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Prenatal phthalate, triclosan, and bisphenol A exposures and child visual-spatial abilities

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ABSTRACT

Introduction: During fetal development, sex steroids influence sexually dimorphic behaviors, such as visual-spatial abilities. Thus, endocrine disrupting chemicals that impact sex steroids during gestation may affect these behaviors.
Objective: We investigated the relationship between prenatal urinary phthalate metabolite, triclosan, and BPA concentrations and visual-spatial abilities in a prospective cohort of 198 mother-child dyads.
Methods: Data are from a prospective cohort in Cincinnati, OH (HOME Study). We measured nine phthalate metabolites, triclosan, and BPA in maternal urine samples collected at 16 and 26 weeks of gestation. We assessed children's visual-spatial abilities at 8 years of age using the Virtual Morris Water Maze (VMWM), a computerized version of the rodent Morris Water Maze. We quantified the covariate-adjusted change in the time or distance to complete the VMWM and time spent in the correct quadrant during a probe trial with an interquartile range increase in chemical concentrations using linear mixed models and linear regression, respectively.
Results: Boys completed the VMWM faster (4.1 s; 95% CI:−7.1, −1.2) and in less distance (1.4 units; 95% CI:−2.8, 0) than girls. Overall, children with higher mono-*n*-butyl (MnBP), mono-benzyl (MBzP), and mono-carboxypropyl phthalate concentrations completed the VMWM in less time and distance than children with lower concentrations. For example, children with higher MnBP concentrations completed the VMWM in 0.9 less distance units (95% CI:−1.8, −0.0). Child sex modified the association between MnBP and VMWM performance. In girls, higher MnBP concentrations were associated with longer time (1.7 s; 95% CI: −0.7, 4.1) and shorter distance (−1.7 units; 95% CI: −2.8, −0.5), whereas in boys, it was associated with shorter time (-3.0 s; 95% CI:−5.6, −0.4), but not distance (−0.1 units; 95% CI:1.4, 1.0). Other phthalate metabolites, triclosan, and BPA were not associated with VMWM performance, and sex did not consistently modify these associations.
Conclusions: In this cohort, greater prenatal urinary concentrations of some phthalate metabolites were associated with improved VMWM performance, particularly among boys. Future studies should confirm these findings and determine if phthalates affect other hormonally sensitive aspects of child neurobehavior.

1. Introduction

Phthalates, triclosan, and bisphenol A (BPA) are endocrine disrupting chemicals used in a multitude of consumer products. Phthalates are a multifunctional class of chemicals used in some personal care and beauty products, polyvinyl chloride plastics,

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food processing or packaging, rainwear, adhesives, and flooring (Braun et al., 2013). Because phthalates are not covalently bound to the products in which they are used, they can leach out and be dermally absorbed, inhaled, or ingested. BPA is used to produce some canned food linings, epoxy resins, polycarbonate plastics, medical equipment, dental sealants, and thermal receipts (von Goetz et al., 2010). Diet is the predominant route of BPA exposure; dermal absorption from handling thermal receipts is another route of exposure. Triclosan is an antimicrobial compound used in personal care products, soaps, cleaning supplies, and medical devices. Triclosan exposure is predominately through oral routes, but dermal absorption may occur from use of soaps or personal care products (Rodricks et al., 2010). Exposure to phthalates, triclosan, and BPA is ubiquitous, including among pregnant women (Woodruff et al., 2011; Calafat et al., 2008a,b; Silva et al., 2004).

In experimental studies, phthalates, triclosan, and BPA affect sex steroid hormone synthesis, metabolism, transport, or action. Prenatal exposure to di-2-ethylhexyl phthalate (DEHP), butylbenzyl phthalate (BBzP), di-n-butyl phthalate (DnBP), and di-isobutyl phthalate (DiBP) reduces Leydig cell testosterone production by decreasing the expression of genes involved in cholesterol biosynthesis and steroidogenic enzymatic pathways (Hannas et al., 2011; Howdeshell et al., 2007). Triclosan can also reduce testosterone production by disrupting cholesterol biosynthesis in Leydig cells (Kumar et al., 2008, 2009). BPA is considered a weak environmental estrogen, but may also reduce testosterone levels and increase estrogen production by inhibiting enzymes involved in sex steroid synthesis and metabolism (Zhang et al., 2011; Matthews et al., 2001).

Experimental studies also show that some aspects of brain development are dependent on the action of sex steroids, particularly testosterone (Arnold, 2009). These hormones have organizational effects on the brain during fetal development that are partly responsible for sexually-dimorphic behaviors (Schulz et al., 2009). In humans, sexual dimorphisms include differences in toy preference, play style, and visual-spatial abilities, as well as differences in the risk of autism and attention-deficit hyperactivity disorder (Boyle et al., 2011; Cohen-Bendahan et al., 2005). Studies of children with congenital adrenal hyperplasia (CAH), a genetic disorder that causes excess fetal androgen production, show that high testosterone exposure during the sensitive prenatal period of brain development can affect human neurodevelopment. Female children with CAH have behavioral profiles that are more "masculine" than unaffected females (Cohen-Bendahan et al., 2005). For example, girls with the most severe form of CAH have visual-spatial abilities that are similar to those of unaffected males and better than those of unaffected girls (Mueller et al., 2008).

Sexually dimorphic behaviors, including visual spatial abilities, may be sensitive to endocrine disrupting chemical exposures that disrupt the organizational effects of sex steroids during fetal development. Thus, we investigated the relationship of prenatal exposures to phthalates, triclosan, and BPA with visual-spatial abilities at 8 years of age in a prospective birth cohort of 198 children from Cincinnati, OH. To facilitate comparisons to prior animal studies investigating the neurotoxicity of these chemicals, we assessed child visual-spatial abilities with a computerized version of the Morris Water Maze (MWM).

2. Materials and methods

2.1. Study participants

The Health Outcomes and Measures of the Environment (HOME) Study is a prospective pregnancy and birth cohort that has followed mothers and their children in the greater Cincinnati, OH metropolitan area from the 2nd trimester of pregnancy (March 2003–January 2006) until their singleton children were 7.5–10 years old (March 2012–July 2014) (Braun et al., 2016). We designed the study to assess the relationship between low-level environmental chemical exposures and child development. Inclusion criteria at enrollment included: 1) 16 ± 3 weeks gestation, 2) $\geq$18 years old, 3) living in a home built before 1978, 4) no history of HIV infection, and 5) not taking medications for seizure or thyroid disorders. All women provided informed consent for themselves and their child's participation. The institutional review boards (IRBs) of Cincinnati Children's Hospital Medical Center and the cooperating delivery hospitals approved this study. The Brown University relinquished IRB authority to Cincinnati Children's Hospital Medical Center with an Interagency Agreement.

2.2. Urinary biomarkers of phthalate metabolites, triclosan, and BPA

We assessed maternal exposure to phthalates, triclosan, and BPA by measuring total (conjugated + free) urinary concentrations of nine phthalate metabolites, triclosan, and BPA. Women provided up to two urine samples in polypropylene cups at prenatal care clinic visits at 16 and 26 weeks of pregnancy. All samples were refrigerated for <24 h until they were processed, after which they were stored at or below −20 °C until shipped on dry ice to the CDC for analysis. CDC staff measured phthalate metabolites, triclosan, and BPA concentrations using previously described analytic chemistry methods (Ye et al., 2008; Silva et al., 2007).

We summed the molar concentrations of mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) to create a summary measure of di(2-ethylhexyl) phthalate (ΣDEHP) exposure. We expressed the ΣDEHP concentrations in μg/L of MECPP by multiplying the molar sum of the individual metabolites by 308 μg/μmol.

We measured urinary creatinine concentrations to account for urine dilution and creatinine-normalized urine concentrations of phthalate metabolites, triclosan, and BPA in units of μg/g creatinine and then $\log_{10}$-transformed concentrations before averaging the 16 and 26 week concentrations. One-hundred ninety one (96%) women provided urine samples at both time-points.

We applied the methods of Varshavsky et al. (2016) to calculate the anti-androgenic weighted daily phthalate intake of BBzP, DnBP, DiBP, and DEHP (Varshavsky et al., 2016). Briefly, we calculated the daily intakes of BBzP, DnBP, DiBP, and DEHP at 16 and 26 weeks gestation using urine concentrations of monobenzyl phthalate (MBzP), mono-n-butyl phthalate (MnBP), mono-iso-butyl phthalate (MiBP), and ΣDEHP, respectively. Then, we weighted each of these intakes by the potency of each phthalate, and took the average of the 16 and 26 week weighted intakes (Mage et al., 2008; Qian et al., 2015). The potencies used to weight each phthalate were based on benchmark doses associated with a 5% reduction in testosterone production in rat experiments (Howdeshell et al., 2008; NRC, 2008).

2.3. Child visual-spatial ability assessment

We assessed child visual-spatial abilities using the Virtual Morris Water Maze (VMWM). The VMWM is a computerized version of the MWM, a rodent test of learning and visual-spatial reference memory (Astur et al., 1998; Morris, 1984). On average, in humans, rats, and mice, males perform better on the MWM and VMWM than females (Newhouse et al., 2007; Jonasson, 2005). In experimental rat studies, female performance on this task is improved after prenatal exposure to androgens; epidemiological