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Pyrethroid Pesticide Exposure and Parental Report of Learning Disability and Attention Deficit/Hyperactivity Disorder in U.S. Children: NHANES 1999–2002

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Short running title: Pyrethroids and neurodevelopment in U.S. children

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Abstract

Background: Usage of pyrethroid insecticides has increased dramatically over the past decade; however, data on their potential health effects, particularly on children, are limited.

Objective: We examined the cross-sectional association between postnatal pyrethroid exposure and parental report of learning disability (LD) and attention-deficit/hyperactivity disorder (ADHD) in children 6-15 years of age.

Methods: Using data from the National Health and Nutrition Examination Survey (1999-2002), we estimated associations of urinary metabolites of pyrethroid insecticides with parent-reported LD, ADHD, and both LD/ADHD in 1659-1680 children using logistic regression.

Results: The prevalence rates of parent-reported LD, ADHD, and both LD/ADHD were 12.7%, 10.0%, and 5.4%, respectively. Metabolite detection frequencies for 3-PBA [3-phenoxybenzoic acid], *cis*-DCCA [*cis*-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid], and *trans*-DCCA [*trans*-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid] were 77.1%, 35.6%, and 33.9%, respectively. The geometric mean 3-PBA concentration was 0.32 µg/L (Median = 0.31 µg/L; IQR = 0.10-0.89 µg/L). *cis*- and *trans*-DCCA 75th percentile concentrations were 0.21 µg/L and 0.68 µg/L, respectively. Log₁₀-transformed 3-PBA concentrations were associated with an adjusted odds ratio (OR) of 1.18 (95% CI: 0.92, 1.51) for parent-reported LD, 1.16 (95% CI: 0.85, 1.58) for ADHD, and 1.45 (95% CI: 0.92, 2.27) for both LD/ADHD. Adjusted ORs remained non-significant and decreased after controlling for creatinine and other environmental chemicals previously linked to altered neurodevelopment. Similarly, no significant associations were observed for *cis*- and *trans*-DCCA.

Conclusions: Postnatal pyrethroid exposure was not associated with parental report of LD and/or ADHD. Given the widespread and increasing use of pyrethroids, future research should evaluate exposures at current levels, particularly during critical windows of brain development.

Introduction

Pyrethroid insecticides account for more than 30% of insecticides used worldwide (Barr et al. 2010). They are used to control pests in residential and agricultural settings, to treat head lice and scabies in humans and fleas in pets, for public health vector control, and for disinsection of commercial aircrafts (USEPA 2013a; 2013b; Wei et al. 2012). These synthetic insecticides act by altering the permeability of sodium ion channels in excited nerve cells, causing repetitive nerve impulses that may vary in intensity depending on the chemical structure of the individual pyrethroid (Barr et al. 2010; Mandhane and Chopde 1997; Nasuti et al. 2003). In humans, pyrethroid insecticides are rapidly metabolized and excreted (elimination half time: ~6-17 hours) (ATDSR 2003).

Usage of pyrethroids, particularly in residential settings, has increased dramatically over the past decade and will likely increase further as they are replacing other pesticides (e.g., organophosphate pesticides) that are considered to have higher mammalian toxicity and linked to adverse health effects in children (Barr et al. 2010; Horton et al. 2011a; USEPA 2013b; Williams et al. 2008). Hence, exposure to pyrethroids in the U.S. general population is widespread (Barr et al. 2010) mostly from diet and indoor residential uses (via ingestion, dermal, and inhalation pathways) (ATDSR 2003; Lu et al. 2009). In the U.S. general population, higher pyrethroid exposures have been reported in children compared with adolescents and adults (Barr et al. 2010). Human exposure to pyrethroids is primarily assessed by measures of non-specific urinary metabolites such as 3-phenoxybenzoic acid (3-PBA), and *cis*- and *trans*-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*- and *trans*-DCCA) (Barr et al. 2010).

Although pyrethroids are considered to be safer than other insecticides, rodent studies suggest that early life and pubertal pyrethroid exposures alter neurobehavioral functioning (Farag et al. 2007; Shafer et al. 2005; Sinha et al. 2006). For example, one study reported that early prenatal and postnatal pyrethroid exposure led to oxidative stress in various areas of the brain, with the hippocampal area exhibiting cholinergic dysfunction. Neurochemical changes were accompanied by impaired learning and memory (Sinha et al. 2006). Another rodent study reported associations of pyrethroid exposure during puberty with spatial learning and memory impairments that were more severe in females, increased anxiety in females, and inhibition of aggressive behavior in males (Meng et al. 2011). Pubertal pyrethroid exposure has also been reported to disrupt testosterone and estradiol synthesis and expression of androgen receptor in the cerebral cortex, which may impair neurobehavioral development (Liu et al. 2011). Select pyrethroids have also shown greater toxicity in neonatal than in adult rats, possibly due to incomplete development of detoxifying enzymes (Cantalamessa 1993).

In humans, symptoms of systemic pyrethroid poisoning resulting from accidental exposure or intentional ingestion are well characterized (ATDSR 2003; Ray and Forshaw 2000; Soderlund et al. 2002). However, data on the human health effects of pyrethroids (particularly neurodevelopmental effects) at the lower environmental doses encountered by the general public are limited, and data on developing children are particularly sparse. Only two studies to date have evaluated the effects of prenatal pyrethroid exposure on children's neurodevelopment. Horton et al. (Horton et al. 2011b) examined the relationship of prenatal exposure to permethrin, a pyrethroid, and piperonyl butoxide (PBO), a synergist commonly formulated with pyrethroids, on children's neurodevelopment at 3 years (n = 230-342). Children in the highest PBO exposure group, as assessed in personal air samples, scored approximately four points lower on the Bayley

Mental Developmental Index than children in the lowest exposure group. Prenatal exposure to permethrin in air and/or blood was not associated with altered neurodevelopment, though authors noted difficulty measuring permethrin in these media (Horton et al. 2011b). Another study reported an inverse association between prenatal pyrethroid exposure, using urinary metabolites, and measures of motor function, social adaptation, and intelligence in one-year-old Chinese infants (n = 497) (Xue et al. 2013). Neither of these studies controlled for postnatal pyrethroid exposures.

Two other studies have considered the potential effects of childhood pyrethroid exposure on neurodevelopment. A study of 7- to 9-year-old Nicaraguan children (n = 110) living in an agricultural community (Rodriguez 2012) found that parent-reported hours of pyrethroid use during the first year of life was associated with poorer perceptual reasoning using the Wechsler Intelligence Scale for Children IV and teacher-reported hyperactivity and attention-deficit/hyperactivity disorder (ADHD) using the revised Conner's Teachers Rating Scale short version. In a subset of these children (n = 74), child's 3-PBA urinary concentration levels were associated with impaired cognition and ADHD in girls, but not boys (Rodriguez 2012). Most recently, a nationally representative Canadian study found an association between urinary concentrations of *cis*-DCCA and parent-reported total behavioral problems on the Strengths and Difficulties Questionnaire in 6- to 11-year-olds (n = 779). However, urine 3-PBA concentrations were not associated with behavioral problems in the study population (Oulhote and Bouchard 2013).

Herein, we examine the cross-sectional association between children's pyrethroid exposures and parent-reported learning disability (LD) and/or ADHD in a representative sample of U.S. 6- to 15-year-olds participating in the National Health and Nutrition Examination Survey (NHANES).

Learning disabilities encompass various difficulties in receptive and expressive language, reading, and mathematics, which may affect scholastic performance (Pastor and Reuben 2002); while ADHD is a neurobehavioral disorder characterized by a persistent pattern of inattention, impulsivity, and/or hyperactivity that interferes with functioning or development (American Psychiatric Association 2013).

Methods

Data source and study population

Data for this analysis were extracted from NHANES, a population-based, cross-sectional survey assessing the health and nutritional status of the U.S. civilian, non-institutionalized population that is conducted by the National Center for Health Statistics (NCHS) of the U.S. Centers for Disease Control and Prevention (CDC). The sample is selected to represent the U.S. population of all ages, using a complex, stratified multistage probability sample design, which oversamples certain subpopulations. Information on study participants was collected via a household interview. A standardized physical examination, consisting of select medical and laboratory tests, was conducted in a mobile examination center. Study activities were approved by the NCHS institutional review board; parents/guardians provided written consent for their minor children (i.e., under 17 years) to participate, and children assented in the presence of the parent/guardian and interviewer (Zipf G et al. 2013). Further details on interviews, examination procedures, and sample collection are available elsewhere (CDC 2014). For this analysis, we used publicly available data for children between the ages of 6 and 15 years who participated in two consecutive NHANES cycles (1999-2000 and 2001-2002). Pyrethroid exposure measurements were conducted on a random subsample of participants ≥ 6 years of age. We selected study

cycles and child age range based on availability of exposure, outcome, and critical covariate data.

Parental report of a LD and ADHD

Our primary outcome variables, parental report of a learning disability (LD) and/or ADHD, were based on parental/guardian response to two NHANES interview questions: “Has a representative from a school or a health professional ever told (you) that (the child) had a learning disability?” and “Has a doctor or health professional ever told (you) that (the child) had attention deficit disorder?” For children who were 12 years of age or older, the same questions about LD and ADHD were asked; however, the words in parentheses were replaced with [the child] and [he/she], respectively. Question phrasing did not differ by cycle year. For this analysis, we classified children as reported with LD, ADHD or with both LD and ADHD.

Pyrethroid exposure assessment

To assess pyrethroid exposure, we used urinary metabolite measurements of 3-PBA, *cis*-DCCA, and *trans*-DCCA in spot urine samples provided by participants during the physical examination. The metabolite 3-PBA represents exposure to permethrin, cypermethrin, deltamethrin, allethrin, resmethrin, fenvalerate, cyhalothrin, fenpropathrin, and tralomethrin and *cis*- and *trans*-DCCA represent exposure to the *cis*- and *trans*- isomers, respectively, of permethrin, cypermethrin, and cyfluthrin (CDC 2009; Barr et al. 2010). Two other pyrethroid metabolites (4-fluoro-3-phenoxybenzoic acid, 4F3PBA, a specific metabolite of cyfluthrin; and *cis*-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, *cis*-DBCA, a specific metabolite of deltamethrin) were also measured; however, they were not included in our analyses due to low detection frequencies ($\leq 3.2\%$). Samples were analyzed at CDC’s National Center for Environmental

Health laboratory and metabolite concentrations were measured through high-performance liquid chromatography/tandem mass spectrometry using validated laboratory methods detailed elsewhere (Baker et al. 2004; Olsson et al. 2004). The limit of detection (LOD) for 3-PBA and *cis*-DCCA was 0.10 µg/L, and 0.40 µg/L for *trans*-DCCA. Urinary creatinine (mg/dL) concentrations were determined using an automated colorimetric method based on a modified Jaffe reaction on a Beckman Synchron AS/ASTRA clinical analyzer (Beckman Instruments, Inc., Brea, CA) at the Fairview University Medical Center (Minneapolis, MN) (Barr et al. 2010).

Of the 4,672 children 6 to 15 years old surveyed during 1999–2002, only 1,861 (39.8%), 1,839 (39.4%), and 1,848 (39.6%) children had urinary 3-PBA, *cis*- and *trans*-DCCA data, respectively; two children with *cis*-DCCA, one child with *trans*-DCCA, and one child with 3-PBA data were missing creatinine data. Compared with children who did not have pyrethroid metabolite data, children who did were more likely to be of other races/ethnicities (i.e., multiracial or of other races/ethnicities besides non-Hispanic white, non-Hispanic black, or Mexican American), to have the head of household with an education level greater than high school, to not have attended a daycare or preschool, and to have no health insurance (see Supplemental Material, Table S1). Of the children with pyrethroid metabolite measurements, two were missing information on LD and five on ADHD.

Prevalence rates of LD, ADHD, and both LD and ADHD were similar between children with and without pyrethroid metabolite data (see Supplemental Material, Table S2). Additionally, outcome prevalence rates did not differ significantly ($p \geq 0.36$) by study cycle (not shown). A total of 1,680 (90.3%), 1,659 (90.2%), and 1,669 (90.3%) children had complete exposure (3-PBA, *cis*- and *trans*-DCCA, respectively), outcome, and covariate data available for multivariable analysis.

Statistical analyses

We computed descriptive statistics (e.g., geometric means (GMs), GM 95th percent confidence intervals (CIs), and weighted percentiles) for 3-PBA concentrations, and given their low detection frequencies, weighted 75th percentiles for *cis*- and *trans*-DCCA. To assess associations between our main exposures, urinary pyrethroid metabolites, and each outcome of interest, we used logistic regression models to estimate crude and adjusted odds ratios and corresponding 95% CIs. We constructed separate models for each metabolite to assess its association with parent-reported LD, ADHD, and both LD and ADHD. Concentrations of 3-PBA were log-normally distributed; thus, we modeled 3-PBA concentrations as a continuous log₁₀-transformed variable in our analyses. Concentrations of 3-PBA below the analytical LOD (23% of values) were imputed at random in R (version 3.1.0) based on a log-normal probability distribution whose parameters were determined by maximum likelihood estimation with the imputed values forced to be below the LOD. This method yields reasonable estimates when detection frequencies are > 70% (Lubin et al. 2004). Concentrations of *cis*- and *trans*-DCCA were dichotomized (detected vs. not detected) given their low detection frequency (35.6% and 33.9%, respectively).

For the multivariable models, children with parental report of the outcome of interest were compared to all others without the specific outcome modeled (e.g., in models of LD, the comparison population would include those with ADHD). We also ran models in which the comparison population did not include either LD or ADHD cases. Results were similar; thus, only the former models are shown.

We examined several variables as potential confounders for inclusion in our models. Variables retained *a priori* in all multivariable models included child's sex, race/ethnicity (non-Hispanic

white, non-Hispanic black, Mexican-American, and other), child's age (years), household reference person's education level (did not graduate high school, graduated high school or received GED, and greater than high school education), and health insurance status (yes/no). Covariates that predicted at least one outcome of interest with $p < 0.20$ in separate bivariate analyses were included in all final models. Covariates considered included: birth weight (at or above 2500g vs. < 2500 g), neonatal intensive care unit admission (NICU; yes/no); preschool or daycare attendance (yes/no); mother's age at child's birth (years); maternal smoking status during pregnancy (yes/no); and poverty income ratio (continuous). Because poverty income ratio (PIR) data were missing on 166 children, we retained health insurance status and household reference person's education level (both associated with PIR at $p < 0.0001$) as proxies for socioeconomic status in all final models to maintain sample size. To ensure the validity of our findings, we ran separate analyses on the subsample with PIR as a covariate in the model; results did not change appreciably (Supplemental Material, Table S3).

To account for the effects of urine dilution on metabolite concentrations, we ran separate models adjusted for creatinine concentration (mg/dL) as a covariate (Barr et al. 2010). We did not model creatinine-corrected metabolite concentrations because creatinine concentrations varied widely in our sample population (Barr et al. 2005). In separate analyses, we also examined potential sex-related differences in the association between urinary 3-PBA concentrations and our outcomes by introducing an interaction term for sex and urinary 3-PBA concentrations to our final models. We also assessed possible interaction between children's age and 3-PBA concentrations, both as continuous variables.

In sensitivity analyses, we also considered in the models exposures to other agents purported to be neurotoxicants including organophosphate pesticides as measured via non-specific urinary

dialkylphosphate metabolites (DAPs, comprised of the molar sum (nmol/L) of dimethyl phosphate, diethyl phosphate, dimethylthiophosphate, diethylthiophosphate, dimethyldithiophosphate, and diethyldithiophosphate) (Bouchard et al. 2010; Bouchard et al. 2011), environmental tobacco smoke as measured using serum cotinine concentrations (ng/mL) (Froehlich et al. 2009), and lead as measured in children's blood ($\mu\text{g/dL}$) (Lanphear et al. 2000). Because concentrations for these environmental contaminants were log-normally distributed in our population, we \log_{10} -transformed these concentrations. Concentrations of these chemicals below their LODs were assigned a concentration value of $\text{LOD}/\sqrt{2}$ (CDC 2009). We controlled for each environmental contaminant in separate models and then altogether in the same model. Inclusion of environmental contaminants decreased our final models' sample size due to missing participant data. To determine if missing participants were impacting estimates, we ran models with and without adjustment for each chemical among only those participants with data on each respective environmental chemical.

Although the main criteria for ADHD was based on parental report alone, we also considered in additional analyses children who were reported to use stimulant medication in order to maximize the likelihood that ADHD cases were diagnosed by a medical professional. Of the 148 children with a parental report of ADHD, 61 children (41.2%) also reported using stimulant medication. Thus, we defined children with ADHD based on (a) parental report of ADHD ($n = 148$ children met this criteria) (our main analyses), and in separate analyses based on (b) parental report of ADHD *or* using stimulant medication ($n = 154$ children met this criteria), and (c) parental report of ADHD *and* using stimulant medication ($n = 61$ children met this criteria). We did not look at children with no parental report but who were using stimulant medication as the sample size was too small ($n = 6$). Information on stimulant medication use was provided by respondents and was

based on National Drug Codes corresponding to: amphetamine aspartate/amphetamine sulfate/dextroamphetamine saccharate/dextroamphetamine sulfate (3700), dextroamphetamine sulfate (17900), methylphenidate hydrochloride (39500), or unspecified ADHD drug medications (82000).

Lastly, we also explored whether cohort effects were present by including a covariate for NHANES survey cycle year in our final adjusted models as part of our sensitivity analyses. With the exception of evaluating cohort effects, sensitivity analyses were limited to 3-PBA given the low detection frequency of *cis*- and *trans*-DCCA.

NCHS-created sampling weights, strata, and primary sampling units were applied in all statistical analyses according to the NCHS guidelines to yield robust SEs and unbiased point estimates, and to account for the complex, stratified multistage probability sample design of NHANES. All data analysis was performed using Statistical Analysis Software (SAS), version 9.3 (SAS Institute Inc., Cary, NC). The threshold for statistical significance in our logistic regression analyses (i.e., p-value on the main exposure variable) was set at $p < 0.05$ and at $p < 0.10$ for interactions.

Results

The metabolite 3-PBA was detected in 77.1% of the urine samples, while lower detection frequencies were observed for *cis*- and *trans*-DCCA (35.6% and 33.9%, respectively). The geometric mean (GM) urinary 3-PBA concentration was 0.32 $\mu\text{g/L}$ (Median = 0.31 $\mu\text{g/L}$; IQR: 0.10 - 0.89 $\mu\text{g/L}$). The weighted 75th percentile concentrations for *cis*- and *trans*-DCCA were 0.21 $\mu\text{g/L}$ and 0.68 $\mu\text{g/L}$, respectively. Among children with detectable pyrethroid metabolite concentrations, we found that *cis*- and *trans*-DCCA concentrations were significantly correlated ($p < 0.0001$) with each other ($r = 0.89$) and with 3-PBA ($r = 0.96$ and $r = 0.88$ for *cis*- and *trans*-

DCCA, respectively), suggesting that 3-PBA was more likely to be a marker of exposure to cypermethrin and/or permethrin (precursor pyrethroid pesticides common to these three urinary metabolites) rather than other pyrethroid insecticides.

Urinary concentrations of 3-PBA by participant characteristics are presented in Table 1. GM urinary 3-PBA concentrations were higher in children who were non-Hispanic black than children of other races/ethnicities (GM = 0.43 $\mu\text{g/L}$ vs. GM \leq 0.34 $\mu\text{g/L}$). Additionally, children in the lowest PIR quartile had higher 3-PBA concentrations than children in the other three PIR quartiles (GM = 0.45 $\mu\text{g/L}$ vs. GM \leq 0.37 $\mu\text{g/L}$). Children living in homes with a head of household with less than a high school education also had higher 3-PBA concentrations than children in homes with a head of household with more education (GM = 0.43 $\mu\text{g/L}$ vs. GM \leq 0.33 $\mu\text{g/L}$). Significant but weak correlations were observed between concentrations of 3-PBA and each of the environmental chemicals considered (3-PBA and DAPs $r = 0.12$; 3-PBA and blood lead $r = 0.12$; and 3-PBA and serum cotinine $r = 0.18$, all $p < 0.001$).

Of the 1,861 children with 3-PBA and creatinine concentrations, there were a total of 223 (12.7%), 148 (10.0%), and 78 (5.4%) with parental report of LD, ADHD, and both LD and ADHD, respectively; similar results were observed for children with *cis*- and *trans*-DCCA data (Supplemental Material, Table S2). Participants with a parental report of LD, ADHD, and both LD and ADHD had higher urinary 3-PBA concentrations than children without these conditions (Table 2). Specifically, non-creatinine standardized GM 3-PBA concentrations were 0.42 $\mu\text{g/L}$ (95% CI: 0.31, 0.57) in children reported having LD vs. 0.30 $\mu\text{g/L}$ (95% CI: 0.25, 0.37) for children without LD ($p = 0.007$); 0.35 $\mu\text{g/L}$ (95% CI: 0.25, 0.49) in children with ADHD vs. 0.31 $\mu\text{g/L}$ (95% CI: 0.26, 0.38) for children without ADHD ($p = 0.38$); and 0.52 $\mu\text{g/L}$ (95% CI:

0.32, 0.83) for children with parent-reported LD and ADHD vs. 0.31 $\mu\text{g/L}$ (95% CI: 0.25, 0.38) for children without both of these conditions ($p = 0.02$).

Results from crude and multivariable logistic regression analyses for 3-PBA are presented in Table 3. For every 10-fold increase in 3-PBA concentrations, there was a 1.24 (95% CI: 1.00, 1.55), 1.26 (95% CI: 0.96, 1.65), and 1.55 (95% CI: 1.04, 2.32) unadjusted odds ratio (OR) of children having a parental report of LD, ADHD, and both LD and ADHD, respectively, compared to children without parental report of these disorders. After taking into account potential confounders, the ORs became slightly attenuated: for every 10-fold increase in 3-PBA concentration, there was a 1.18 (95% CI: 0.92, 1.51), 1.16 (95% CI: 0.85, 1.58), and a 1.45 (95% CI: 0.92, 2.27) adjusted odds ratio (aOR) of children having a parental report of LD, ADHD, and both LD and ADHD, respectively, compared to children without parental report of these disorders. Results were further attenuated when creatinine concentrations were included in the models (Table 3). No interactions between 3-PBA exposure and sex or child age (i.e., all interaction p -values > 0.10) were observed (data not shown).

When we controlled for other environmental chemicals (i.e., total DAPs, serum cotinine, and blood lead concentrations) in our sensitivity analyses, results remained similar albeit slightly attenuated (Supplemental Material, Table S3).

Additionally, results remained non-significant when we extended our definition of ADHD to include those children with parent-reported ADHD *or* taking stimulant medication (aOR = 1.16; 95% CI: 0.85, 1.58; aOR including creatinine as a covariate, aOR_{creatinine} = 1.07; 95% CI: 0.77, 1.50), and to include children with parent-reported ADHD *and* taking stimulant medication (aOR = 0.79; 95% CI: 0.42, 1.51; aOR_{creatinine} = 0.72; 95% CI: 0.36, 1.42).

No significant associations were observed between any of our outcomes and detection of *cis*- and *trans*-DCCA (Table 3). As with 3-PBA, adjusted associations became further attenuated when creatinine was taken into account.

Lastly, although we did not observe significant differences in GM 3-PBA concentrations between the two cycle years (1999-2001 vs. 2001-2002), we did observe significantly higher detection rates ($p < 0.01$) of *cis*- and *trans*-DCCA during the 1999-2001 cycle compared to the subsequent cycle (Supplemental Table 4). When we included survey cycle year in our final models, aORs generally increased slightly; however, results remained similarly non-significant for all three metabolites and the survey cycle year covariate was not significant in any of the models (not shown).

Discussion

Neurodevelopmental disorders like LD and ADHD affect between 400,000 and 600,000 children in the U.S. each year. The disabilities caused by such neurodevelopmental disorders may persist throughout adulthood and place economic burdens on society (Landrigan et al. 2012). Previous studies indicate that environmental factors may play a role in the etiology of these disorders (Bouchard et al. 2010; Braun et al. 2008; Ciesielski et al. 2012; Froehlich et al. 2009; London et al. 2012). In this study, concentrations of 3-PBA were not associated with significantly higher odds of parent-reported LD and/or ADHD in children ages 6-15 years from a representative sample of the U.S. general population after controlling for covariates; this finding remained after controlling for creatinine and other environmental chemicals previously linked to altered neurodevelopment. Also, detection of *cis*- and *trans*-DCCA was not associated with any of our outcomes.

To our knowledge, only two studies have assessed the association between postnatal pyrethroid exposure and children's neurodevelopment. Similar to a recent Canadian population-based study in 6-11 year old children (Oulhote and Bouchard 2013), no significant associations were observed between 3-PBA concentrations and parent-reported behavior problems in our population. Although, contrary to the Canadian study, which reported an association between urinary concentrations of *cis*-DCCA and parent-reported behavioral problems (as assessed using a validated questionnaire encompassing various dimension scales such as emotional symptoms, conduct problems, hyperactivity/inattention, and peer problems) (Oulhote and Bouchard 2013), we did not observe significant associations between detection of *cis*-DCCA and parent-reported LD and/or ADHD in our study population. However, there were key differences in these two studies that may limit comparisons: detection frequencies in the Canadian study were higher (35.6% in NHANES vs. 97.3% in the Canadian children) due to lower limits of detection (LOD = 0.10 µg/L in NHANES vs. 0.007 µg/L in Canadian study), the years during which samples were collected were later in the Canadian study (1999–2002 for NHANES vs. 2007–2009 in the Canadian study), and the outcomes assessed differed. In the Nicaraguan study (Rodriguez 2012), which reported an association between 3-PBA concentrations and ADHD in girls, the children lived in an agricultural community where pyrethroid exposures were over seven times higher than those reported in our population (GM = 2.8 µg/gCre; n = 74 vs. GM = 0.37 µg/gCre; n = 1861, respectively), sampling of urine samples occurred at a later time (2008 vs. 1999-2002 in NHANES), and the mixture of pyrethroids used may have differed (Rodriguez 2012).

Despite the scarcity of epidemiologic data on associations between pyrethroid insecticides and children's neurobehavioral development, animal studies have suggested potential mechanisms of action. Behavioral changes and neurochemical changes to cholinergic, dopaminergic, and

catecholaminergic pathways have been reported at subacute and subchronic doses in rodent studies for select pyrethroid insecticides (Aziz et al. 2001; Elwan et al. 2006; Eriksson and Fredriksson 1991; Lazarini et al. 2001; Shafer et al. 2005). A systematic review of developmental *in vivo* toxicity studies of pyrethroids by Shafer et al. (Shafer et al. 2005) highlights changes in motor activity and in muscarinic acetylcholine receptor density in rodents. One study by Sinha et al. (Sinha et al. 2006) reported that pyrethroid exposure in rat pups during the prenatal, perinatal, and early postnatal period at doses meant to simulate those experienced during human residential use led to significant oxidative stress, an increase in lipid peroxidation, and a decrease in antioxidants, glutathione, superoxide dismutase and catalase in various brain areas. The hippocampus was the most affected region. A decrease in learning and memory performance was reported to accompany these neurochemical changes, suggesting that pyrethroid exposure during early life adversely affects the developing brain by causing cholinergic dysfunction and leading to deficits in learning and memory (Sinha et al. 2006). Significant and persistent memory and learning deficiencies were also linked to muscarinic receptor binding in young rats after low-level prenatal pyrethroid exposure (Aziz et al. 2001). Additionally, significant dopaminergic changes have been reported from early-life exposure to multiple pyrethroids in rodents (Carloni et al. 2013; Elwan et al. 2006) and recent experimental studies suggest that dopamine may play a role in ADHD through abnormal functioning of dopaminergic receptors (Wu et al. 2012). High-dose pubertal pyrethroid exposure has also been linked to hormone disruption (testosterone and estradiol synthesis) and expression of androgen receptor in the cerebral cortex in mice; such changes in the developing brain may be deleterious for neurobehavioral development (Liu et al. 2011).

The present study has several limitations. The main limitation is that NHANES is a cross-sectional survey, thus it is not possible to determine the temporal sequence of exposure and outcome and to establish causality. Reverse causality is conceivable, that is, children with behavioral problems such as ADHD by virtue of these problems may become more exposed; e.g., their higher activity levels may lead to more exposure. It also remains possible that uncontrolled confounding may be biasing our results towards the null (e.g., if a more educated parent were more likely to have their child diagnosed and less likely to use pyrethroids at home). Although we tried to adjust for several important confounders, including education and health insurance status, we are limited by the variables available to us in this national survey.

There are also a number of limitations with the biomarkers of exposure used. Pyrethroids are non-persistent and thus rapidly metabolized in the body, so the use of single spot urine samples may not accurately reflect typical exposure levels. Additionally, the use of non-specific urinary metabolites to assess pyrethroid exposure and the fact that metabolite concentrations in urine may also reflect direct exposure to the preformed breakdown products (i.e., the metabolites themselves) present in the environment (CDC 2009; Starr et al. 2008) prevents us from determining associations with specific pyrethroid insecticide(s). Also, the high detection limits and low detection frequencies of *cis*- and *trans*-DCCA did not allow us to assess the association between actual metabolite concentrations and our outcomes. Low percent of detectable concentrations for these metabolites limited our sample size in that stratum and thus, our ability to find an association. Additionally, exposure to pyrethroids during a potentially critical period of vulnerability, prenatally and during infancy, was not assessed in NHANES.

Urine collection in our study population took place before the residential phase-out of other pesticides that are being replaced by pyrethroid insecticides; thus, residential usage of

pyrethroids may not have been widespread. In fact, the geometric mean concentration for 3-PBA for U.S. children 6-15 years of age using data from the NHANES 2007-2008 (CDC 2013) was higher than that observed during 1999-2002, the period of the present study (GM = 0.42 $\mu\text{g/L}$ vs. GM = 0.32 $\mu\text{g/L}$, respectively). Unfortunately, data on our outcomes of interest were not available for 2007-2008. Higher 3-PBA levels were also noted in two later children's studies: one 2003-2004 study conducted in Seattle, WA of children 3-11 years of age reported a median 3-PBA concentration nearly four times higher than our study population (1.2 $\mu\text{g/L}$ vs. 0.31 $\mu\text{g/L}$) (Morgan 2012) and another study in 2009 of farmworker children 2-8 years of age found 3-PBA concentrations five times higher than in our study population (GM = 1.97 $\mu\text{g/gCre}$ vs. GM = 0.37 $\mu\text{g/gCre}$, respectively) (Trunnelle et al. 2014).

Another study limitation is that "diagnosis" of LD and ADHD was based on parental/guardian report of clinical or teacher evaluation rather than based on school or medical records or a neuropsychological assessment. Though to increase the likelihood that a child was indeed an ADHD case, we also included children who were taking stimulant medication and results remained non-significant (i.e., ORs were further attenuated). Furthermore, we examined only the association between pyrethroid exposure and LD and ADHD, but there may be other childhood behavioral disorders (e.g., anxiety, aggression) that are related to pyrethroid exposure that we are not able to investigate with the available data.

Despite these limitations, our analyses have certain strengths. First, we conducted our analyses on a large, representative sample of children from the U.S. general population. Thus, results are generalizable to U.S. children 6 to 15 years of age during the time period assessed. To our knowledge, this is also the first study in the U.S. to assess the association between postnatal pyrethroid exposure and altered neurodevelopment in children. We also took into account

numerous potential confounders as well as other environmental exposures previously linked to the outcomes of interest.

In conclusion, postnatal pyrethroid exposure was not significantly associated with parent-reported LD, ADHD, or both LD and ADHD in children ages 6-15 participating in NHANES during 1999–2002. However, the cross-sectional nature of this study does not allow us to establish causality nor to examine exposure during the potentially more critical prenatal period. We found that a large percentage of children participating in NHANES in 1999-2002 had detectable levels of the pyrethroid metabolite 3-PBA; however, recent studies indicate that pyrethroid use has increased since the residential phase-out of certain organophosphate insecticides (Gan 2008; Horton et al. 2011a; Power and Sudakin 2007) and higher urinary pyrethroid concentrations have been reported in samples collected after 2002 (Morgan 2012; Trunelle et al. 2014). Given that pyrethroid use is increasing and is widespread, future research should evaluate the safety of current levels of pyrethroid insecticide exposures during critical windows of brain development.

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Table 1. Urinary 3-PBA concentrations ($\mu\text{g/L}$) based on study participants' characteristics (NHANES 1999-2002; n = 1861).^{a,b}

Children's characteristic	N (%) ^b	3-PBA concentration ($\mu\text{g/L}$) GM (95% CI) ^b	P-value ^c
Gender			
Male	899 (51.7)	0.34 (0.28, 0.42)	0.24
Female	962 (48.3)	0.39 (0.32, 0.48)	
Missing	0		
Race/ethnicity			
Non-Hispanic White	466 (58.5)	0.30 (0.23, 0.40)	0.001
Non-Hispanic Black	596 (14.6)	0.43 (0.36, 0.53)	
Mexican American	625 (11.3)	0.25 (0.20, 0.31)	
Other	174 (15.7)	0.34 (0.27, 0.42)	
Missing	0		
Poverty Income Ratio (PIR)			
< 0.94	549 (22.9)	0.45 (0.36, 0.56)	0.001
0.94-1.80	441 (24.0)	0.37 (0.27, 0.50)	
1.81-3.56	387 (25.9)	0.33 (0.25, 0.45)	
> 3.56	318 (27.2)	0.19 (0.15, 0.26)	
Household reference person's education level			
< High school	688 (24.7)	0.43 (0.35, 0.53)	0.002
High school	438 (25.9)	0.33 (0.24, 0.44)	
> High school	663 (49.4)	0.27 (0.21, 0.35)	
Missing	72		
Low birth weight (< 2500g)			
Yes	160 (7.4)	0.31 (0.19, 0.51)	0.96
No	1629 (92.6)	0.32 (0.26, 0.39)	
Missing	72		
Age			
6-7 years	333 (19.8)	0.34 (0.22, 0.52)	0.12
8-9 years	377 (20.4)	0.32 (0.25, 0.42)	
10-11 years	339 (18.3)	0.27 (0.20, 0.35)	
12-13 years	411 (20.6)	0.37 (0.27, 0.49)	
14-15 years	401 (20.9)	0.29 (0.22, 0.40)	
Neonatal intensive care unit			
Yes	205 (12.0)	0.37 (0.25, 0.53)	0.38
No	1641 (88.0)	0.31 (0.25, 0.38)	
Missing	15		
Attended daycare/preschool			
Yes	1229 (71.6)	0.30 (0.24, 0.38)	0.22
No	630 (28.4)	0.36 (0.27, 0.47)	
Missing	2		
Health insurance			
Yes	1492 (82.8)	0.31 (0.25, 0.37)	0.32
No	348 (17.2)	0.37 (0.24, 0.56)	
Missing	21		
Total DAPs (mol/L)			
< 3.15×10^{-8}	517 (30.5)	0.25 (0.19, 0.32)	0.04
3.15×10^{-8} to 1.14×10^{-7}	594 (31.1)	0.34 (0.27, 0.43)	
> 1.14×10^{-7}	718 (38.4)	0.36 (0.27, 0.47)	
Missing	32		

Children's characteristic	N (%) ^b	3-PBA concentration (µg/L) GM (95% CI) ^b	P-value ^c
Blood lead level (µg/dL)			
0.2-1.2 µg/dL	771 (50.1)	0.27 (0.22, 0.34)	0.003
1.3-2.1 µg/dL	522 (31.4)	0.41 (0.31, 0.54)	
> 2.1 µg/dL	369 (18.5)	0.45 (0.32, 0.63)	
Missing	199		
Serum cotinine (ng/mL)			
Bottom 50% (< 0.06 ng/mL)	650 (41.2)	0.21 (0.17, 0.28)	< 0.0001
Upper 50% (0.06-9.99 ng/mL)	910 (55.7)	0.47 (0.39, 0.55)	
≥ 10 ng/mL	35 (3.1)	0.43 (0.23, 0.80)	
Creatinine (mg/dL)			
< 92 mg/dL	685 (38.8)	0.17 (0.14, 0.22)	< 0.0001
92-164 mg/dL	717 (39.3)	0.40 (0.30, 0.53)	
> 164 mg/dL	459 (21.8)	0.56 (0.43, 0.73)	
Missing	0		
Mother's age at child's birth			
< 25 years	861 (41.8)	0.37 (0.35, 0.46)	0.1
25-34 years	836 (49.2)	0.29 (0.22, 0.37)	
35+ years	135 (9.0)	0.22 (0.13, 0.38)	
Missing	29	--	
Mother smoked during pregnancy			
Yes	265 (18.7)	0.37 (0.28, 0.49)	0.12
No	1566 (81.3)	0.31 (0.25, 0.38)	
Missing	30		

^aIncludes children with 3-PBA and creatinine data. ^bPercentages, geometric means (GM) and 95% CIs are weighted to reflect sampling. ^cT-test *p*-value comparing GMs among subgroups.

Table 2. Geometric mean 3-PBA concentrations ($\mu\text{g/L}$) based on outcomes for study participants ($n = 1861$).^{a,b,c,d}

Neurodevelopmental outcome	N (%)	3-PBA concentration ($\mu\text{g/L}$) GM (95% CI)	P-value ^c
LD			
Yes	223 (12.7)	0.42 (0.31, 0.57)	0.007
No	1636 (87.3)	0.30 (0.25, 0.37)	
ADHD			
Yes	148 (10.0)	0.35 (0.25, 0.49)	0.38
No	1708 (90.0)	0.31 (0.26, 0.38)	
LD+ADHD			
Yes	78 (5.4)	0.52 (0.32, 0.83)	0.02
No	1776 (94.6)	0.31 (0.25, 0.38)	

^aIncludes children with 3-PBA and creatinine data. ^bPercentages, geometric means (GM) and 95% CIs are weighted to reflect survey sampling. ^cP-value comparing GMs between groups.

^dStudy participants with missing data for LD ($n = 2$), ADHD ($n = 5$), and LD + ADHD ($n = 7$).

Table 3. Association between pyrethroid exposure and parent-reported LD, ADHD, and both LD and ADHD.^{a,b}

Neurodevelopmental outcome	cOR (95% CI)	p	aOR ^c (95% CI)	p	aOR _{creat} ^c (95% CI)	p
3-PBA (n = 1680)						
LD	1.24 (1.00, 1.55)	0.05	1.18 (0.92, 1.51)	0.19	1.11 (0.83, 1.48)	0.49
ADHD	1.26 (0.96, 1.65)	0.10	1.16 (0.85, 1.58)	0.35	1.06 (0.76, 1.48)	0.72
LD+ADHD	1.55 (1.04, 2.32)	0.03	1.45 (0.92, 2.27)	0.11	1.31 (0.82, 2.09)	0.26
cis-DCCA (n = 1659)						
LD	1.58 (0.97, 2.58)	0.07	1.43 (0.83, 2.48)	0.20	1.36 (0.76, 2.43)	0.31
ADHD	1.08 (0.71, 1.64)	0.72	1.00 (0.64, 1.57)	0.99	0.90 (0.56, 1.45)	0.68
LD+ADHD	1.30 (0.59, 2.84)	0.51	1.23 (0.56, 2.70)	0.61	1.08 (0.47, 2.46)	0.86
trans-DCCA (n = 1669)						
LD	1.54 (0.92, 2.60)	0.10	1.46 (0.85, 2.50)	0.17	1.36 (0.74, 2.49)	0.33
ADHD	1.46 (0.86, 2.48)	0.16	1.29 (0.72, 2.33)	0.40	1.15 (0.62, 2.13)	0.65
LD+ADHD	2.04 (0.97, 4.29)	0.06	1.84 (0.88, 3.84)	0.11	1.62 (0.74, 3.56)	0.23

Abbreviations: cOR = Crude odds ratio; aOR = Adjusted odds ratio; aOR_{creat} = Adjusted odds ratio including creatinine in the model.

^aModel sample sizes were based on the number of children with complete information on exposure, outcome, and covariates. Thus, estimates provided (i.e., crude OR, aOR, and aOR_{creat}) are based on the same set of observations indicated for each metabolite. ^bPyrethroid exposure was modeled as follows: for 3-PBA we used log₁₀-transformed concentrations; for *cis*- and *trans*-DCCA we used a dichotomous variable (detected vs. not detected) given their low detection frequencies. Number of children with LD, ADHD, and LD+ADHD and a detectable concentration of *cis*-DCCA were N = 88, N = 49, N = 26, respectively. Number of children with LD, ADHD, and LD+ADHD and a detectable concentration of *trans*-DCCA were N = 81, N = 54, N = 32, respectively. ^cModels adjusted for sex, age, race/ethnicity, household reference education level, low birth weight status, maternal age at child's birth, NICU admission, maternal smoking during pregnancy, daycare/preschool attendance, and health insurance.