to grow and is currently estimated to represent more than 5% of all births (CDC, 2009; Schieve et al., 2009). Concentrations of phthalate metabolites in our study were also within the ranges reported in the U.S. National Health and Nutrition Examination Survey (CDC, 2015) and associations were observed at concentrations found in the general U.S. population.

Our study had several limitations including the inability to account for co-exposure to other chemicals of potential concern to neurodevelopmental outcomes. Phthalate exposure may also be a surrogate for other unmeasured lifestyle factors that might be associated with child neurobehavior. While we accounted for several important factors by adjusting for both maternal and paternal age, BMI, and smoking, maternal education, and infertility diagnosis, we were unable to adjust for other potentially relevant factors including parental behavior, the quantity and quality of caregiving environment, and postnatal phthalate exposures. Our study sample also included twins and triplets; it is possible that the inclusion of multiple gestations through its association with preterm birth may have influenced the neurological outcomes we examined if gestational age was a causal intermediate. However, when we restricted our sample to only singletons (n = 111), our results remained largely consistent with our total sample; although power was more limited and confidence intervals were wider. This suggests that there is little bias with the inclusion of twins and triplets in the original analysis. However, it is possible that our reported results showing decreased internalizing behaviors among boys in relation to maternal preconception MEP concentration may have been influenced by gestational age or some other aspect of multiple gestation. Our child behavioral assessment was also based on mailed parental questionnaires, and while the BASC-2 parent assessment has been validated, assessment from teachers may provide more information about child behavior. Furthermore, our study had a relatively modest sample size that may have limited our statistical power, particularly when examining sex-specific associations, and we also conducted multiple comparisons, which can increase the likelihood of chance findings.

Assessment of exposure to phthalates is a challenge in most observational studies due to their short half-lives and the frequent and episodic nature of exposure. However, a major strength of our study was that we had multiple urine samples for each of the exposure windows examined for the vast majority of participants, including at least one urine sample

from both men and women from the menstrual cycle of conception. As such we were able to partially account for the within-person variability by using the average concentration of multiple urines samples provided in the preconception and prenatal periods. Nevertheless, we need to consider the possibility of misclassification of exposure, and that such misclassification may differ by mode of conception and by paternal vs. maternal preconception window of exposure. Couples conceiving with IVF may take longer to achieve pregnancy. We do not account for differential time to pregnancy and its potential impact on our study findings: couples taking longer to conceive a pregnancy would have more preconception urine samples collected and therefore less within-person variability. Women also provide two urine samples per cycle (compared to only one sample per cycle for men); therefore we would have better exposure measurement in the maternal preconception window compared to the paternal window. However, it is unlikely that the number of urine samples collected is related to urinary phthalate metabolite concentrations and neurobehavioral test results. Finally, future studies should also consider using other statistical methods to identify potential periods of vulnerability during the preconception period (Sanchez et al., 2011). Nonetheless, our study design allowed us to comprehensively assess exposure in men and women before conception, as well as women during pregnancy, permitting us to adjust for and carefully examine the preconception period independent of prenatal exposure in relation to neurobehavior in children.

5. Conclusion

In this cohort, paternal and maternal preconception concentrations of some phthalate biomarkers were associated with specific aspects of child behavior, even after adjustment for prenatal concentrations. Although additional research is warranted to confirm these results, our findings suggest that the preconception period of exposure may be a critical window for offspring neurodevelopment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Parental characteristics from 134 Mothers and 100 Fathers Participating in the Environment and Reproductive Health (EARTH) neurodevelopmental follow-up study.

Parental characteristic	Mothers N = 134	Fathers N = 100
Age (years)		
Mean (min-max)	34.8 (24–43)	35.9 (26–49)
Age > 35, n (%)	56 (42%)	59 (59%)
Race, n (%)		
White	119 (92%)	94 (94%)
Black	–	–
Asian	7 (5%)	4 (4%)
Other	3 (2%)	2 (2%)
Body Mass Index (BMI Kg/m^2)		
Mean (min-max)	23.7 (16–36)	27.0 (20–45)
BMI ≥ 25, n (%)	40 (29%)	70 (70%)
Education, n (%)		
< College	3 (2%)	10 (13%)
College Graduate	50 (39%)	29 (36%)
Graduate Degree	75 (59%)	41 (51%)
missing	6	20
Smoking Status, n (%)		
Never	97 (72%)	71 (71%)
Former	37 (28%)	28 (28%)
Current	–	1 (1%)
Infertility Diagnosis, n (%)		
Male Factor	41 (31%)	31 (31%)
Female Factor	38 (28%)	27 (27%)
Unexplained	55 (41%)	42 (42%)
Primiparous, n (%)		
Yes	111 (83%)	–

Table 2

Birth characteristics of 166 children participating in the Environment and Reproductive Health (EARTH) neurodevelopmental follow-up study.

Child characteristics	All children N = 166	Boys n = 85	Girls n = 81
Mean child's age at testing (min-max)	4.7 (2.1–8.9)	4.9 (2.3–8.9)	4.6 (2.1–8.1)
Male, n (%)	85 (51%)	–	–
Multiples, n (%)	55 (33%)	16 (19%)	39 (48%)
Mean birth weight in grams (min-max)	3043 (1130–5040)	3086 (1230–5040)	2996 (1130–4165)
Mean gestational age at birth in weeks (min-max)	38.3 (29–42)	38.2 (29–42)	38.4 (29–42)
Conception Mode			
Natural	36 (22%)	14 (16%)	22 (27%)
IUI	16 (9%)	6 (7%)	10 (12%)
IVF	114 (69%)	65 (76%)	49 (60%)
BASC-2 T-Scores[a]			
Mean BSI (SD)	46.6 (7.6)	48.0 (8.0)	45.1 (6.8)
Mean INZ (SD)	48.0 (8.4)	48.2 (9.6)	47.8 (7.0)
Mean EXT (SD)	46.6 (7.5)	47.8 (8.0)	45.4 (6.8)

Abbreviations: BASC-2: Behavioral Assessment System for Children-2; BSI: behavioral symptoms index scores; INZ: internalizing problems composite scores; EXT: externalizing problems composite scores.

[a]Total number of participants (N) with BSI (N = 156), boys (n = 80), girls (n = 76); INZ (N = 155), boys (n = 80), girls (n = 75); EXT (N = 158), boys (n = 81), girls (n=77).

Table 3

Difference in BASC-2 behavioral symptoms index (BSI), internalizing (INZ), and externalizing (EXT) T-Scores with $\log_e$-unit increase in maternal preconception urinary phthalate concentrations: All children, and stratified by sex, adjusted analysis.

Phthalate metabolites	Maternal preconception[a,b]		Boys		Girls		Sex × phthalate EMM p-value
	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value	
BSI							
ΣDEHP	− 0.26 (− 1.35, 0.84)	0.65	− 1.51 (− 3.40, 0.37)	0.12	− 0.42 (− 1.54, 0.70)	0.47	0.09
MnBP	0.34 (− 1.05, 1.72)	0.63	0.10 (− 1.72, 1.91)	0.92	0.51 (− 0.90, 1.92)	0.48	0.57
MiBP	1.13 (− 0.20, 2.46)	0.09	1.74 (0.27, 3.21)	0.02	0.90 (− 0.48, 2.27)	0.20	0.12
MBzP	0.28 (− 1.09, 1.65)	0.69	0.27 (− 1.63, 2.18)	0.78	0.30 (− 1.07, 1.67)	0.67	0.97
MEP	0.38 (− 0.64, 1.41)	0.47	0.50 (− 0.77, 1.76)	0.44	0.29 (− 0.76, 1.34)	0.58	0.68
MCPP	0.32 (− 1.02, 1.66)	0.64	1.79 (0.04, 3.53)	0.04	0.25 (− 1.05, 1.55)	0.71	0.01
INZ							
ΣDEHP	− 2.00 (− 3.24, −0.75)	0.002	− 4.97 (− 9.29, − 0.64)	0.02	− 2.23 (− 3.66, − 0.79)	0.002	0.14
MnBP	− 0.43 (− 2.06, 1.21)	0.61	0.87 (− 1.31, 3.05)	0.43	− 0.75 (− 2.37, 0.88)	0.38	0.06
MiBP	− 0.27 (− 1.94, 1.40)	0.75	0.78 (− 1.06, 2.63)	0.41	− 0.74 (− 2.39, 0.92)	0.38	0.001
MBzP	0.22 (− 1.26, 1.70)	0.77	− 1.41 (− 5.96, 3.14)	0.54	− 0.09 (− 1.92, 1.73)	0.92	0.43
MEP	− 0.63 (− 1.87, 0.61)	0.32	− 2.94 (− 4.72, − 1.17)	0.001	− 0.55 (− 1.95, 0.83)	0.43	0.0001
MCPP	− 0.99 (− 2.50, 0.51)	0.20	0.06 (− 3.24, 3.36)	0.97	− 1.01 (− 2.45, 0.43)	0.17	0.49
EXT							
ΣDEHP	0.34 (− 1.02, 1.69)	0.63	− 0.74 (− 2.77, 1.30)	0.48	0.23 (− 1.07, 1.52)	0.73	0.19
MnBP	0.46 (− 1.04, 1.96)	0.54	0.71 (− 1.30, 2.71)	0.49	0.49 (− 0.98, 1.96)	0.52	0.77
MiBP	1.12 (− 0.25, 2.49)	0.11	2.14 (0.48, 3.80)	0.01	0.65 (− 0.73, 2.03)	0.36	0.01
MBzP	0.35 (− 1.05, 1.76)	0.62	0.35 (− 1.65, 2.36)	0.73	0.37 (− 1.05, 1.79)	0.61	0.98
MEP	0.34 (− 0.75, 1.44)	0.54	0.11 (− 1.30, 1.53)	0.88	0.29 (− 0.84, 1.42)	0.62	0.71
MCPP	0.54 (− 0.76, 1.84)	0.41	1.48 (− 0.32, 3.28)	0.11	0.52 (− 0.84, 1.88)	0.45	0.15

Abbreviations: ΣDEHP: molar sum of di(2-ethylhexyl) phthalate metabolites; MnBP: mono-n-butyl phthalate; MiBP: mono-isobutyl phthalate; MBzP: monobenzyl phthalate; MEP: monoethyl phthalate; MCPP: mono(3-carboxypropyl) phthalate; BASC-2: Behavioral Assessment System for Children-2; BSI: behavioral symptoms index scores; INZ: internalizing problems composite scores; EXT: externalizing problems composite scores; EMM: effect measure modification.

[a]Total maternal preconception sample (N): BSI (N = 146), boys (n = 78), girls (n = 68); INZ (N = 145), boys (n = 78), girls (n = 67); EXT (N = 148), boys (n = 79), girls (n = 69).

[b] Maternal models adjusted for: maternal (continuous), maternal BMI (continuous), maternal education (< college, college, graduate degree), maternal smoking (ever vs. never), and IVF treatment vs. non-IVF treatment.

Table 4

Difference in BASC-2 behavioral symptoms index (BSI), internalizing (INZ), and externalizing (EXT) T-Scores with natural $\log_e$-unit increase in paternal preconception urinary phthalate concentrations: All children, and stratified by sex, adjusted analysis.

Phthalate metabolites	Paternal preconception[a,b]		Boys		Girls		Sex × phthalate EMM p-value
	Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value	
BSI							
ΣDEHP	− 0.96 (− 2.20, 0.27)	0.13	− 1.33 (− 3.36, 0.70)	0.20	− 1.00 (− 2.26, 0.25)	0.12	0.66
MnBP	− 1.68 (− 2.96, −0.41)	0.009	− 2.32 (− 3.66, − 0.99)	0.0007	− 1.21 (− 2.90, 0.47)	0.16	0.24
MiBP	− 0.48 (− 2.00, 1.03)	0.53	− 0.60 (− 2.77, 1.58)	0.59	− 0.66 (− 2.05, 0.73)	0.35	0.92
MBzP	− 0.52 (− 1.87, 0.83)	0.45	− 1.31 (− 3.15, 0.53)	0.16	− 0.10 (− 1.54, 1.34)	0.88	0.19
MEP	− 0.22 (− 1.38, 0.95)	0.72	− 0.53 (− 2.47, 1.40)	0.59	− 0.34 (− 1.54, 0.85)	0.57	0.76
MCPP	− 0.06 (− 1.81, 1.69)	0.95	1.30 (− 1.41, 4.01)	0.35	− 0.28 (− 2.04, 1.49)	0.76	0.06
INZ							
ΣDEHP	− 1.77 (− 3.13, − 0.40)	0.01	− 3.11 (− 5.86, − 0.36)	0.03	− 1.59 (− 3.14, − 0.03)	0.05	0.27
MnBP	− 0.91 (− 2.94, 1.12)	0.38	2.05 (− 1.25, 5.35)	0.22	− 4.50 (− 7.96, − 1.04)	0.01	0.01
MiBP	− 0.86 (− 3.08, 1.35)	0.44	2.14 (− 0.50, 4.78)	0.11	− 2.40 (− 5.20, 0.41)	0.09	< 0.0001
MBzP	0.48 (− 1.49, 2.47)	0.63	1.78 (− 0.78, 4.35)	0.17	− 0.59 (− 3.04, 1.86)	0.64	0.09
MEP	0.40 (− 0.98, 1.78)	0.57	0.51 (− 5.20, 6.21)	0.86	0.31 (− 1.11, 1.73)	0.67	0.94
MCPP	− 0.46 (− 2.32, 1.40)	0.63	0.97 (− 2.41, 4.34)	0.58	− 0.63 (− 2.67, 1.41)	0.54	0.31
EXT							
ΣDEHP	− 0.43 (− 1.69, 0.84)	0.51	0.15 (− 1.53, 1.83)	0.86	− 0.53 (− 1.67, 0.62)	0.37	0.25
MnBP	− 1.20 (− 2.46, 0.06)	0.06	− 1.42 (− 2.84, − 0.001)	0.05	− 1.01 (− 2.63, 0.61)	0.22	0.64
MiBP	− 0.37 (− 1.78, 1.03)	0.60	− 0.37 (− 2.36, 1.62)	0.71	− 0.47 (− 1.88, 0.95)	0.52	0.90
MBzP	− 0.38 (− 1.72, 0.96)	0.58	− 0.86 (− 2.45, 0.73)	0.29	− 0.15 (− 1.62, 1.32)	0.84	0.41
MEP	− 0.24 (− 1.32, 0.85)	0.67	− 0.66 (− 2.30, 0.98)	0.43	− 0.33 (− 1.46, 0.81)	0.57	0.54
MCPP	0.47 (− 1.37, 2.30)	0.62	1.54 (− 1.15, 4.22)	0.26	0.33 (− 1.49, 2.15)	0.72	0.13

Abbreviations: ΣDEHP: molar sum of di(2-ethylhexyl) phthalate metabolites; MnBP: mono-n-butyl phthalate; MiBP: mono-isobutyl phthalate; MBzP: monobenzyl phthalate; MEP: monoethyl phthalate; MCPP: mono(3-carboxypropyl) phthalate; BASC-2: Behavioral Assessment System for Children-2; BSI: behavioral symptoms index scores; INZ: internalizing problems composite scores; EXT: externalizing problems composite scores; EMM: effect measure modification.

[a]Total paternal preconception sample (N): BSI (N = 107), boys (n = 56), girls (n = 51); INZ (N = 105), boys (n = 55), girls (n = 50); EXT (N = 107), boys (n = 56), girls (n = 51).

[b] Paternal models adjusted for maternal and paternal age (continuous), paternal BMI (continuous) maternal education (< college, college, graduate degree), maternal and paternal smoking (ever vs. never), and IVF treatment vs. non-IVF treatment.