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Urinary organophosphate insecticide metabolite concentrations during pregnancy and children's interpersonal, communication, repetitive, and stereotypic behaviors at 8 years of age: The home study

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ABSTRACT

Background: Prenatal exposure to organophosphate insecticides may be associated with autism spectrum disorders and related behaviors. This association may be modified by single nucleotide polymorphisms in the paraoxonase (*PON1*) enzyme.

Objective: We examined the relationship of prenatal organophosphate insecticide biomarkers with reciprocal social, repetitive, and stereotypic behaviors in 8-year old children, and modification of this relationship by child *PON1* polymorphisms.

Methods: Among 224 pregnant women, we quantified concentrations of six nonspecific dialkyl phosphate (DAP) metabolites of organophosphate insecticides in two urine samples collected at ~16 and ~26 weeks gestation. When children were eight years old, we administered the Social Responsiveness Scale (SRS), a continuous measure of various dimensions of interpersonal behavior, communication, and repetitive/stereotypic behaviors. We estimated the association between a 10-fold increase in the sum of six DAP concentrations (ΣDAP) and SRS scores. We examined whether child $PON1_{192}$ and $PON1_{-108}$ genotypes modified this association.

Results: After covariate adjustment, ΣDAP concentrations were not associated with SRS scores [β = –1.2; 95% confidence interval (CI): –4.0, 1.6]. Among children with the $PON1_{-108TT}$ genotype, ΣDAP concentrations were associated with 2.5-point higher (95% CI: −4.9, 9.8) SRS scores; however, the association was not different from the 1.8-point decrease (95% CI: −5.8, 2.2) among children with $PON1_{-108CT/CC}$ genotypes (ΣDAP × $PON1_{-108}$ p-value = 0.54). The association between ΣDAP concentrations and SRS scores was not modified by $PON1_{192}$ (ΣDAP × $PON1_{192}$ p-value = 0.89).

Conclusions: In this cohort, prenatal urinary DAP concentrations were not associated with children's social behaviors; these associations were not modified by child *PON1* genotype.

1. Introduction

Autism spectrum disorder (ASD), a condition characterized by impaired interpersonal behavior or communication and repetitive or stereotypic behaviors, affects approximately 1% of children in the United States (American Psychiatric Association 2013; Centers for Disease Control and Prevention (CDC) 2013). While the autistic phenotype develops and manifests in early childhood, the abnormal neural circuitry behind ASDs most likely develops *in utero* (London et al., 2007). Gestational exposures play an important role in ASD risk (Mendelsohn and Schaefer, 2008; Chomiak et al., 2013), but few genetic or environmental risk factors have been found to contribute definitively to ASD risk (Kalkbrenner et al., 2014).

An *in utero* environmental exposure that may increase the risk of

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ASDs is the class of insecticides known as organophosphates (OP). Although the use of OP insecticides has declined by 45% in the United States since 1980, 75 million pounds of OP insecticides were used in agricultural and residential settings during 2001 (US EPA, 2004). Once ingested, about 75% of OP insecticides are metabolized into dialkyl phosphates (DAPs) by the enzyme paraoxonase 1 (PON1). DAPs do not inhibit acetylcholinesterase, have longer biological half-lives than the parent insecticide, and are excreted in the urine (Davies et al., 1997; Franklin et al., 1981). Thus, urinary DAP concentrations are suitable biomarkers for OP pesticide exposures in epidemiological studies. One limitation of using DAPs as OP pesticide exposure biomarkers is that the metabolites can be present in food due to environmental degradation of the parent insecticides.[15] Thus, DAPs may not specifically reflect exposure to the parent insecticides.

Because these neurotoxic insecticides can cross the placenta, fetal neurodevelopment may be impacted by the mother's exposure during pregnancy (Eskenazi et al., 2007). In addition, genetic variations in the efficiency of PON1 may modify the association between OP insecticide exposure and neurodevelopment (Eskenazi et al., 2010; Engel et al., 2011). Despite evidence from animal studies demonstrating that *in utero* OP insecticide exposure affects neurodevelopment (Levin et al., 2010; Oliveri et al., 2015), few epidemiological studies have examined the relationship between prenatal OP insecticide exposure and ASD diagnosis or autistic behaviors (Eskenazi et al., 2007; Furlong et al., 2014; Shelton et al., 2014). We are not aware of any studies that have investigated whether child *PON1* polymorphisms modify the association between OP insecticide exposure and ASD.

To address this research gap, we used a prospective pregnancy and birth cohort of 224 mothers and their children to examine the association between maternal urinary OP insecticide metabolite concentrations during pregnancy and social behaviors linked with ASD at 8 years of age, and determine if this association was modified by child *PON1* polymorphisms.

2. Methods

2.1. Study participants

We used data collected from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective birth cohort from the greater Cincinnati, Ohio, metropolitan area designed to investigate the relationship between low-level environmental chemical exposures and children's growth and development. Details about eligibility, subject recruitment, data collection, and follow-up for the HOME Study are described elsewhere (Braun et al., 2016). Briefly, we identified and contacted pregnant women from nine prenatal clinics associated with three hospitals in the Cincinnati area from March 2003 to January 2006. Eligibility criteria at enrollment included: *a*) age ≥18 years, *b*) 16 ± 3 weeks of pregnancy, *c*) residence in a home built before 1978, *d*) no history of HIV infection, and *e*) no chemotherapy or radiation treatments nor medications taken for thyroid disorders or seizure. We obtained written informed consent from all women for themselves and their children after the study protocols had been explained. The institutional review boards (IRBs) of Cincinnati Children's Hospital Medical Center (CCHMC) and all involved delivery hospitals approved this study. The Centers for Disease Control and Prevention (CDC) IRB relied on the determinations made by the CCHMC IRB.

Of 1263 eligible women, 468 enrolled in the study, and 67 women dropped out of the study before delivery. The remaining 401 women gave birth to 389 live singleton infants, 9 sets of twins, and 3 still-born infants. Of the 389 women with singletons, 228 completed follow-up when their children were 8 years old (range =7.5–10 years) and 224 (57%) of these women and their children had complete exposure and covariate data.

2.2. Measurement of insecticide exposure

Maternal urine samples were shipped to the CDC for analysis. We quantified six OP insecticide metabolite concentrations in urine: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) using a modification of the analytical method of Bravo et al. (2004) that employs gas chromatography-tandem mass spectrometry with isotope dilution calibration. These dialkyl phosphate metabolites (DAPs) are common metabolites of about 75% of the OP insecticides used in the United States.

Quality control (QC) materials, prepared at the CDC from spiked pooled urine, were analyzed with standards, blanks, and study samples. The QC concentrations were evaluated using standard statistical probability rules (Caudill et al., 2008). The limits of detection (LODs) varied depending on the metabolite and ranged from 0.2 μg/L for DMTP to 0.6 μg/L for DMP. We used the reported value for nonzero concentrations below the LOD, and we imputed DAP concentrations that were reported as zero by choosing a random number between zero and the lowest nonzero value for that metabolite.

We converted the metabolite concentrations to molar concentrations (nmol/L) and summed them to obtain overall concentrations of diethyl alkyl phosphates (ΣDEs: DEP, DETP, and DEDTP), dimethyl alkyl phosphates (ΣDMs: DMP, DMTP, DMDTP), and a sum of all six of the DAPs (ΣDAP). To control for individual variation in urine dilution, we measured urinary creatinine concentrations and calculated creatinine-standardized ΣDAP concentrations by dividing metabolite concentrations by creatinine concentration (Larsen, 1972). Because ΣDAP concentrations varied widely between 16- and 26-weeks gestation, we averaged the $\log_{10}$-transformed creatinine-adjusted ΣDAP concentrations across the two samples.

2.3. Reciprocal social, repetitive, and stereotypic behaviors

Mothers completed the Social Responsiveness Scale (SRS) (Constantino, 2005) in our study clinic when their children were 8 years of age. The SRS is a valid and reliable measure of interpersonal behaviors, communication, and repetitive or stereotypic behaviors (Bölte et al., 2008; Constantino, 2005). An advantage to the SRS is that it assesses autistic behaviors along a continuum, rather than a binary yes/no diagnosis, using 65 Likert-scale questions that are summed and transformed into a total *T*-score (with mean ± SD, 50 ± 10 in the normative sample). Higher scores indicate more autistic behaviors. *T*-scores ≥60 are considered to be indicative of clinically significant deficiencies in reciprocal social behavior, and *T*-scores ≥75 are consistent with a clinical diagnosis of ASDs. However, the SRS cannot be used as the sole instrument to diagnose children with ASD. Instead, it provides a useful tool with which to examine ASD behaviors along a continuum.

2.4. Confounding variables

We adjusted for potential confounding factors that may be associated with both OP insecticide exposure and autistic behaviors based on biological plausibility and prior knowledge. We obtained maternal sociodemographic and perinatal factors, including maternal age at delivery, race, marital status, education, parity, insurance status, household income, prenatal vitamin use, maternal serum cotinine concentration, and children's sex using structured interviews and chart reviews conducted by trained research staff. We measured maternal depressive symptoms during the second trimester with the Beck Depression Inventory-II (Beck et al., 1996). The frequency of fresh fruit and vegetable consumption during pregnancy was measured using a standardized questionnaire administered by trained research staff.