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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 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 (Σ DEHP) 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,

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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