Environment International 70 (2014) 125–131

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood ☆

Melissa A. Furlong [a,*], Stephanie M. Engel [a], Dana Boyd Barr [b], Mary S. Wolff [c]

[a] Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
[b] Rollins School of Public Health, Emory University, Atlanta, GA, United States
[c] Department of Preventive Medicine, Mount Sinai School of Medicine, New York, NY, United States

ARTICLE INFO

Article history:
Received 8 December 2013
Accepted 12 May 2014
Available online 13 June 2014

Keywords:
Social Responsiveness Scale
Organophosphate pesticides
Environmental exposures
Neurodevelopment

ABSTRACT

Prenatal exposure to organophosphate pesticides (OPs) has been associated with adverse neurodevelopmental outcomes in childhood, including low IQ, pervasive developmental disorder (PDD), attention problems and ADHD. Many of these disorders involve impairments in social functioning. Thus, we investigated the relationship between biomarkers of prenatal OP exposure and impaired reciprocal social behavior in childhood, as measured by the Social Responsiveness Scale (SRS). Using a multi-ethnic urban prospective cohort of mother–infant pairs in New York City recruited between 1998 and 2002 (n = 404) we examined the relation between third trimester maternal urinary levels of dialkylphosphate (ΣDAP) OP metabolites and SRS scores among 136 children who returned for the 7–9 year visit. Overall, there was no association between OPs and SRS scores, although in multivariate adjusted models, associations were heterogeneous by race and by sex. Among blacks, each 10-fold increase in total diethylphosphates (ΣDEP) was associated with poorer social responsiveness (β = 5.1 points, 95% confidence interval (CI) 0.8, 9.4). There was no association among whites or Hispanics, or for total ΣDAP or total dimethylphosphate (ΣDMP) biomarker levels. Additionally, stratum-specific models supported a stronger negative association among boys for ΣDEPs (β = 3.5 points, 95% CI 0.2, 6.8), with no notable association among girls. Our results support an association of prenatal OP exposure with deficits in social functioning among blacks and among boys, although this may be in part reflective of differences in exposure patterns.

1. Introduction

Organophosphate pesticides (OPs) are acutely neurotoxic at high doses. The primary mechanism of action at these doses is inhibition of acetylcholinesterase, which leads to an accumulation of acetylcholine at the neuronal junction (Sultatos, 1994). At low doses, OPs have several suspected mechanisms of action, including disruption of nuclear transcription factors (Dam et al., 2003), interference with neural cell development and neurotransmitter systems (Aldridge et al., 2005b), and altered synaptic formation (Qiao et al., 2003). Fetuses and babies are thought to be highly susceptible to OP exposure, due to the ready transmission of OPs through the placenta, and the immaturity of metabolic pathways required to process and excrete these compounds (Landrigan, 1999; Whyatt et al., 2005). OPs were removed from the USA market for most residential uses in the early 2000s, although exposure in the general population can still occur through dust reservoirs and ingestion from approved agricultural uses. (Stout et al., 2009).

OP exposure has been negatively associated with a wide range of childhood cognitive and behavioral outcomes. Three independent birth cohort studies in the USA found prenatal OP exposure to be associated with lowered IQ in childhood (Bouchard et al., 2011; Engel et al., 2011; Eskenazi et al., 2007; Rauh et al., 2011), although the specific cognitive domains most strongly associated with exposure varied among populations. Two different cohort studies have found an association between prenatal OP exposure and attention-deficit hyperactivity disorder (ADHD)-like behaviors at 3 (Rauh et al., 2006) and 5 (Marks et al., 2010) years of age, although no association was found at 2 years (Eskenazi et al., 2007). Using a parent report instrument, the Child Behavior Checklist, these same studies also report associations with pervasive developmental disorder (PDD), an umbrella term which includes

Abbreviations: OP, organophosphate pesticide; SRS, Social Responsiveness Scale; ASD, autism spectrum disorders; ADHD, attention-deficit-hyperactivity disorder; CBCL, Child Behavior Checklist; MRI, magnetic resonance imaging; PDD, pervasive developmental disorder; DAPs, ∑ dialkylphosphates; DEPs, ∑ diethylphosphate; DMPs, ∑ dimethylphosphates.

☆ Funding: This work was supported by the National Institute of Environmental Health Sciences/U.S. Environmental Protection Agency Children's Center grants ES09584 and R827039, the New York Community Trust, and the Agency for Toxic Substances and Disease Registry/Centers for Disease Control and Prevention (CDC)/Association of Teachers of Preventive Medicine. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC. M. Furlong was supported partly by the University of North Carolina Graduate School's Merit Fellowship. MF also partly supported by NIEHS institutional training grant ES07018.

* Corresponding author at: University of North Carolina at Chapel Hill, Box 7435, Chapel Hill 27599, United States.
E-mail address: furlongm@med.unc.edu (M.A. Furlong).

http://dx.doi.org/10.1016/j.envint.2014.05.011
0160-4120/

autism spectrum disorders (ASDs) (Eskenazi et al., 2007; Rauh et al., 2006), although in both studies the number of putative cases was quite small.

The Social Responsiveness Scale (SRS) is a parent/caregiver survey designed to quantify impairments in reciprocal social behaviors (Constantino and Gruber, 2005). While these impairments are useful in distinguishing autism spectrum disorder (ASD) from other child psychiatric conditions (Constantino and Gruber, 2005), they are not necessarily specific to ASD, in that deficits in social reciprocity may also be found in conditions such as ADHD, language problems, maladaptive behavior, social anxiety, and mood disorders (Constantino et al., 2003; Hus et al., 2013; Pine et al., 2008; Reiersen et al., 2007). Therefore, higher scores on the SRS may highlight a neurobehavioral social impairment common to multiple neuropsychiatric conditions (Hus et al., 2013). Given that a number of recent studies have linked prenatal OP exposure with neuropsychiatric conditions that involve impaired social functioning (ADHD, PDD), we explored whether exposure to OPs in utero was associated with a continuous measure of impaired social responsiveness in childhood, and whether associations varied according to race/ethnicity or child sex.

2. Methods

2.1. Cohort enrollment and follow-up

The Mount Sinai Children's Environmental Health Study is a prospective multiethnic cohort of primiparous women with singleton pregnancies who delivered at the Mount Sinai Hospital between May 1998 and July 2001 (Berkowitz et al., 2003, 2004). Women were recruited at either the Mount Sinai Diagnostic and Treatment Center, which serves a predominantly minority East Harlem population, or at one of two private practices on the Upper East Side of Manhattan. Of the 479 mother–infant pairs that were successfully recruited, 75 were excluded for reasons described elsewhere (Engel et al., 2007), for a final cohort of 404 mother–infant pairs for whom birth data were available. During approximately the third trimester of pregnancy, maternal urinary specimens were obtained and questionnaires were administered to participants to obtain information on sociodemographic characteristics, medical history, and lifestyle factors. Delivery characteristics and birth outcomes were obtained from a perinatal database. Women were invited to return with their child for follow-up visits at ages 1, 2, 4, 6 and 7–9 years. At the 7–9 year evaluation, mothers completed the SRS ($n = 136$).

2.2. Reciprocal social behavior outcomes

The SRS is a 65-item caregiver rating scale of social behaviors characteristic of autism spectrum and related disorders for children ages 4 to 18 (Constantino and Gruber, 2005). Each item rates the frequency of a behavior on a 4 point Likert scale, with higher scores indicating more symptoms of impairment (Constantino and Gruber, 2005). Raw scores are sex-standardized to T-scores with a mean of 50 and a standard deviation of 10, and are calculated separately for boys and girls. The SRS has good test–retest temporal stability, parent–parent and parent–teacher interrater agreement and discriminate and concurrent validity (Constantino and Gruber, 2005). T-scores over 60 are described as possible indicators of mild/moderate impairment, and T-scores over 75 are possible indicators of severe impairment.

2.3. Pesticide metabolite measurements

Maternal urine samples were collected between 25 and 40 weeks' gestation (mean of 31.2 weeks, sd of 3.7) and were analyzed by the Centers for Disease Control and Prevention (Atlanta, Georgia) for six dialkylphosphate (DAP, in nm/L) metabolites in two batches using lyophilization, derivatization to form chloropropyl phosphate esters and analysis using gas chromatography–tandem mass spectrometry. Isotope dilution quantification was performed with 10% quality control samples included in each run (Bravo et al., 2004).

Metabolite levels that were missing due to analytic interference were imputed using regression analysis to predict the missing metabolite from the other non-missing metabolites measured for that woman ($n = 6/136$ (4.4%) of DEP metabolites, $n = 0$ of DMP metabolites), as has been previously described (Engel et al., 2007). Prior to imputation, samples below the limit of detection were assigned a value of the LOD/$\sqrt{2}$. Diethyl- (DEP) and dimethylphosphate (DMP) metabolites were then summed on a molar basis (as nm/L) to respectively obtain total diethylphosphates (ΣDEP) and total dimethylphosphates (ΣDMP) (Barr et al., 2011). These were together summed to obtain total dialkylphosphates (ΣDAPs). Overall, approximately 97%, 89%, and 90% of the cohort had detectable levels of DAP, DEP and DMP metabolites, respectively.

2.4. Statistical analyses

All analyses were performed in SAS version 9.3 (SAS Institute, Inc, Cary, NC). ΣDAPs, ΣDEPs, and ΣDMPs were transformed using log base 10 to approximate a normal distribution. Very dilute urine samples containing less than 10 mg/dL of creatinine ($n = 1$) were excluded from statistical analyses, consistent with methods previously described (Engel et al., 2007; Eskenazi et al., 2004). OP metabolite biomarker levels (nm/L) were examined continuously on a $\log_{10}$ basis and using creatinine-corrected tertiles (nm/gC).

Multivariate linear regression was used to analyze relationships between $\log_{10}$ ΣDAPs and the dependent variable, continuous SRS total scores. Potential covariates for the model were selected after examining a directed acyclic graph (DAG) for confounding. Variables that the DAG identified as likely mediators or colliders were not considered for inclusion in the model (Rothman et al., 2008). After constructing our DAG, and due to our small sample size, we used a backward elimination method to obtain the most parsimonious model (Weng et al., 2009). We eliminated covariates that did not change the estimate of the main effect by more than 20% unless they improved the precision of the model. Analyzing the DAG yielded a potential covariate list that included maternal age, maternal education (dichotomous for high school or less), race/ethnicity (disjoint class variable for White, Black, Hispanic), marital status (dichotomous for married/living with partner or single), child sex, any smoking during pregnancy, breastfeeding, housing status (categorical indicators for public housing, private rentals, and ownership), child age in months at the time of testing, and urine creatinine. After backward selection, final included variables were maternal education, race/ethnicity, marital status, child age, housing status, and natural log transformed creatinine. Five mothers had missing data on either education or marital status and were excluded from analyses, while two mothers had missing $\sum$ DEP data and were excluded from models examining $\sum$ DEPs and $\sum$ DAPs. Previous studies have suggested that organophosphate pesticide associations may be differential by sex (Marks et al., 2010; Rauh et al., 2012), and previous studies in this population have suggested that effects may also be differential by race (Engel et al., 2011). Thus, additional models testing effect measure modification by child sex and race/ethnicity were also explored (interaction $\alpha < 0.20$), and effects were estimated for each race and sex strata regardless of interaction p-values due to this a priori hypothesis. Although an alpha of 0.20 increases the type I error rate, we accepted this rate in exchange for investigating potentially meaningful associations by strata. We could not simultaneously test interactions by race and sex due to small cell size. Finally, we examined F-tests in ANOVA analyses and trend tests of tertiles in multivariate linear regression analyses of the creatinine-adjusted $\log_{10}$ total metabolites by sex and by race to assess linearity of the dose–response curve, in addition to analyzing restricted cubic splines of the dose–response function in exploratory analyses (data not shown).