HHS Public Access

Author manuscript

Neurotoxicology. Author manuscript; available in PMC 2018 September 01.

Published in final edited form as:

Neurotoxicology. 2017 September ; 62: 231–238. doi:10.1016/j.neuro.2017.08.005.

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 1998–2002. 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

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that prenatal exposure to 3-PBA and *cis*-DCCA may be associated with a variety of behavioral and executive functioning deficits.

Keywords

pyrethroids; pesticides; behavior; neurodevelopment; executive functioning; depression

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.

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; Barr et al., 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, ΣDMP 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, ΣDMPs, and visit in the DAG. We included ΣDMPs and not ΣDEPs because prior studies in our population only implicated ΣDMPs in behavioral outcomes (Furlong et al., under review), 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, Hispanic, black/other, HOME scores, ΣDMPs (logged and continuous sum of dimethylphosphate, dimethyldithiophosphate, and dimethylthiophosphate), maternal marital status at follow-up (married, single, living with partner), maternal education at follow-up (high school or less vs some college or higher), child sex, creatinine, and visit. We assumed all missing covariate data was missing at random. In sensitivity analyses we excluded the organophosphate pesticides (OPs) from the models, assessed a binary variable for detection of malathion (an OP) as a confounder, and excluded individuals with BASC F Index scores >4, which indicate unreliable answering patterns. We also assessed interactions between 3-PBA and race, and between 3-PBA and sex for each scale, and set an alpha of 0.10 to denote presence of a notable interaction.

All analyses were performed in R v3.3.1.

3. Results

The population was primarily unmarried and young at enrollment, with low levels of educational attainment (Table 1). Characteristics at enrollment generally did not differ by follow-up status, although participants at follow-up were slightly more likely to have pyrethroid metabolite values below the LOD than those who did not return for follow-up (Table 1).

168 mothers returned for follow-up. 162 mother/child pairs with complete covariate data returned for at least one BASC follow-up at the 4–5, 6, or 7–9 year visit. One additional child had the BRIEF (n=163) but not the BASC. Across the three visits, there were 328 total observations for the BRIEF, and 327 total observations for the BASC. Of participants with complete covariate data, 84 returned for first time period, 76 participants returned for second time period, and 92 participants returned for third time period. Only 32 participants returned for all 3 time points. 152 mother-child pairs were used to assess associations with the two BASC subscales that were not assessed at the 4 year visit (Conduct Problems and Leadership), based on 240 observations across the two later visits.

Detection frequencies and levels for the three pyrethroid metabolites were low, and detection varied significantly by follow-up status (Tables 1 and 2). The three metabolites were correlated; the Spearman's correlation coefficient for 3-PBA detection and *c*-DCCA detection was 0.48, 3-PBA detection and *t*-DCCA detection were correlated at 0.56, and *c*-DCCA and *t*-DCCA were correlated at 0.74.

Women with detectable levels of the 3-PBA metabolite during pregnancy were more likely to have children with worse scores on the BASC's Internalizing Composite (β −4.50, 95% CI −8.05, −0.95), the Depression subscale of the Internalizing Composite (β −3.21, 95% CI −6.38, −0.05), and the Somatization subscale of the Internalizing Composite (β −5.07, 95% CI −8.62, −1.51) (Table 3). 3-PBA was also associated with worse scores on the BRIEF'S Behavioral Regulation Index (BRI) (β −3.59, 95% CI −6.97, −0.21), the Emotional Control subscale of the BRI (β −3.35, 95% CI −6.58, −0.12), the Shifting subscale of the BRI (β −3.42, 95% CI −6.73, −0.11), and the Monitoring subscale of the Metacognition Index (β −4.08, 95% CI −7.07, −1.08). These suggest a pattern of pyrethroid-associated problems with Internalizing Behaviors from the BASC and Behavioral Regulation from the BRIEF.

Women with detectable levels of the *cis*-DCCA metabolite during pregnancy were also more likely to have children that suffered from Behavioral Regulation Problems on the BRIEF, although these associations were paired with associations with Externalizing Problems, rather than Internalizing Problems, from the BASC (Table 4). *cis*-DCCA was associated with worse scores on the BASC's Externalizing Composite (β −4.74, 95% CI −9.37, −0.10), and the Conduct Problems subscale of the Externalizing Composite (β −5.35, 95% CI −9.90, −0.81). *cis*-DCCA was also associated with worse scores on the BRIEF's Behavioral Regulation Index (β −6.42, 95% CI −11.39, −1.45), the Emotional Control subscale of the

BRI (β −4.74, 95% CI −9.52, 0.03), and the Inhibitory Control subscale of the BRI (β −7.20, 95% CI −12.00, −2.39).

The *trans*-DCCA metabolite was not associated with adverse performance for any composite or subscale, although it was associated with better performance on the BASC's Anxiety subscale (β 4.61, 95% CI 0.05, 9.16) (Table 5).

There were no interactions with race or sex at the 0.10 level. Sensitivity analyses that excluded participants with BASC F Index scores >4 (n=1) did not materially change results. Excluding OPs from the analysis did not change results beyond producing a slight strengthening in some effect estimates. Including a binary variable for detectable levels of malathion, an OP metabolite that belongs to the DMP class of OPs, also did not change results.

4. Discussion

This population, the majority of whom were enrolled prior to the phase-out of organophosphorous pesticides for residential pest control, overall had low exposure to pyrethroids. Fewer than 30% of the enrolled population had detectable metabolite concentrations in pregnancy. Despite the low detection frequency, we identified several associations between detectable levels of 3-PBA and *cis*-DCCA pyrethroid metabolites during pregnancy and adverse behavioral and executive functioning outcomes during childhood. Specifically, we report associations between 3-PBA and internalizing behaviors and behavioral regulation, and between *cis*-DCCA and externalizing behaviors and behavioral regulation. The magnitude of these associations should be interpreted cautiously, given that any detectable exposures were grouped together.

These findings are generally supported by the available evidence, although the long-term consequences of prenatal exposure to pyrethroids in the United States have not been extensively investigated. Biomarker studies, in particular, are quite limited. One study of prenatal exposure to pyrethroids based on a geospatial assessment of exposure reported that exposure to pyrethroids in the third trimester was associated with elevated odds of autism spectrum disorders (ASD) (Shelton et al., 2014). This study, however, is the only longitudinal assessment of the association between prenatal exposure to pyrethroids and childhood behavior. Several cross-sectional studies have indicated a possible relationship between concurrent pyrethroid metabolite levels and behavioral problems. A cross-sectional study in NHANES reported associations between 3-PBA and ADHD and hyperactive-impulsive symptoms (Wagner-Schuman et al., 2015) while another study in NHANES reports null associations with ADHD (Quirós-Alcalá et al., 2014). Another cross-sectional study reports associations with ASD (Domingues et al., 2016), and yet another reports associations between *cis*-DCCA biomarker levels and adverse performance on the Strengths and Difficulties Questionnaire (Oulhote and Bouchard, 2013). These previously reported adverse findings are consistent with the results we present here, where detectable levels of prenatal 3-PBA was associated with worse behavioral regulation, emotional control, shifting, internalizing behaviors, depression, and somatization, and *cis*-DCCA was associated with worse behavioral regulation, emotional control, inhibition, externalizing problems, and

conduct problems. These symptom clusters are consistent with generalized behavioral disorders in childhood, including ADHD or ASD. However, we do not report associations with the hyperactivity or attention subscales, both of which are theoretically hallmarks of an ADHD diagnosis. We also do not report associations with the social skills subscale, which is a hallmark of an ASD diagnosis. However, in this study, prenatal pyrethroids are associated with a suite of behavioral problems that commonly present in both disorders, and pyrethroids may be an important contributing factor in the development of these pathologies.

Our reported findings are also supported by toxicology studies. Of the common pyrethroid pesticides, 3-PBA is a metabolite of permethrin, cypermethrin, deltamethrin, allethrin, resmethrin, fenvalerate, and others. *cis*-DCCA and *trans*-DCCA display some overlap with 3-PBA and are both metabolites of their respective isomers of permethrin, cypermethrin, and cyfluthrin. Although the DCCA metabolites are derived from the same parent pesticides, different exposure routes may result in relatively higher *trans*-DCCA concentrations relative to *cis*-DCCA concentrations (Leng et al., 1997; Woollen et al., 1992), and differing pharmacokinetics for the two isomers may result in varying toxicities (Liu et al., 2004; Zhang et al., 2008). For instance, oral administration of equal parts *cis*- and *trans*-cypermethrin may result in higher urinary *trans*-DCCA concentrations relative to *cis*-DCCA, while dermal administration results in a 1:1 concentration ratio of *trans* to *cis* DCCA isomers (Leng et al., 1997). Additionally, administration of the *cis* isomer of cypermethrin results in higher fat storage and a longer half-life of approximately 13 days for the pesticide, relative to the *trans* isomer (Hutson et al., 1981). This is supported by other studies indicating the *trans* cypermethrin isomer is excreted more rapidly than the *cis* isomer (Eadsforth and Baldwin, 1983; Liu et al., 2004). Thus, the *cis* isomer of cypermethrin may result in higher toxicity relative to the *trans* isomer due to continued release from fat storage over a weeks-long period, which is supported by several studies indicating higher toxicities of the *cis* isomer, including several in mice (Jin et al., 2012; Liu et al., 2004; Zhang et al., 2008), one study in humans (Wagner-Schuman et al., 2015), and our current study.

One important difference between our study and prior studies is the prevalence of exposure in our population based on the 3-PBA biomarker. We report that only approximately 25% of the population had detectable levels of 3-PBA, and the limit of detection was 0.25 μg/L. However, in NHANES, the LOD was 0.10 μg/L, and 66% of NHANES participants had detectable levels (Barr et al., 2010). The LOD in our study, 0.25 μg/L, corresponded to the 50^{th} percentile for exposure in 1999–2000 NHANES. It is thus likely that a substantial proportion of our population may have had low levels of pyrethroid exposure, but exposure was not detected in our analyses. Still, participants with detectable values reflect a group with higher levels than those with undetectable values, and the binary comparison can be considered a group of highly exposed (the top quartile) versus those with less exposure. Unfortunately, these low detection frequencies, in concert with the relatively small sample size, resulted in a loss of precision. Many of the confidence intervals associated with our results were somewhat wide, lending more support to the idea that these results should be replicated in other populations before definitive conclusions about the relationship between prenatal pyrethroid exposures and behavior may be drawn.

There are several other biomarker-based limitations to this study. We were unable to assess linear or dose response relationships due to the small numbers of participants with biomarker values above the LOD. We were also unable to assess varying windows of exposure during pregnancy, since biomarkers were analyzed from a single spot urine sample in the third trimester. Exposures during early pregnancy may disrupt cell proliferation patterns with downstream effects. Additionally, the pyrethroid biomarkers we used here are not specific to the parent pesticides. The DCCA metabolites are derived from three pesticides (permethrin, cypermethrin, cyfluthrin), while the 3-PBA metabolite is derived from several parent pesticides (Barr et al., 2010). Most of the metabolites are non-toxic or low-toxicity. The parent pesticides degrade into the 3-PBA and DCCA metabolites in photolysis situations (Holmstead et al., 1978), and people may be exposed directly to the non-toxic metabolites on fruits and vegetables. Thus, like DAP biomarkers for OPs, a pyrethroid biomarker does not distinguish between toxic and non-toxic exposure. However, in the late 1990s, New York City began a city-wide spraying program of the pesticide Anvil to limit the spread of West Nile Virus. Anvil's active ingredient is sumithrin, which breaks down into 3-PBA, and exposure to this parent pesticide may be at least partially responsible for some of the detected 3-PBA metabolites in our NYC population. Finally, in this population, those with detectable levels of pyrethroids may also represent a sub-population with generally high pesticide loads. Thus, although we controlled for the DMP class of OPs, and specifically for malathion in sensitivity analyses, residual confounding by other pesticides may be present.

Another drawback is that the Type 1 error rate may be inflated due to multiple testing. However, the pattern of associations across scales was consistent for 3-PBA and *cis*-DCCA: 3-PBA was associated with multiple Internalizing Composite subscales and multiple Behavioral Regulation subscales, while *cis*-DCCA was associated with multiple Externalizing Composite subscales and Behavioral Regulation subscales. Thus, we report a pattern of associations rather than an association with a single, random subscale. We do report a single association between *trans*-DCCA and improved anxiety, which may be a result of the inflated Type I error rate, particularly since *trans*-DCCA was not associated with any other scale.

Finally, our study had substantial loss-to-follow-up. Although none of the demographic characteristics differed by follow-up status, having a pyrethroid biomarker <LOD was associated with increased likelihood of returning for follow-up. Women with higher levels of 3-PBA, *cis*-DCCA, and *trans*-DCCA were all less likely to return for follow-up. If pyrethroid exposure causes worse behavioral problems, returning for follow-up may have been more difficult for mothers of children with behavioral problems. This would have biased our results towards the null, as our study excluded this set of children with worse behavioral outcomes. An unknown third factor could influence both pyrethroids and follow-up, although this factor would have to be unassociated with any of the demographic characteristics we assessed, as no other characteristics were associated with follow-up. Future studies must account for potential missingness due to loss-to-follow-up, and our reported associations should be confirmed in other birth cohort studies. The loss-to-follow-up also influenced power to assess age-specific associations; the limited number of

participants that returned at each visit precluded our ability to estimate whether associations between prenatal pyrethroids and neurodevelopment varied by age.

Our study also had several strengths. Primary strengths include longitudinal follow-up, a statistical design that accounted for the repeated outcome measures, and a multi-ethnic study population. This is the first published biomarker study of prenatal pyrethroids and childhood behavior, with substantial support from cross-sectional studies, and one prior geospatial study that reported an association between prenatal exposure and ASD. Another advantage of this study is that we were able to control for OP pesticide metabolites, which has previously been associated with Internalizing Behaviors (Furlong et al., under review)

The public health impact of prenatal pyrethroid exposure may be substantial. Although pyrethroids are consistently touted as displaying low mammalian toxicity, occupational pyrethroid exposure during adulthood has been linked to Parkinson's disease (Furlong et al., 2015), prenatal exposures have been associated with ASD (Shelton et al., 2014), postnatal associations have been associated with ADHD (Wagner-Schuman et al., 2015), and piperonyl butoxide, a synergist commonly added to pyrethroids, has been associated with decrements in Mental Development (Horton et al., 2011). Permethrins are not only commonly used home and agricultural insecticides, but they are common ingredients in flea and tick medicine for dogs, and are readily available at sporting goods stores for treating fabrics as a flea and tick deterrent. The Department of Defense currently requires all soldier's uniforms to be treated with permethrin, and wearing these uniforms does confer higher biomarker levels of pyrethroids (Proctor et al., 2014). Although pregnant soldiers are exempt from wearing these uniforms when the probability of vector-borne disease transmission is low, women who are unaware they are pregnant will continue to wear these uniforms during the first trimester. A recent study estimated that 50–60% of pregnancies among active-duty women were unplanned (Custer et al., 2008), placing a disproportionate number of fetuses at risk in the first trimester. Additionally, pregnant women must wear permethrin-treated uniforms when their Commanders deem treated uniforms necessary to reduce the risk of insect-borne hazards. The DOD adopted these policies based on Environmental Protection Agency guidance that permethrin treated uniforms are safe with no known health effects, despite the lack of epidemiological evidence supporting this claim. Further research should be done to determine whether prenatal exposure to pyrethroids poses a risk to child development.

Acknowledgments

This work was supported by the National Institute of Environmental Health Sciences/U.S. Environmental Protection Agency Children's Center [ES09584 and R827039], the New York Community Trust, the Agency for Toxic Substances and Disease Registry/Centers for Disease Control and Prevention (CDC)/Association of Teachers of Preventive Medicine, and the National Institute of Environmental Health Sciences [P30ES023515]. M. Furlong was supported in part by NIEHS institutional training grant [T32ES007018].

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Highlights

- We investigated associations between prenatal pyrethroids and childhood behavior
- 3-PBA was associated with worse internalizing behaviors and behavioral regulation
- *cis*-DCCA was associated with worse externalizing behavior and behavioral regulation

Table 1

Demographic Characteristics of Study Population

Characteristic	Enrollment (n=361) N (%)	Follow-Up (n=162) N (%))	No Follow-Up (N=199) N (%)	Difference by follow-up status (chi^2 p value)*
Maternal Marital Status at Enrollment				
Married	101 (28.0)	38 (23.5)	63 (31.7)	
Living with Partner	91 (25.2)	38 (23.5)	53 (26.6)	
Single	169 (46.8)	86 (53.1)	83 (41.7)	0.08
Maternal Education				
High School or Less	264 (73.1)	119 (73.5)	145 (72.9)	
Some College or Higher	95 (26.3)	42 (25.9)	53 (26.6)	0.98
Maternal Age at Enrollment				
<20	129 (35.7)	52 (32.1)	77 (38.7)	
20–25	112 (31.0)	58 (35.8)	54 (27.1)	
>25	120 (33.2)	52 (32.1)	68 (34.2)	0.19
Race/Ethnicity				
Non-Hispanic White	71 (19.7)	31 (19.1)	40 (20.1)	
Hispanic	189 (52.4)	87 (53.7)	102 (51.3)	
Black	95 (26.3)	41 (25.3)	54 (27.1)	
Other	6 (1.7)	3 (1.9)	3 (1.5)	0.90
Average HOME scores [mean (sd)]	36.2 (6.3)	36.4 (6.0)	36.0 (6.7)	0.62
Sex				
Male	202 (56.0)	89 (54.9)	113 (56.8)	
Female	159 (44.0)	73 (45.1)	86 (43.2)	0.81
Alcohol During Pregnancy				
Ever	48 (13.3)	25 (15.4)	23 (11.6)	
Never	306 (84.8)	134 (82.7)	172 (86.4)	
Missing	7 (1.9)	3 (1.9)	4 (0.20)	0.36
Smoking During Pregnancy				
Any	61 (16.9)	27 (16.7)	34 (17.1)	
None	300 (83.1)	135 (83.3)	163 (82.9)	0.99
Pyrethroid Biomarker				
3-PBA >LOD*	107 (29.6)	39 (24.1)	68 (34.2)	
3-PBA <LOD	254 (70.4)	123 (75.9)	131 (65.8)	0.05
cis-DCCA >LOD*	52 (14.4)	15 (9.3)	37 (18.6)	
cis-DCCA <LOD	309 (85.6)	147 (90.7)	162 (81.4)	0.01
trans DCCA >LOD*	78 (21.6)	22 (13.6)	56 (28.1)	
trans DCCA <LOD	283 (78.4)	140 (86.4)	143 (71.9)	0.02

*Differences by follow-up status for categorical variables were assessed with chi squared tests.

Differences in mean HOME scores (continuous variable) was estimated with a t-test.

Table 2

Pyrethroid Biomarker Characteristics

	% Detected at enrollment	Limit of Detection (LOD) μg/L	$50^{th}/75^{th}/95^{th}$ percentiles μg/L
3-PBA	29.6	0.25	ND/0.34/2.98
cis-DCCA	14.4	0.20	ND/ND/1.91
trans-DCCA	21.6	0.20	ND/ND/3.37

Table 3

Associations between Detectable Levels of Urinary 3-PBA During Pregnancy and Childhood Behavior and Executive Functioning

BASC Scales (N=162)	3-PBA β (95% CI)	BRIEF Scales (N=163)	3-PBA β (95% CI)
Internalizing Composite	−4.50 (−8.05, −0.95)	**Behavioral Regulation Index**	−3.59 (−6.97, −0.21)
Anxiety	−2.31 (−5.91, 1.29)	Emotional Control	−3.35 (−6.58, −0.12)
Depression	−3.21 (−6.38, −0.05)	Inhibit	−2.57 (−5.89, 0.75)
Somatization	−5.07 (−8.62, −1.51)	Shift	−3.42 (−6.73, −0.11)
Externalizing Composite	−0.90 (−4.04, 2.24)	**Metacognition Index**	−2.05 (−5.16, 1.05)
Aggression	−1.37 (−4.38, 1.65)	Initiate	−1.52 (−4.44, 1.39)
Conduct Problems[1]	−2.04 (−5.16, 1.09)	Monitor	−4.08 (−7.07, −1.08)
Hyperactivity	−1.04 (−4.74, 2.66)	Organization of Materials	−1.11 (−4.27, 2.06)
Adaptive Skills Composite	−2.62 (−0.58, 5.82)	Plan/Organize	−1.86 (−5.09, 1.37)
Adaptability	−1.82 (−5.18, 1.54)	Working Memory	−1.15 (−4.80, 2.50)
Leadership[1]	−2.29 (−5.77, 1.19)	**Global Executive Composite**	−2.70 (−5.87, 0.47)
Social Skills	−2.98 (−6.26, 0.29)		
Behavioral Symptoms Index	−2.43 (−5.84, 0.98)		
Aggression*	−1.37 (−4.38, 1.65)		
Anxiety*	−2.31 (−5.91, 1.29)		
Attention	−0.43 (−3.68, 2.82)		
Atypicality	−0.16 (−2.85, 2.53)		
Conduct Problems*	−2.04 (−5.16, 1.09)		
Depression*	−3.21 (−6.38, −0.05)		
Hyperactivity*	−1.04 (−4.74, 2.66)		
Other Problems			
Atypicality*	−0.16 (−2.85, 2.53)		
Withdrawal	−0.90 (−4.04, 2.24)		

BASC models are based on 327 observations across 162 individuals and 3 visits. BRIEF models are based on 328 observations across 163 individuals and 3 visits. All mixed models adjusted for the fixed effects of race/ethnicity, quality of the home environment HOME scores, log ΣDMPs (organophosphorus pesticide metabolites), mother's marital status, mother's education, log creatinine, visit, and child sex, with random effects for subject. 3-PBA is dichotomized at the limit of detection, where 1 indicates a detect. Neurobehavioral t-scores are scaled so that positive values reflect positive outcomes and negative values reflect adverse outcomes.

*Indicates a subscale which is included in a prior composite scale

[1]Conduct Problems and Leadership are only available for the 6 and 7–9 year visit. These models are based on 240 total observations for 152 individuals and 2 visits

Table 4

Associations between Detectable Levels of Urinary *cis*-DCCA During Pregnancy and Childhood Behavior and Executive Functioning

BASC Scales (N=162)	***cis-DCCA* β (95% CI)**	**BRIEF Scales (N=163)**	***cis*-DCCA β (95% CI)**
Internalizing Composite	2.08 (−3.23, 7.40)	**Behavioral Regulation Index**	−6.42 (−11.39, −1.45)
Anxiety	3.19 (−2.11, 8.48)	Emotional Control	−4.74 (−9.52, 0.03)
Depression	0.42 (−4.30, 5.14)	Inhibit	−7.20 (−12.00, −2.39)
Somatization	0.84 (−4.53, 6.22)	Shift	−3.57 (−8.49, 1.35)
Externalizing Composite	−4.74 (−9.37, −0.10)	**Metacognition Index**	−1.21 (−5.80, 3.39)
Aggression	−2.09 (−6.54, 2.35)	Initiate	−2.16 (−6.46, 2.14)
Conduct Problems	−5.35 (−9.90, −0.81)	Monitor	−3.80 (−8.37, 0.76)
Hyperactivity	−4.54 (−9.96, 0.89)	Organization of Materials	−2.24 (−6.90, 2.42)
Adaptive Skills Composite	−3.71 (−8.45, 1.03)	Plan/Organize	−2.97 (−7.88, 1.93)
Adaptability	−1.50 (−6.47, 3.48)	**Global Executive Composite**	−3.41 (−8.09, 1.28)
Leadership	−3.62 (−8.78, 1.53)		
Social Skills	−3.99 (−8.85, 0.86)		
Behavioral Symptoms Index	−2.62 (−7.66, 2.43)		
Aggression *	−2.09 (−6.54, 2.35)		
Anxiety *	3.19 (−2.11, 8.48)		
Attention	−3.37 (−8.14, 1.39)		
Atypicality	−0.73 (−4.61, 3.14)		
Conduct Problems *	−5.35 (−9.90, −0.81)		
Depression *	0.42 (−4.30, 5.14)		
Hyperactivity *	−4.54 (−9.96, 0.89)		
Other Problems			
Withdrawal	0.11 (−4.52, 4.75)		
Atypicality	−0.73 (−4.61, 3.14)		

BASC models are based on 327 observations across 162 individuals and 3 visits. BRIEF models are based on 328 observations across 163 individuals and 3 visits. All mixed models adjusted for the fixed effects of race/ethnicity, quality of the home environment HOME scores, log ΣDMPs (organophosphorus pesticide metabolites), mother's marital status, mother's education, log creatinine, visit, and child sex, with random effects for subject. *cis*-DCCA is dichotomized at the limit of detection, where 1 indicates a detect. Neurobehavioral t-scores are scaled so that positive values reflect positive outcomes and negative values reflect adverse outcomes.

* Indicates a subscale which is included in a prior composite scale

1 Conduct Problems and Leadership are only available for the 6 and 7–9 year visit. These models are based on 240 total observations for 152 individuals and 2 visits

Table 5

Associations between Detectable Levels of Urinary *trans*-DCCA During Pregnancy and Childhood Behavior and Executive Functioning

BASC Scales (N=162)	***trans-DCCA* β (95% CI)**	**BRIEF Scales (N=163)**	***trans*-DCCA β (95% CI)**
Internalizing Composite	1.85 (−2.77, 6.47)	**Behavioral Regulation Index**	−2.71 (−7.08, 1.67)
Anxiety	4.61 (0.05, 9.16)	Emotional Control	−2.41 (−6.59, 1.76)
Depression	0.42 (−4.30, 5.14)	Inhibit	−2.78 (−7.05, 1.49)
Somatization	−0.55 (−5.23, 4.12)	Shift	−1.17 (−5.47, 3.13)
Externalizing Composite	−1.72 (−5.75, 2.32)	**Metacognition Index**	−0.27 (−4.26, 3.73)
Aggression	−0.26 (−4.14, 3.62)	Initiate	−1.03 (−4.78, 2.73)
Conduct Problems	−3.19 (−7.15, 0.76)	Monitor	−2.26 (−6.24, 1.72)
Hyperactivity	−1.37 (−6.11, 3.36)	Organization of Materials	−0.83 (−4.89, 3.22)
Adaptive Skills Composite	−3.17 (−7.28, 0.95)	Plan/Organize	−1.46 (−5.69, 2.77)
Adaptability	−0.90 (−5.22, 3.42)	Working Memory	−1.05 (−5.73, 3.64)
Leadership	−3.89 (−8.30, 0.52)	**Global Executive Composite**	−1.22 (−5.31, 2.87)
Social Skills	−3.68 (−7.89, 0.53)		
Behavioral Symptoms Index	−0.48 (−4.87, 3.92)		
Aggression *	−0.26 (−4.14, 3.62)		
Anxiety *	4.61 (0.05, 9.16)		
Attention	−1.47 (−5.64, 2.69)		
Atypicality	−0.35 (−3.77, 3.07)		
Conduct Problems *	−3.19 (−7.15, 0.76)		
Depression *	0.42 (−4.30, 5.14)		
Hyperactivity *	−1.37 (−6.11, 3.36)		
Other Problems			
Withdrawal	0.19 (−3.84, 4.23)		
Atypicality *	−0.35 (−3.77, 3.07)		

BASC models are based on 327 observations across 162 individuals and 3 visits. BRIEF models are based on 328 observations across 163 individuals and 3 visits. All mixed models adjusted for the fixed effects of race/ethnicity, quality of the home environment HOME scores, log ΣDMPs (organophosphorus pesticide metabolites), mother's marital status, mother's education, log creatinine, visit, and child sex, with random effects for subject. *trans*-DCCA is dichotomized at the limit of detection, where 1 indicates a detect. Neurobehavioral t-scores are scaled so that positive values reflect positive outcomes and negative values reflect adverse outcomes.

* Indicates a subscale which is included in a prior composite scale

1 Conduct Problems and Leadership are only available for the 6 and 7–9 year visit. These models are based on 240 total observations for 152 individuals and 2 visits.