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Prenatal exposure to pyrethroid pesticides and childhood behavior and executive functioning

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ABSTRACT

Several previous studies of pyrethroid biomarkers and behavior have reported associations between concurrent pyrethroid levels and adverse behavioral problems in children. One geospatial study reported associations between prenatal exposure to pyrethroids and autism. However, the association between prenatal pyrethroid biomarkers and childhood behavior is unknown. The Mount Sinai Children's Environmental Health Center is a prospective birth cohort with urinary pyrethroid biomarkers during pregnancy and behavioral measurements at 4, 6, and 7–9 years of age. Primiparous women were enrolled between 1998 and 2002. 162 mother/child pairs with complete exposure and behavioral outcomes data were used to investigate associations between detectable levels of prenatal pyrethroid metabolites and scores on the Behavioral Assessment System for Children and the Behavior Rating Inventory of Executive Function. Overall, detection frequencies of pyrethroid metabolites were low (<30%). In longitudinal mixed models, detectable levels of 3-PBA during pregnancy were associated with worse Internalizing (β −4.50, 95% CI −8.05, −0.95), Depression (β −3.21, 95% CI −6.38, −0.05), Somatization (β −3.22, 95% CI −6.38, −0.06), Behavioral Regulation (β −3.59, 95% CI −6.97, −0.21), Emotional Control (β −3.35, 95% CI −6.58, −0.12), Shifting (β −3.42, 95% CI −6.73, −0.11), and Monitoring (β −4.08, 95% CI −7.07, −1.08) scales. Detectable levels of *cis*-DCCA were associated with worse Externalizing (β −4.74, 95% CI −9.37, −0.10), Conduct Problems (β −5.35, 95% CI −9.90, −0.81), Behavioral Regulation (β −6.42, 95% CI −11.39, −1.45), and Inhibitory Control (β −7.20, 95% CI −12.00, −2.39). Although detection frequencies of pyrethroid metabolites were low, we found suggestive evidence that prenatal exposure to 3-PBA and *cis*-DCCA may be associated with a variety of behavioral and executive functioning deficits.

1. Introduction

Pyrethroids were the second most commonly used class of insecticides for the home and garden market in 2007 (Grube et al., 2011). Permethrin, a common pyrethroid, is the active ingredient in several flea and tick medicines for dogs, and is also used to treat clothing for tick resistance. Use has grown in the past two decades as the use of organophosphate pesticides has declined (Grube et al., 2011). However, recent evidence suggests that exposure to pyrethroids during pregnancy or childhood may be associated with childhood behavioral problems. A geospatial study of pyrethroid sprays during pregnancy reported associations with autism spectrum disorders (ASD) and developmental delay (Shelton et al., 2014). Cross-sectional studies also implicate pyrethroids in ASD (Domingues et al., 2016) and Attention Deficit Hyperactivity Disorder (Wagner-Schuman et al., 2015). While spatial methods are advantageous in characterizing direct exposure to parent pesticides near the residence, these methods may be subject to exposure misclassification as they do not account for time spent away from the residence, or for dietary or occupational sources of pesticide exposure. Additionally, interpreting etiology from cross-sectional studies is challenging due to a number of biological and statistical obstacles.

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Chirality of pyrethroid isomers may also be important in determining pyrethroid toxicity. Several toxicology studies and a cross-sectional human study suggest that the *cis* isomers of some pyrethroid pesticides may result in stronger toxicity than the *trans* isomers (Jin et al., 2012; Liu et al., 2004; Wagner-Schuman et al., 2015; Zhang et al., 2008). This distinction could be critical in formulating pesticides to have a minimal impact on human health. Despite limitations of prior studies and the implications of stereo-isomeric specificity for toxicity, no cohort studies in the United States have yet linked biomarkers of permethrin exposure and/or their specific isomers during pregnancy to behavioral outcomes in childhood. In this study, we use a longitudinal birth cohort to investigate associations between pyrethroid metabolites and their *cis* and *trans* isomers during pregnancy and behavioral outcomes during childhood.

2. Methods

2.1. Study population

The Mount Sinai Children's Environmental Health Center enrolled 404 primiparous women in late pregnancy (mean = 31.2 weeks) from 1998 to 2001. Women were recruited from either the Mount Sinai Diagnostic and Treatment Center, which serves a predominantly East Harlem population, or one of two private practices on the Upper East Side of Manhattan. Mothers were primiparous with singleton pregnancies and delivered at the Mount Sinai Hospital between May 1998 and July 2001 (Berkowitz et al., 2003). Exclusion criteria have been detailed elsewhere (Berkowitz et al., 2003; Engel et al., 2007). During the third trimester, participants completed questionnaires about home, demographics, and behavioral characteristics during pregnancy. Mothers were re-contacted when their children were 1, 2, 4, 6, and 7–9 years. The Home Observation for Measurement of the Environment (HOME scale) was assessed at the 1 and 2 year visits, while demographics and neurodevelopment were assessed at the 4, 6, and 7–9 year visits.

2.2. Biomarkers

Participants provided a spot urine sample during the 3rd trimester. Spot urine samples were analyzed for the pyrethroid metabolites 3-PBA, *trans*-DCCA, and *cis*-DCCA by the Centers for Disease Control and Prevention using methods described elsewhere (Baker et al., 2004; Barr et al., 2010). Briefly, an internal standard mixture of isotopically labeled 3-phenoyxbenzoic acid (3-PBA) and *trans*-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*trans*-DCCA) was used to spike 2 ml of urine, which was then incubated with *beta*-glucuronidase/sulfatase to liberate the conjugated metabolites. Hydrolysates were extracted with OASIS HLB mixed-mode solid-phase extraction cartridges, which were then washed with 5% methanol in a 0.1% acetic acid solution. Metabolites were eluted with methanol. High performance liquid chromatography-tandem mass spectrometry was used to analyze the extracts. Analytes were quantified using isotope dilution calibration (Barr et al., 2010). Samples were also analyzed for the organophosphorous pesticide (OP) metabolites diethyldithiophosphate, diethylphosphate, diethylthiophosphate, dimethyldithiophosphate, dimethylphosphate, and dimethylthiophosphate (DEDP, DEP, DETP, DMDP, DMP, DMTP) (Engel et al., 2007). Quality control and laboratory methods have been published previously (Baker et al., 2004; Barr et al., 2005, 2010; Bravo et al., 2004). Creatinine was measured using a standard enzymatic colorimetric reaction with detection on a Roche/Hitachi cobas c311 auto-analyzer.

2.3. Neurodevelopmental assessments

The Behavior Assessment System for Children (BASC) is a parent-report assessment of children's adaptive and problem behaviors in the home and community setting (Sandoval and Echandia, 1995). Test-retest reliabilities and internal consistencies are good (Cronbach's alphas average 0.80 across scales and ages, mean $r_s = 0.85$ for preschool, mean $r_s = 0.87$ for children ages 6–11) (Sandoval and Echandia, 1995). Composite indices include Externalizing Behaviors (comprised of the subscales Aggression, Hyperactivity, Conduct Problems), Internalizing Behaviors (subscales include Anxiety, Depression, Somatization), Adaptive Skills (subscales include Adaptability, Leadership, Social Skills), and the Behavioral Symptoms Index (subscales include Aggression, Hyperactivity, Anxiety, Depression, Attention, Conduct Problems, Atypicality). Leadership and Conduct Problems are not assessed for 4 year olds. Scores are age-normed and reported as T-scores.

The Behavior Rating Inventory of Executive Functioning (BRIEF) is a parent-report assessment of children's problems with executive functioning over the past 6 months (Bodnar et al., 2007). Internal consistency and reliability are high (mean rs = 0.81 for parents across scales, Cronbach's alphas range from 0.80-0.98 across scales) (Gioia et al., 1996). Indices include the Behavioral Regulation Index (subscales include Inhibit, Shift, Emotional Control) and the Metacognition Index (subscales include Initiate, Working Memory, Plan/Organize, Organization of Materials, and Monitor), which are age normed and combined to form the overall Global Executive Composite.

The BASC and the BRIEF were both completed at the 4, 6, and 7–9 year visits. We used the t-scores at all visits.

2.4. Statistical methods

We estimated demographic characteristics of the study population at enrollment and at follow-up, and assessed whether characteristics at enrollment were different by follow-up status with chi-square tests for categorical variables, and a *t*-test for the continuous variable. Etiological analyses were based on a complete case analysis. Thus, we report demographic characteristics for those participants who both returned for follow-up and had complete covariate data.

Associations between the pyrethroid metabolites (3-PBA, *cis*-DCCA, and *trans*-DCCA) and each of the composites and subscales were estimated with linear mixed models, with random effects for subject. Two subscales on the BASC (Conduct Problems and Leadership) were not assessed for four year olds, and these models were assessed based on only the 6 and 7–9 year visit. Outcomes were scaled so that positive values represent better outcomes and negative values represent more adverse outcomes. The pyrethroid metabolites displayed a low detection frequency in our population and were dichotomized to indicate values above or below the limit of detection (LOD).

Directed acyclic graphs (DAGs) were constructed to identify possible colliders and mediators (Rothman et al., 2008), and included maternal education, maternal marital status at follow up, race/ethnicity, quality of the home environment, maternal IQ, $\sum$DMP pesticide use, smoking during pregnancy, alcohol during pregnancy, creatinine, and preterm birth. We adjusted for the minimally sufficient set and variables that were hypothesized to be highly predictive of the outcome, after noting adjustment for creatinine, $\sum$DMPs, and visit in the DAG. We included $\sum$DMPs and not $\sum$DEPs because prior studies in our population only implicated $\sum$DMPs in behavioral outcomes (Furlong et al., 2017), and the two are highly correlated and may present statistical issues when controlling for both in frequentist models. The final adjustment set for all models included race/ethnicity (non-Hispanic white,