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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

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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.

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* 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).

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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).

3. Results

3.1. Demographics and characteristics

Mothers were primarily young (59% <22 years), unmarried (57%) minorities (85% non-white) who had achieved a high school education or less (63%). Approximately 18% reported smoking at any time during pregnancy (Table 1). OP metabolite concentrations are summarized in Table 2. The geometric mean (GM) level of ΣDAPs was 76.9 nm/L (GSD 4.6), while the GM for ΣDEPs was 17.4 nm/L (GSD 4.63), and the GM for ΣDEPs was 41.7 (GSD 6.06). The average SRS total t score was 52.9 (SD 10.6), with 30 children scoring above 60 (the cut-off for mild/moderate impairment), and 6 children scoring above 75 (the cut-off for severe impairment) (Table 2).

3.2. Organophosphate pesticide metabolites and SRS scores

Overall, there were no associations between prenatal ∑DAPs, ∑DEPs, or ∑DMPs and total SRS t-scores (Table 3). However, associations between prenatal total diethylphosphate metabolite levels and SRS total score were heterogeneous by race and by sex (∑DEP p-interaction = 0.06 for race and ∑DEP p-interaction = 0.12 for sex) in multivariate adjusted models. Stratum-specific estimates indicated stronger associations between ∑DEP and SRS total scores among boys than girls, and stronger associations among blacks than whites and Hispanics. Because the stratum specific effects for whites and Hispanics were similar (data not shown), we combined these strata to improve our overall power.

In multivariate adjusted models including a race interaction term, increasing ∑DEP biomarker levels were associated with poorer scores on the SRS among blacks (β = 5.1 points, 95% CI 0.8, 9.4), with no effect among whites or Hispanics (β = 0.2 points, 95% CI −2.8, 3.2). Likewise, associations were stronger among boys (β = 3.5 points, 95% CI 0.2, 6.8), than girls (β = −0.4 points, 95% CI −4.1, 3.3) (Table 3 and Fig. 1a). In both cases, due to our small sample size, confidence intervals were wide; however the overall ∑DEP estimate also supported the suggestion of poorer SRS scores with increasing metabolite levels (β = 1.8 points, 95% −0.7, 4.3). ∑DEP tertiles (nm/gC) suggested a dose-dependent effect on total SRS scores among blacks and among boys, although the trend test and F-test were only significant for boys (p for trend boys = 0.01 and F-test for boys = 0.03; p for trend blacks = 0.21, F-test for blacks = 0.43). Additionally, tertiles 1 and 2 for males were not different from each other (p = 0.76), although both were different from tertile 3 (tertile 1 vs tertile 3 p = 0.02, tertile 2 vs tertile 3 p = 0.03), which may be suggestive of non-linearity among boys (Fig. 2).

Neither overall, nor within strata of race/ethnicity or child sex did we see any notable associations of prenatal total ∑DAP or ∑DMP with total SRS score (Table 3 and Fig. 1b). In general, effects hovered around the null (Fig. 1b).

We tested response bias with Satterthwaite pooled t-tests to determine whether mothers with SRS follow-up data were different from mothers who did not return for the SRS follow-up visit, with respect to maternal education, prenatal smoking, alcohol consumption during pregnancy, marital status, and mother's age at birth. There was no evidence of response bias except for marital status, in that mothers who were married or living with the baby's father at enrollment were significantly less likely to return for a follow-up visit than unmarried mothers.

Table 1
Characteristics of study population (n = 136).[a]

Characteristic	Category	N	%
Maternal education	High school or less	82	63
	Some college or higher	49	37
	Missing	5	
Marital status	Married or living with baby's father	58	43
	Not married or living with partner	76	57
	Missing	2	
Race/ethnicity	Hispanic	71	52
	Black	45	33
	White	20	15
	Missing	0	
Sex	Male	68	50
	Female	68	50
	Missing	0	
Any smoking during pregnancy	No	112	82
	Yes	24	18
	Missing	0	
Mother's age	<22 years	80	59
	22–34 years	45	33
	35 years and older	11	8

[a] Excludes subjects with creatinine ≤ 10 mg/dL.

4. Discussion

4.1. Findings

This study adds to the growing body of literature investigating the neurobehavioral consequences of prenatal OP exposure. Although we found little evidence of any overall association of dialkylphosphate metabolite concentrations with social-reciprocal behavioral deficits, there was some suggestion of an association between increasing prenatal ∑DEP metabolite concentrations and adverse SRS scores among blacks and among boys. One of DEPs parent compounds is chlorpyrifos, which has previously been implicated in studies of the association between prenatal exposure to pesticides and child neurodevelopment (Rauh et al., 2006, 2011), and was widely used in New York City before the year 2000 (Thier et al., 1998).

4.2. Previous research

Unfortunately, there are few cohort studies of prenatal exposure to organophosphate pesticides and social behavior in childhood, and thus the literature is in large part attributable to a limited number of relatively small cohorts that have been studied intensively over a period of years, principally in the Salinas valley of California (Eskenazi et al., 2003), or in urban (Berkowitz et al., 2003; Perera et al., 2002) and semi-urban (Rauch et al., 2012) environments. The nature and extent of exposure in these populations are likely to vary substantially, given differences in parent compound applications, source, and time period. Nonetheless, some troubling patterns have begun to emerge. In a primarily Hispanic agricultural cohort, the CHAMACOS study, Eskenazi et al. (2007) reported a relationship between prenatal exposure to total ΣDAPs and ΣDMPs and parent-report based PDD in 2 year olds (measured by the Child Behavior Checklist), with no association found for ΣDEPs (Eskenazi et al., 2007). Notably, reported geometric means in the agricultural cohort for ΣDMPs were approximately twice the geometric mean in our cohort, and these differential levels of exposure may account for some of the differences in findings. It is also possible that the associations of ∑DAPs in the CHAMACOS cohort may be driven by the dimethylphosphate metabolites, since there was no association for ∑DEPs and the amount of ∑DMPs was considerably greater than the amount of ∑DEPs. In the same cohort, Marks et al. reported a relationship between prenatal exposure to total ΣDAPs and ΣDEPs and a derived variable for ADHD based on several instruments used to evaluate behavior (Marks et al., 2010). They further reported sex-specific effects for several of the outcome variables, including a sex-specific effect in males for the derived composite ADHD indicator. Rauh et al. (2006) reported an association between prenatal exposure to chlorpyrifos (which devolves into DEPs) and PDD in 2–3 year olds in an inner city cohort of blacks and Dominicans in New York City. They also reported associations between prenatal exposure to chlorpyrifos and attention problems and ADHD problems, as measured by the CBCL.

Table 2
Descriptive statistics of dialkylphosphate metabolite biomarker levels and Social Responsiveness Scale (SRS) total score (n = 136).

		N	Geometric mean (GSD)	Min	Max	IQR	5/95 percentile
∑DAP nm/L	All	134	76.9 (4.59)	0.50	4990	6.09	8.45/7112
	Boys	68	72.5 (3.27)	0.50	1240	6.45	8.63/475
	Girls	66	81.7 (6.16)	0.50	4990	7.78	0.49/956
	Blacks	44	90.5 (6.14)	0.50	4990	6.91	7.48/1240
	Whites & Hispanics	90	71.0 (3.92)	0.50	814	5.52	8.48/475
∑DEP nm/L	All	134	17.4 (4.63)	0.50	345	5.56	0.49/118
	Boys	68	14.5 (4.78)	0.50	208	5.16	0.49/104
	Girls	66	21.0 (4.43)	0.50	345	7.06	0.49/120
	Blacks	44	15.7 (4.90)	0.50	345	5.90	0.49/135
	Whites & Hispanics	90	18.3 (4.53)	0.50	223	6.14	0.49/113
∑DMP nm/L	All	135	41.7 (6.06)	0.50	4900	12.92	0.49/755
	Boys	68	38.1 (4.83)	0.50	1240	9.49	3.96/444
	Girls	67	45.7 (7.50)	0.50	4900	16.04	0.49/908
	Blacks	45	49.8 (8.50)	0.50	4900	14.09	0.49/1240
	Whites & Hispanics	90	38.2 (5.02)	0.50	788	9.46	3.96/415
Creatinine mg/dL	All	136	69.3 (2.19)	12.7	342	3.31	16.7/246
	Boys	68	69.0 (2.29)	12.7	342	3.26	16.2/241
	Girls	68	69.6 (2.10)	13.7	271	3.02	20.9/246
	Blacks	45	75.6 (2.38)	12.7	342	4.07	14.5/271
	Whites & Hispanics	91	66.4 (2.09)	14.4	270	3.08	18.0/194
SRS total score[1]	All	136	52.9 (10.6)	38.0	84.0	15.0	39.0/74.0
	Boys	68	52.4 (10.4)	38.0	84.0	16.5	38.0/72.0
	Girls	68	53.4 (10.8)	38.0	82.0	14.5	41.0/75.0
	Blacks	45	53.9 (11.9)	38.0	82.0	14.0	40.0/76.0
	Whites & Hispanics	91	52.4 (9.90)	38.0	84.0	15.0	39.0/72.0

[1] Arithmetic means and standard deviations, not geometric means, are reported for SRS total scores.

While our findings generally do not support evidence of an association with total ΣDAPs or ΣDMPs in any strata, they do provide evidence of an association with ΣDEP metabolites and social reciprocal deficits among blacks and among boys in our urban, inner-city population. The differences in associations between ∑DEPs, ∑DMPs, and ∑DAPs and behavior across studies may in part reflect the non-specificity of these biomarkers of exposure, as they arise from multiple different parent compounds of varying toxicity. ∑DEPs in our population, for instance, may reflect a different mixture of parent compounds than ∑DEPs in the CHAMACOS cohort. The difference in associations we observed according to race/ethnicity may reflect varying sources of exposure, different genotype frequencies for genes that govern metabolism and detoxification of organophosphate pesticides (Thyagarajan et al., 2008), and/or unmeasured social confounding. While the mean levels of ∑DEPs for whites and blacks were within one standard deviation of each other (Table 2), blacks had more variability in measured levels of ∑DEPs. The white population in this study is likely to have been primarily exposed to OPs through diet, which includes a mixture of parent compound and preformed metabolite exposure, the latter of which would not be toxic. Exposure through the diet is further complicated by co-exposures to potentially beneficial nutrients, such as folate consumption during the preconceptual and early pregnancy period, which has been found to be associated with lower risk of ASD (Schmidt et al., 2011), improved social competence scores, and fewer symptoms of inattention (Julvez et al., 2009). These co-exposures may compete with the toxic effects of exposure to parent OP compounds. Alternatively, a high proportion of the black and Hispanic populations studied here lived in low-income housing (Engel et al., 2011) and may have been exposed to indoor pest treatments, although we controlled for housing in our models. The lack of an effect in Hispanics may be due to differing genotypes, or to protective exposures from different dietary patterns. There is suggestive evidence that paraoxonase 1 (PON1) phenotype may modify associations of OPs with neurobehavior (Eskenazi et al., 2010). Unfortunately, our sample size is too small to reliably test interactions with *PON1* genotype while also assessing interactions with race and/or sex, although genetic susceptibility may be an important source of variability. Unmeasured confounding by social factors may also play a role; although we considered Medicaid status, housing type, maternal education, and marital status in our models, there may still be residual confounding by other social factors that are associated with race, prenatal pesticide exposure, and social responsiveness.

Our study found stronger associations of ΣDEP with social deficits among boys than girls. Boys are almost five times more likely than girls to be diagnosed with autism (Baio, 2012), and are more than twice as likely as girls to be diagnosed with ADHD (Visser et al., 2010). This could be indicative of greater environmental sensitivities in boys, or it could be due to a diagnostic or reporting bias if social deficits have a higher degree of recognition in boys. This finding could also be spurious. However, previous studies have found sex-specific effects of organophosphate pesticides on attention in boys (Marks et al., 2010), and animal studies suggest there may be greater neurological effects in males in response to chlorpyrifos (Slotkin and Seidler, 2005), or other sex-specific effects of organophosphate pesticides (Ricceri et al., 2006; Slotkin et al., 2008); thus our results seem plausible and are in line with the evolving literature.

Prenatal exposure to the organophosphate pesticide chlorpyrifos has also been found to result in structural changes in the brain (Rauh et al., 2012). In a structural MRI study of 5–11 year olds exposed prenatally to chlorpyrifos, higher levels of chlorpyrifos were associated with bilateral enlargement of temporal lobes, unilateral enlargement in the right hemisphere of the frontal lobe, and enlargement in the cuneus and precuneus (both of which are in the occipital lobe) (Rauh et al., 2012). Structural brain differences have also been found in children with autism and ADHD. Autistic 2–4 year old children have been found to have enlarged frontal and temporal lobes, and enlarged amygdala and hippocampi (Carper and Courchesne, 2005; Courchesne et al., 2007; Sparks et al., 2002). Children with ADHD also have unilateral enlargement in the right frontal hemisphere, specifically the prefrontal cortex (Krain and Castellanos, 2006). Animal models of in utero exposure to organophosphates provide further evidence that they play a role in depression and anxiety, which are also associated with higher SRS scores (Pine et al., 2008). Mouse and rat models support the hypothesis that in utero exposure to low-levels of chlorpyrifos alters serotoninergic functioning in the absence of cholinergic effects. Serotonin is a critical neurotransmitter in the regulation of anxiety and depression, and OP exposure also increases behaviors associated with anxiety and depression (Aldridge et al., 2005a; Venerosi et al., 2010). Animal models have also shown that adult exposure to low-levels of chlorpyrifos

Table 3
Dialkylphosphate metabolite associations with total Social Responsiveness Scale score, by race and by sex.

	Log_{10} ∑DAP Unadjusted[c] β (95% CI)	Log_{10} ∑DAP Adjusted β (95% CI)	Log_{10} ∑DEP Unadjusted[c] β (95% CI)	Log_{10} ∑DEP Adjusted β (95% CI)	Log_{10} ∑DMP Unadjusted β (95% CI)	Log_{10} ∑DMP Adjusted β (95% CI)
All n = 129	−0.9 (−3.6, 1.7)	0.3 (−2.3, 2.9)	1.1 (−1.6, 3.8)	1.8 (−0.7, 4.3)	−1.0 (−3.3, 1.3)	−0.2 (−2.4, 2.0)
Black[a] n = 42	−0.6 (−4.5, 3.4)	1.8 (−2.2, 5.8)	2.3 (−2.2, 6.8)	**5.1** (**0.8**, **9.4**)	−0.9 (−4.2, 2.4)	0.4 (−2.8, 3.7)
Whites and Hispanics n = 87	−1.3 (−5.0, 2.3)	−1.0 (−4.3, 2.3)	0.5 (−2.7, 3.8)	0.2 (−2.8, 3.2)	−1.2 (−4.3, 1.9)	−1.1 (−3.9, 1.7)
p interaction	0.78	0.29	0.54	**0.06**	0.89	0.48
Boys[b] (n = 66)	0.7 (−4.0, 5.5)	1.6 (−3.0, 6.1)	2.4 (−1.2, 6.1)	**3.5** (**0.2**, **6.8**)	−0.2 (−3.9, 3.5)	0.7 (−2.8, 4.2)
Girls (n = 63)	−1.7 (−4.9, 1.5)	−0.4 (−3.6, 2.8)	−0.5 (−4.4, 3.4)	−0.4 (−4.1, 3.3)	−1.6 (−4.5, 1.3)	−0.8 (−3.7, 2.0)
p interaction	0.41	0.50	0.28	**0.12**	0.56	0.52

Bolded estimates indicate $p < 0.05$ for stratum-specific effects and $p < 0.20$ for interaction p-values.
[a] Race interaction models adjusted for education, marital status, sex, housing status, child age, and creatinine.
[b] Sex interaction models adjusted for education, marital status, creatinine, housing status, child age, and three category race variable (black, Hispanic, white).
[c] "Unadjusted" models adjusted for log_e creatinine.

impairs attention and increases impulsivity in rats (Middlemore-Risher et al., 2010).

While it may be tempting to equate deficits in social responsiveness with ASD, there is no evidence actually associating prenatal exposure to organophosphate pesticides with development of ASD. Although previous studies have linked OP exposure to CBCL scores that are indicative of PDD, an umbrella diagnosis that includes ASD, these studies likely suffered from a relatively high degree of outcome misclassification. Population-based studies suggest the frequency of ASD in the general population to be approximately 1/88 children (Baio, 2012). Rauh et al. (2006) reported that 4.7% of the NYC cohort met the cut-off for PDD, which yielded 11 cases and an OR estimate of 5.39 with a 95% confidence interval of 1.2, 24.11. This large confidence interval suggests sparse data. Additionally, Eskenazi et al. (2007) reported that 14.4% of the CHAMACOS cohort met criteria for clinically significant PDD, which is much higher than the expected ~1% prevalence of autism in the general population, indicating a likelihood that, as with the SRS, the CBCL's clinically significant cut-off may have a low positive predictive value. However, even with misclassification in the precise clinical diagnosis, these studies and ours are suggestive of a relationship between prenatal organophosphate pesticide exposure and social impairments, which are common across multiple neuropsychiatric conditions, including ADHD, autism, depression, and mood disorders.

4.3. Study limitations

Finally, there are several limitations to our study that should be considered while interpreting our results. Although the sample size at birth (n = 404) is comparable in size to other longitudinal birth cohorts in the United States, the substantial loss-to-follow-up has resulted in a relatively small sample at ages 7 to 9 for this study. In our study, loss-to-follow-up appeared to only be associated with marital status, which was adjusted for in this analysis. Even so, it is possible that other unmeasured covariates are associated with loss-to-follow-up, which could result in residual uncontrolled confounding. In addition, the small sample size available for analysis results in reduced power for statistical associations and interaction analyses. It is possible that the heterogeneity in associations we observed according to race is attributable in part to residual confounding by social factors that we cannot account for with our existing covariates. Additionally, we lack postnatal exposure measurements in the children that would allow us to consider whether exposure during other periods of development might impact social functioning. Although one study that examined associations between prenatal organophosphate pesticide exposure and IQ in childhood found that postnatal exposure was not an important predictor of the outcome when prenatal exposure was accounted for (Bouchard et al., 2011), it is still possible that postnatal exposure to organophosphate pesticides accounts for some of the variability in childhood neurobehavioral development. Nonetheless we would not expect postnatal exposure to bias any prenatal exposure associations given that it would temporally follow prenatal exposure. Still, future studies should consider whether later exposure windows are relevant to child social functioning and neurobehavioral development.

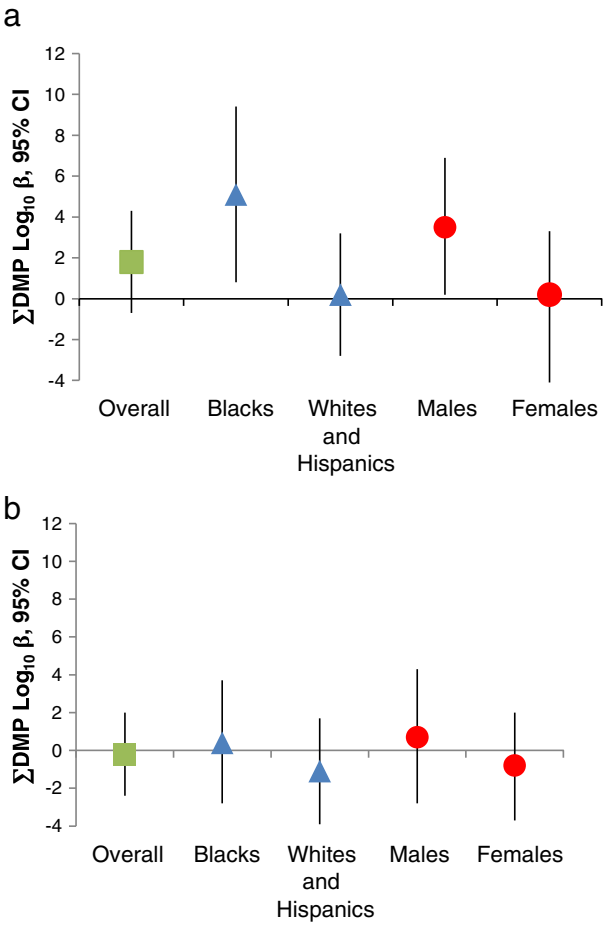

Fig. 1. Overall and stratum-specific associations of log_{10} dialkylphosphate metabolite levels and total SRS score. Fig. 1a shows the association between increasing log_{10} ∑DEPs and total SRS t-scores according to race and sex. Stronger adverse associations with SRS score were found for blacks and boys, with no associations among Whites/Hispanics and girls (Race p-interaction = 0.06; Sex p-interaction = 0.12). Fig. 1b shows null associations between log_{10} ∑DMPs and SRS total t-scores overall, within strata of Race/ethnicity and sex, and no interaction (Race p-interaction = 0.48, Sex-interaction = 0.52). Models adjusted for log creatinine, mother's marital status, mother's education, housing status, child age, race, and sex.

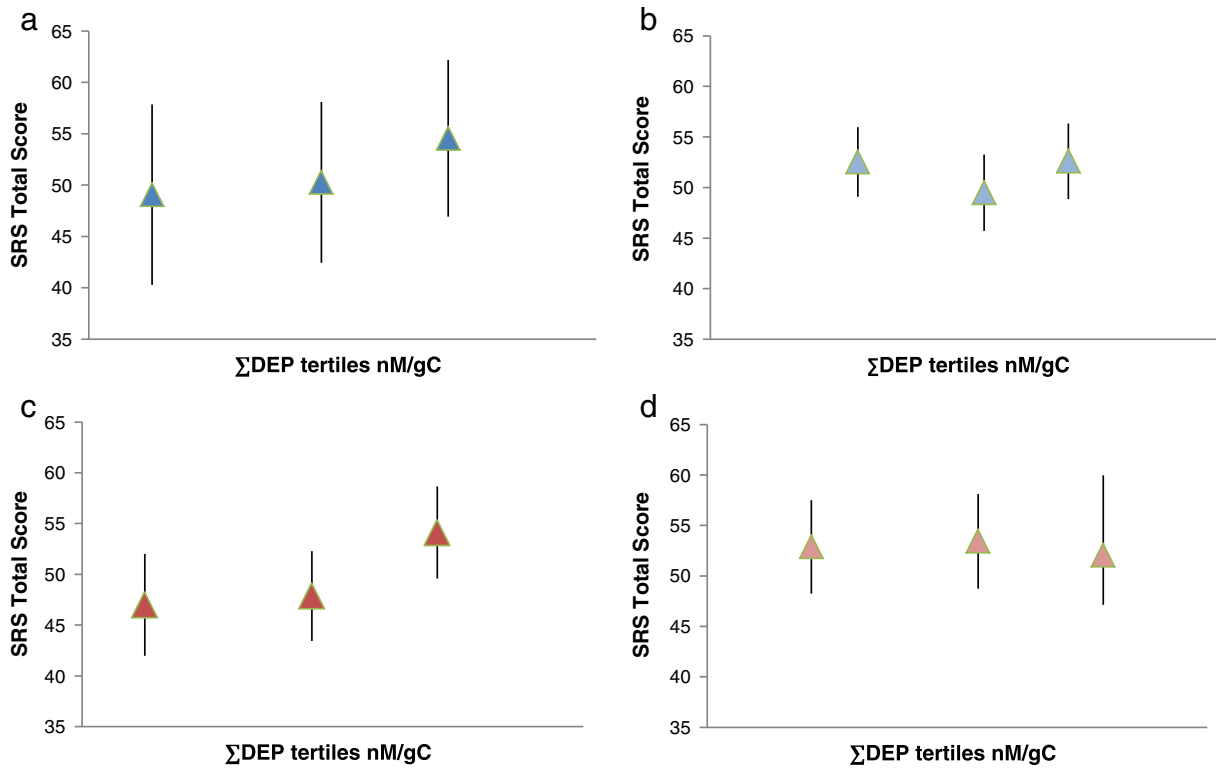

Fig. 2. Associations of ∑ DEP tertiles with predicted SRS total scores within strata of race/ethnicity and sex. Tertiles suggest a weak dose–response relationship among Blacks (p-trend = 0.12) and no association among Whites/Hispanics (p-trend = 0.71). Race-specific tertiles as a group did not predict total SRS t-scores (Type 3 F-test p for tertile: Blacks = 0.43; Whites/Hispanics = 0.36). In sex-specific analysis, tertiles suggest an elevated association in the third tertile among boys (p-trend = 0.01) and no relationship among girls (p-trend = 0.79). Sex-specific tertiles as a group predicted SRS total scores among boys, but not among girls (Type 3 F-test p for tertile: Boys = 0.03; Girls = 0.90).

5. Conclusions

We report an association between prenatal biomarkers of ΣDEPs and poorer scores on the Social Responsiveness Scale among blacks and possibly among boys in New York City. Race-specific effects may be due to varying sources of exposure or other susceptibility factors, while sex-specific effects may be due to enhanced environmental sensitivity of boys. These results indicate a possible relationship between prenatal organophosphate pesticide use and social impairment in childhood, which is a common component of multiple prevalent neuropsychiatric conditions of childhood.

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