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Prenatal Organophosphate Pesticide Exposure and Traits Related to Autism Spectrum Disorders in a Population Living in Proximity to Agriculture

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Background: Prenatal exposure to organophosphate (OP) pesticides has been linked with poorer neurodevelopment and behaviors related to autism spectrum disorders (ASD) in previous studies, including in the Center for Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, a birth cohort living in the agricultural Salinas Valley in California.

Objectives: To investigate the association of prenatal exposure to OP pesticides with traits related to ASD, in childhood and adolescents in CHAMACOS.

Methods: We assessed OP exposure during pregnancy with measurements of dialkyl phosphates (DAP) metabolites in urine, and residential proximity to OP use during pregnancy using California's Pesticide Use Reporting (PUR) data and estimated associations with ASD-related traits using linear regression models. We measured traits reported by parents and teachers as well as the child's performance on tests that evaluate the ability to use facial expressions to recognize the mental state of others at 7, 10½, and 14 years of age.

Results: Prenatal DAPs were associated with poorer parent and teacher reported social behavior [e.g., a 10-fold DAP increase was associated with a 2.7-point increase (95% confidence interval (CI): 0.9, 4.5) in parent-reported Social Responsiveness Scale, Version 2, T-scores at age 14]. We did not find clear evidence of associations between residential proximity to OP use during pregnancy and ASD-related traits.

Conclusions: These findings contribute mixed evidence linking OP pesticide exposures with traits related to developmental disorders like ASD. Subtle pesticide-related effects on ASD-related traits among a population with ubiquitous exposure could result in a rise in cases of clinically diagnosed disorders like ASD. https://doi.org/10.1289/EHP2580

Introduction

There is increasing recognition that modifiable environmental factors play an important role in the etiology of neurodevelopmental disorders such as autism spectrum disorders (ASD) (Hallmayer et al. 2011). Traits related to ASD, such as poor social communication and interaction, restricted interests, and repetitive behaviors exhibit a normal distribution in the general population, with individuals with disorders such as ASD falling at the extreme end (Constantino 2011). More severe ASD-related traits can result in lifelong educational, vocational, adaptive functioning, and mental health challenges among individuals with and without a clinically diagnosed disorder. Assessing exposure-related associations with quantitative dimensional traits of ASD offers significant advantages over studying clinically diagnosed disorders, which include reduced outcome misclassification, identification of more specific exposure-related biologic pathways, and greater sensitivity to detect associations with ubiquitous environmental chemical exposures (Sagiv et al. 2015a).

Organophosphate (OP) pesticides are cholinesterase-inhibiting insecticides currently used primarily in agriculture. Although use of OPs in the United States has declined considerably over the past several decades, OPs are still among the most widely used pesticides in the United States. In addition, 539,232 pounds of OPs were applied in the Salinas Valley in 2000, the prenatal period for children in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study (California Department of Pesticide Regulation 2002). Low-dose exposure to OPs can adversely affect human neurodevelopment, particularly if exposure occurs during the prenatal period when the fetal brain is undergoing rapid development and the ability to detoxify OPs is not yet mature (Eskenazi et al. 1999).

CHAMACOS is a longitudinal birth cohort study initiated to investigate pesticide and other environmental exposures in relation to the health and development of children living in agricultural communities in the Salinas Valley, California. As previously reported, OP levels in CHAMACOS, measured as dialkyl phosphate (DAP) metabolites in urine collected during pregnancy, were considerably higher than levels among women of childbearing age in the general U.S. population, as would be expected in a community living in close proximity to agriculture (Bradman et al. 2005). In CHAMACOS, we previously reported associations of prenatal DAPs with lower IQ (Bouchard et al. 2011), poorer attention (Marks et al. 2010), and a higher likelihood for scoring above the 97th percentile of the national normative sample on the pervasive developmental disorder scale (which includes symptoms consistent with ASD) for the maternally reported Child Behavior Checklist at 24 mo (10-fold increase in DAPs OR = 2.3; 95% confidence interval (CI): 1.0, 5.2) (Eskenazi et al. 2007). In addition, residential proximity to pesticide use during pregnancy, estimated with California Pesticide Use Reporting (PUR) data (California Department of Pesticide Regulation 2002), was associated with childhood IQ in CHAMACOS, but not behaviors related to attention deficit hyperactivity (ADHD), including inattention and hyperactivity. Other epidemiologic studies have reported mixed associations of exposure to OP pesticides with neurodevelopment, including traits related to ASD (Furlong et al. 2014; Shelton et al. 2014).

Our main objective was to investigate the association of prenatal exposure to OP pesticides with ASD-related traits, measured in childhood and adolescence in the CHAMACOS birth cohort. Rich measures of prenatal OP pesticide exposure,

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measured in urine collected during pregnancy and estimated using residential proximity to agricultural OP pesticide use data, and of ASD-related traits in children ages 7, 10½, and 14 y, allowed us to thoroughly examine this association in a population living in proximity to agriculture.

Methods

Study Sample

Pregnant women in California's Salinas Valley were recruited between October 1999 and October 2000. Eligible pregnant women (≥18 years old, <20 weeks gestation, Spanish- or English-speaking, qualifying for low-income health insurance, and planning to deliver at the public hospital) were enrolled via the community clinics at which they received prenatal care. A total of 601 women who were enrolled in the study went on to deliver 536 live-born infants, including five twin sets, who remained in the study at birth. Women were interviewed twice during pregnancy ($n=601$), after delivery ($n=536$), and when children were ages 6 mo ($n=433$), 1 y ($n=441$), 2 y ($n=414$), 3½ y ($n=365$), 5 y ($n=350$), 7 y ($n=353$), 9 y ($n=337$), 10½ ($n=328$), 12 y ($n=345$), and 14 y ($n=333$).

Written informed consent was obtained from all mothers. Children provided verbal assent at ages 7 y, 10½ y, and 12 y, and provided written assent at 14 y. Study activities were approved by the University of California, Berkeley Committee for the Protection of Human Subjects.

Organophosphate Urinary Metabolites

We assessed prenatal exposure to organophosphate pesticides using measures of dialkyl phosphate (DAP) metabolites in maternal urine collected at the early pregnancy interview (13 wk) and later pregnancy interview (26 wk). Urine samples were aliquoted and stored at −80°C until they were shipped on dry ice to the Centers for Disease Control and Prevention, where they were analyzed using gas chromatography-tandem mass spectrometry and quantified using isotope dilution calibration (Bravo et al. 2002). Details of collection, analysis and quality control were described previously (Bradman et al. 2005). We quantified 6 nonspecific DAP urinary metabolites: 3 dimethyl (DM) phosphate metabolites (dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate) and 3 diethyl (DE) phosphate metabolites (diethylphosphate, diethylthiophosphate, and diethyldithiophosphate). Values below the limit of detection (LOD) were randomly imputed based on a log-normal probability distribution whose parameters were estimated using maximum-likelihood estimation. We used a commercially available diagnostic enzyme method to measure urinary creatinine concentrations (Vitros CREA slides; Ortho Clinical Diagnostics).

Geographic-based Residential Proximity to OP Pesticide Use

We previously reported our methods for estimating prenatal residential proximity to agricultural OP pesticide use (Gunier et al. 2016). Briefly, using a geographic information system to combine California Pesticide Use Reporting (PUR) data and latitude and longitude coordinates from the maternal residence during pregnancy, we estimated the amount (kilograms) of agricultural OP pesticide use within a 1-km radius of the residence during each mother's pregnancy (California Department of Pesticide Regulation 2002). We included children whose prenatal residential location was known for at least 75 d per trimester during two or more trimesters of pregnancy and computed the average pesticide use during pregnancy by summing the trimester-specific values and dividing by the number of trimesters included. We selected a 1-km buffer distance for this analysis because it best captures the spatial scale most strongly correlated with measured agricultural pesticide concentrations in house-dust samples (Harnly et al. 2009; Gunier et al. 2011). We replaced PUR data outliers that had unusually high application rates (>2 standard deviation (SD) above the mean application rate, likely data-entry errors) with the median application rate for that pesticide and crop combination (Gunier et al. 2001). We examined pesticide use in several ways: as the use of the four most common individual OP pesticides (chlorpyrifos, diazinon, malathion, and oxydemeton-methyl); the sum of all OP pesticide use in the region (15 total); and as a toxicity-weighted sum of OPs using relative potency factors that are the ratio of the relevant toxicological dose of each individual OP pesticide to chlorpyrifos (U.S. EPA 2006), and then summed as the kg-equivalents of chlorpyrifos (Gunier et al. 2016).

Assessment of ASD-related Traits

Participants were administered several instruments to directly assess or capture parent or teacher reports on children's traits related to ASD. Parents reported their child's social behavior with the Social Responsiveness Scale, Version 2 (SRS-2) at age 14 y and their child's social skills with the Behavioral Assessment Scale for Children, Version 2 (BASC-2) at ages 7, 10½, and 14 y. Teachers also reported the child's social skills in the school setting at age 7 using the BASC-2. In addition, study staff tested children directly with two tests that evaluate the ability to use facial expressions to recognize the mental state of others. Staff administered the Evaluación Neuropsicológica Infantil (ENI) Facial Expression Recognition Test at age 9 y and the NEPSY-II Affect Recognition subtest at age 12 y. These tests assess theory of mind, which involves the ability to infer mental states, including thoughts, feelings, and intentions of other individuals (Premack and Woodruff 1978). Assessments were performed by bilingual psychometricians. Mothers were interviewed in their dominant language (Spanish or English), teachers completed surveys in English, and children were assessed in their dominant language at age 9 y and in English at age 12 y (all had demonstrated English proficiency by that point). All tests are described in detail below and validation information for each test is reported in Supplemental Material.

Social responsiveness scale version 2 (SRS-2). The SRS-2 is a 65-item rating scale developed to assess quantitative traits related to autism in large research samples (Constantino et al. 2003; Constantino and Gruber 2012; Constantino et al. 2000). The SRS has been well validated for use among general population samples (Constantino et al. 2003), where it shows a broad distribution of scores (Constantino and Todd 2003). We used the school-aged parent-report form for children age 4–18 y, which tracks well across this wide age range (Constantino and Gruber 2012). Parents were asked to respond about the frequency in which their child exhibited specific behaviors over the past 6 mo (1 = not true, 2 = sometimes true, 3 = often true, and 4 = almost always true). Examples of behavioral items include: "Avoids social interactions with peers or adults" and "Has repetitive, odd behaviors such as hand flapping or rocking." Items reflect core autism-related traits and quantify severity ratings for impairment in social behavior, social communication, and stereotypic behavior/restricted interests. Items are categorized into five subscales: social awareness, social cognition, social communication, social motivation, and autistic mannerisms, which are used to compute a sex-standardized SRS Total T-Score (M = 50, SD = 10), as well as T-scores for two DSM-V compatible scales: the Social Communication and Interaction (SCI) and Restricted Interest and Repetitive Behavior (RRB) scales. This scale has shown cross-cultural validity (Bölte et al. 2008;

Constantino and Gruber 2012). The publisher (Western Psychological Services) provided a Spanish translation of the SRS-2 for Spanish-speaking mothers.

Behavioral assessment scale for children second edition (BASC-2). The BASC-2 is a multidimensional assessment that evaluates positive as well as negative aspects of behavior in children ages 2 to 18 y (Reynolds and Kamphaus, 2004). Parents completed the English or Spanish version of the BASC-2 at child ages 7 y ($n = 336$ with DAP measures), 10½ y ($n = 319$), and 14 y ($n = 330$), and the child's primary teacher completed the BASC-2 when the child was age 7 y ($n = 285$). Parents and teachers were asked to report the frequency with which their child exhibited specific behaviors over the past several months (N = never occurs, S = sometimes occurs, O = often occurs, and A = almost always occurs). We examined the Social Skills subscale from these tests, both of which are age- and sex-standardized (T-scores; M = 50, SD = 10). Examples of behavioral items from the Social Skills subscale include: "Congratulates others when good things happen to them" and "Shows interests in others' ideas."

Evaluación neuropsicológica infantil (ENI) facial-expression recognition test. When the children were 9 y old, we administered the ENI Facial Expression Recognition Test, a subtest of the Children's Neuropsychological Assessment (Matute et al. 2007). For this test, children were shown photographs of children's faces and instructed to identify the emotion associated with the expression of the child in the photo (e.g., happy, sad, angry). Children were shown eight different photographs, given a score of 1 for correctly identifying the expression and a score of 0 for incorrectly identifying the expression; participants could achieve a maximum score of 8.

NEPSY-II affect recognition test. Children were administered the Affect Recognition subtest of the NEPSY-II Social Perception domain (Korkman et al. 2007). Though similar to the ENI, the NEPSY-II Affect Recognition Test is a more complex and difficult test and uses the following four different tasks to assess the child's ability to recognize affect: 1) identify whether two photographs depict faces with the same affect; 2) select two faces with the same affect from three to four photographs; 3) select one of four faces that depict the same affect as the face at the top of the page; and 4) view a photograph of a face briefly and select two faces that depict the same affect as the face previously viewed. Participants are scored 0 (incorrect) or 1 (correct) on a total of 35 items across the four tasks and could achieve a maximum score of 35.

Covariate Assessment

We obtained sociodemographic data from in-person maternal interviews at all visits between enrollment and child age 14 y. Mothers were administered the Peabody Picture Vocabulary Test (PPVT) (Dunn 1997) to assess maternal verbal intelligence twice, at child age 6 mo and again at 9 y old; the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff 1977) to assess maternal depression when the child was 1, 3, 7, and 9 y old; and the Home Observation for the Measurement of the Environment-Short Form (HOME-SF) (Caldwell and Bradley 1984) to assess the home learning environment when the child was 3, 7, 9, and 10½ years of age.

Statistical Analysis

To offset some of the exposure measurement error due to rapid metabolism of OPs in the body (Bradman et al. 2005), we averaged 13- and 26-wk urinary pregnancy DAP, DM, and DE metabolite concentrations. We fit linear regression models for outcome measures (residuals for all variables were homoscedastic) and $\log_{10}$ transformed urinary DAPs (nanomoles per liter) and OP pesticide use (kg) within 1 km of maternal residence. Coefficients represented the change in outcome score for a 10-fold increase in prenatal DAP concentration or OP pesticide use. We adjusted all DAP metabolite measures for urinary creatinine.

We used a directed acyclic graph (DAG) to determine covariate inclusion in multivariable models (Figure S1). All models included maternal age at delivery (categorical: age 18 to 24, 25 to 29, 30 to 34, 35 to 45 y), education at delivery (<6th grade, 7–12th grade, completed high school), country of birth (United States, Mexico, or other), years in the United States at delivery (≤1, 2–5, 6–10, and 11 + years), marital status at delivery (not married to/living with child's father, married to/living with child's father), parity (nulliparous, 1 +), depression (CES-D ≥ 16, CES-D < 16) (Lewinsohn et al. 1997) at the 9-y visit, and quality of the home environment at the 10½-y visit (continuous HOME score, standardized in our sample using z-scores). We also included child's sex, age at assessment (continuous), and language in which the questionnaire was administered to the mother (Spanish or English); though these variables were not associated with pesticide exposure, we included these variables because they explain variability in the outcome and may therefore improve precision of our estimates. We replaced missing data on HOME score (3%), language of questionnaire administration (2%), and maternal depression (8%) with values for these covariates at earlier or later time points. If there were values from more than one time point, we chose the value that was most proximal in time to the variable we were replacing. Missing data with no replacement values were left missing, and a complete case analysis was conducted.

We conducted sensitivity analyses to examine confounding by several additional covariates, including maternal verbal intelligence (PPVT score <74, 75–99, and 100 +) at the 9-y visit; family poverty level at 10½-y visit (<poverty level, ≥200% of poverty level); breastfeeding duration (never breastfed, ≤1 mo, 1–6 mo, 6–12 mo); whether or not the child attended preschool; and child's intelligence at the 10½-y visit (WISC-IV Full Scale IQ score <74, 75–99, 100 +). These variables were not included in the final models in the interest of parsimony of multivariable models, because they were not strong candidate confounders based on the DAG, because their inclusion did not change effect estimates for exposure-outcome associations, and because most of these variables had missing data, even after imputation using values collected at other time points.

We examined potential confounding by other measured environmental contaminants that have been linked with poorer neurodevelopment in CHAMACOS participants in sensitivity analyses, including prenatal polybrominated diphenyl ethers (PBDEs) and p,p′-dichlorodiphenyldichloroethylene (DDE) (Eskenazi et al. 2013; Eskenazi et al. 2006; Gaspar et al. 2015; Sagiv et al. 2015b); we did not include these variables *a priori* because their inclusion would have reduced our sample size. In addition, we conducted a sensitivity analysis to examine residential proximity to agricultural OP use within a larger radius of the participants' homes (3-km radius).

We checked for linearity of exposure–outcome associations with semiparametric splines. To analyze repeated measures for BASC-2 parent report of social skills (collected when the child was age 7, 10½, and 14 y), we fit linear regression models with generalized estimating equations using an unstructured covariance matrix. We assessed effect modification by sex on the additive scale by including an interaction term between OP exposure (DAPs and residential proximity to OP use) and sex in the multivariable linear regression models and computing sex-specific effect estimates.

Results

We present summary statistics for the tests of ASD-related traits in Table S1. Of the 534 live births with prenatal total DAP measures, 247 had age 14 parent-reported SRS-2 T-scores. As reported in Table 1, participant characteristics were very similar for these two groups, though the subset of children with total DAPs and SRS-2 T-scores ($n = 247$) were more likely to be female, to have breastfed longer, and to have had mothers who were slightly older, were married to or living with the child's father, were parous, had lived in the United States longer, had scored higher on verbal intelligence, and had fewer depressive symptoms. Overall, most CHAMACOS mothers were born in Mexico (87% of those with DAPs and SRS-2 T-scores), were administered questionnaires in Spanish (88%), did not complete high school (79%), were below the poverty level (70%), were married or cohabitating with the child's father at delivery (83%), and were multiparous (68%). In addition, 54% of mothers had verbal intelligence scores below 100, and 28% were classified as having elevated depressive symptoms (CES-D $\geq$ 16). Most children were breastfed for more than 1 mo (65%), attended preschool (71%), and had Full Scale IQ scores below 100 at the 10-y visit (79%). SRS-2 T-scores were generally higher (indicating more ASD-related behavior) for children with mothers who were older at delivery ($\geq$35 years), were born outside of the United States, were living in the United States $\leq$10 years at baseline, were administered the questionnaire in Spanish, had lower educational attainment, were not married or cohabitating with the child's father at delivery, had lower verbal intelligence (<74), were classified as having elevated depressive symptoms at the 9-y visit, and were below the poverty level (Table 1). SRS-2 T-scores were also mildly to moderately higher among children who were male, breastfed >6 months, did not attend preschool and had lower Full Scale IQ scores (Table 1).

Prenatal maternal urinary DAP ($n = 247$), DE ($n = 247$), and DM ($n = 248$) metabolite concentrations, as well as residential proximity to agricultural OP use are presented in Table 2. Detection limits ranged from 0.05 μg/L for DEs to 1.2 μg/L for DMs. Concentrations of urinary DMs were considerably higher than urinary DEs in CHAMACOS participants, and DMs were more highly correlated with total DAPs (Spearman Correlation Coefficient = 0.96 vs. 0.56 for DAPs and DEs). The most heavily used OP within a 1-km radius of maternal residences was diazinon (22 kg), followed by oxydemeton-methyl (8.7 kg), chlorpyrifos (8.0 kg), and malathion (4.1 kg). Agricultural use of individual OPs were correlated with each other (0.26–0.61), but were not correlated with DAPs, DEs or DMs (0.02 for DAPs and total PUR-estimated OPs).

OP Pesticide Urinary Metabolites and ASD-related Traits

Prenatal DAPs were associated with higher scores on the SRS-2, indicating more ASD-related behavior (Table 3). For example, a 10-fold increase in total prenatal urinary DAPs was associated with a 2.7- point increase, which is just over a quarter of a standard deviation increase in the SRS-2 Total T-score (SD = 10). We used splines to examine DAPs and SRS-2 T-scores, and we determined that the association was consistent with a linear exposure–response relationship (data not shown). Though in the same direction, strength of associations for DAPs were slightly stronger for the DSM-V compatible SCI scale of the SRS-2 ($\beta = 2.8$; 95% CI: 0.9, 4.6 per 10-fold increase) in comparison with the RRB ($\beta = 2.2$; 0.3, 4.0). Associations with DMs were generally consistent with DAPs and stronger than associations with DEs. We found no consistent differences of DAP and SRS associations across sex (Table S2).

We also saw consistent inverse associations of DAPs with parent and teacher report of BASC-2 Social Skills T-scores (Table 4) (in contrast with the SRS, lower BASC-2 Social Skills T-scores indicate poorer social skills). Associations with DMs were consistent with total DAPs, but associations with DEs were null. We observed null associations of DAPs with affect recognition measured at 9 y with the ENI and 12 y with the NEPSY-II and found no consistent associations across sex for any of the outcomes (Table S2).

Residential Proximity to OP Pesticide Use and ASD-related Traits

Associations of residential proximity to OP use from Pesticide Use Reporting (PUR) data with all measures of ASD-related traits, including the SRS-2, the BASC-2 Social Skills subscale, and affect-recognition measures, were close to the null (Tables 3 & 4). There were suggestive associations for malathion with more restricted and repetitive behaviors on the SRS-2 ($\beta = 0.9$; −0.3, 2.1 per 10-fold increase in malathion use within 1 km of home during pregnancy) and poorer teacher-reported social skills on the BASC-2 ($\beta = -1.7$; −5.4, 2.0), but these associations were not statistically significant. Diazinon, on the other hand, was associated with better NEPSY-II affect-recognition scores ($\beta = 0.9$; 0.2, 1.6). When we examined agricultural use of OPs within a larger radius (3 km) of participants' homes (Table S3), associations with the SRS-2 bounced around the null, with no meaningful differences. As with OP urinary metabolites, sex differences for residential proximity to total OP use and outcome scores were inconsistent and imprecise (Table S4).

Sensitivity analyses adjusting for additional covariates, including maternal verbal intelligence, family poverty level, breastfeeding duration, child preschool attendance, child's intelligence, and prenatal PBDE and DDE concentrations did not affect any of the associations of OPs with SRS-2 Total T-scores (Table S5).

Discussion

We found associations of prenatal urinary DAP metabolites with ASD-related traits at age 14 y in CHAMACOS using the SRS-2. These findings were supported by associations of prenatal DAPs with teacher and parent reports of poorer social skills assessed from age 7 to 14 y. However, we did not find associations of DAPs with facial affect recognition tests administered to the child at ages 9 and 12 y. In addition, estimated associations of residential proximity to OP pesticide use during pregnancy with ASD-related traits were all essentially null.

In CHAMACOS, we previously reported associations of prenatal DAPs with lower IQ (Bouchard et al. 2011), poorer attention (Marks et al. 2010), and a higher likelihood for scoring above the 97th percentile of the national normative sample on the pervasive developmental disorder scale (which includes symptoms consistent with ASD) for the maternally reported Child Behavior Checklist at 24 mo (10-fold increase in DAPs OR = 2.3; 95% CI: 1.0, 5.2) (Eskenazi et al. 2007). This association of DAPs with traits of PDD, a category that encompasses ASD, is consistent with our current findings of DAPs with ASD-related traits in later childhood and adolescence using different study instruments (SRS-2 and the BASC-2). We have also reported associations of DAPs with other ASD-related traits in CHAMACOS, including inattention at age 5 and poorer verbal comprehension, processing speed and working memory at age 7 (Bouchard et al. 2011; Marks et al. 2010).

A smaller longitudinal cohort study of 136 mother-infant pairs in New York City, with measures most similar to the

Table 1. Sociodemographic characteristics for children with measured prenatal urinary DAP metabolites who were followed up until the birth ($n = 534$) and those with measured prenatal DAPs and 14-year SRS-2 T-scores ($n = 247$), and medianT-scores across these characteristics in the CHAMACOS study population, enrolled 1999–2000 in Salinas Valley, California.

Characteristic	$n = 534$ with DAPs	$n = 247$ with DAPs and SRS-2[a]	
	n (%)	*n* (%)	T-score Median (Q1, Q3)
Maternal/family factors			
Maternal age at child delivery			
18–24	263 (49.3)	111 (44.9)	55.0 (51.0, 59.0)
25–29	157 (29.4)	74 (30.0)	56.0 (48.0, 61.0)
30–34	78 (14.6)	42 (17.0)	55.0 (50.0, 58.0)
35–45	36 (6.7)	20 (8.1)	58.0 (51.0, 61.5)
Maternal country of birth			
U.S.	70 (13.1)	30 (12.2)	53.0 (49.0, 59.0)
Mexico	452 (84.6)	214 (86.6)	55.0 (51.0, 60.0)
Other	12 (2.3)	3 (1.2)	56.0 (41.0, 69.0)
Maternal years in U.S. at child delivery			
≤1	129 (24.2)	51 (20.7)	55.0 (49.0, 60.0)
2–5	144 (27.0)	63 (25.5)	57.0 (51.0, 60.0)
6–10	116 (21.7)	70 (28.3)	57.0 (52.0, 61.0)
11 + (includes entire life)	145 (27.2)	63 (25.5)	53.0 (48.0, 58.0)
Maternal Language for 14 y questionnaire			
Spanish	316 (87.3)	216 (87.5)	55.0 (51.0, 60.0)
English	46 (12.7)	31 (12.6)	52.0 (48.0, 58.0)
Missing	172		
Maternal education at child delivery			
<6th grade	233 (43.6)	106 (42.9)	56.0 (51.0, 61.0)
7–12th grade	192 (36.0)	90 (36.4)	55.5 (49.0, 60.0)
Completed high school	109 (20.4)	51 (20.7)	54.0 (48.0, 59.0)
Maternal marital status at child delivery			
Not married to/living with child's father	102 (19.1)	42 (17.0)	56.5 (49.0, 61.0)
Married to/living with child's father	432 (80.9)	205 (83.0)	55.0 (50.0, 60.0)
Parity at child delivery			
Nulliparous	184 (34.5)	78 (31.6)	55.0 (49.0, 59.0)
1 +	350 (65.5)	169 (68.4)	55.0 (51.0, 60.0)
Maternal verbal intelligence (PPVT score) at 9-year visit[b]			
<74	118 (25.5)	47 (19.1)	57.0 (51.0, 61.0)
75–99	154 (33.3)	86 (35.0)	55.0 (50.0, 60.0)
100 +	190 (41.1)	113 (45.9)	55.0 (49.0, 59.0)
Missing	72	10	
Maternal Depression (≥16 on CES-D) at 9-year visit[b]			
No	308 (69.8)	177 (72.0)	54.0 (49.0, 59.0)
Yes	133 (30.2)	69 (28.1)	58.0 (54.0, 65.0)
Missing	93	1	
Family poverty at 10½-year visit[b]			
<Poverty level	246 (72.1)	170 (70.3)	56.0 (51.0, 60.0)
Within 200% of poverty level	93 (27.3)	71 (29.3)	54.0 (49.0, 58.0)
>200% of poverty level	2 (0.6)	1 (0.4)	61.0 (61.0, 61.0)
Missing	193	5	
Child factors			
Sex			
Male	264 (49.4)	113 (45.8)	56.0 (50.0, 60.0)
Female	270 (50.6)	134 (54.3)	55.0 (50.0, 60.0)
Breastfeeding duration[b]			
Never breastfed	68 (14.7)	33 (13.8)	52.0 (48.0, 58.0)
≤1 month	118 (25.5)	50 (20.9)	54.0 (48.0, 60.0)
1–6 months	79 (17.1)	33 (13.8)	53.0 (51.0, 57.0)
>6 months	197 (42.6)	123 (51.5)	57.0 (52.0, 61.0)
Missing	72	5	
History of preschool attendance			
Did not attend	108 (31.0)	65 (28.8)	56.0 (52.0, 62.0)
Attended preschool	241 (69.1)	161 (71.2)	55.0 (50.0, 59.0)
Missing	185	21	
Intelligence at 10½-year visit (WISC-IV FSIQ)			
≤79	49 (15.7)	35 (14.8)	58.0 (51.0, 64.0)
80–99	195 (62.3)	152 (64.1)	55.0 (51.0, 60.0)
100 +	69 (22.0)	50 (21.1)	53.0 (47.0, 57.0)
Missing	221	10	
	Mean ± SD	Mean ± SD	
Child's age at SRS assessment	14.18 ± 0.27	14.16 ± 0.23	
Missing	207	0	
HOME total *z*-score (10.5 years)	−0.02 ± 1.07	0.01 ± 1.01	
Missing	89	0	

DAPs, dialkyl phosphates; SRS-2, Social Responsiveness Scale, Version 2; PPVT, Peabody Picture Vocabulary Test; CES-D, Center for Epidemiological Studies-Depression Scale; WISC-IV FSIQ, Wechsler Intelligence Scale for Children, Version IV Full Scale IQ.

[a]T-score standardized mean = 50 (SD = 10); higher SRS score indicates more ASD-related traits.

[b]Includes observations with missing data that we imputed with values collected on these variables at earlier or later time points.

Table 2. Pregnancy maternal urinary DAP metabolite concentrations and residential proximity to OP pesticide use during pregnancy using pesticide use reporting (PUR) data among participants with a 14-year SRS-2 score in the CHAMACOS study population, enrolled 1999–2000 in Salinas Valley, California.

OP pesticide exposure	*n*	GM	(95% CI)
Urinary metabolites (nmol/L)[a]			
Total DAPs	247	124.6	(109.0, 142.5)
Total DEs	247	20.3	(17.8, 23.2)
Total DMs	248	92.6	(79.7, 107.6)
Creatinine-adjusted urinary metabolites (nmol/g creatinine)[a]			
Total DAPs	247	144.1	(125.7, 165.1)
Total DEs	247	24.0	(20.9, 27.6)
Total DMs	248	106.0	(91.1, 123.3)
PUR data (kg within 1 km)			
Chlorpyrifos	236	8.0	(6.6, 9.8)
Diazinon	236	22.4	(19.0, 26.3)
Malathion	236	4.1	(3.4, 5.1)
Oxydemeton-methyl	236	8.7	(7.2, 10.4)
Total OP pesticides	236	69.5	(56.5, 85.3)
Toxicity-weighted OP pesticides[b]	236	172.3	(128.3, 231.3)

OP, organophosphate; DAPs, dialkyl phosphates; Des, diethyl phosphates; DMs, dimethyl phosphates; GM, geometric mean; CI, confidence interval.
[a]Values for individual urinary metabolites were averaged over of samples collected at 13 and 26 weeks of gestation, after randomly imputing values <LOD in individual samples using a lognormal distribution. Detection frequency ranges for the individual DEs (sum of diethylphosphate, diethylthiophosphate, and diethyldithiophosphate metabolites) at 13 weeks were 45–55%, DEs at 26 weeks were 25–99%, DMs (sum of dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate metabolites) at 13 weeks were 51–65% and DMs at 26 weeks were 5–99%. DM metabolite concentrations were available for all participants ($n = 248$), DE metabolite concentrations were missing for one participant, resulting in 247 values for total DEs and total DAPs.
[b]OP toxicity-weighted use in kg-equivalents of chlorpyrifos.

current study, found null associations with prenatal DAPs and the SRS at age 7–9 y ($\beta = 0.3$; 95% CI: −2.3, 2.9 for a 10-fold increase in total DAPs) (Furlong et al. 2014). This study did find associations of DEs with the SRS among specific subgroups, including among males ($n = 66$) ($\beta = 3.5$; 95% CI: 0.2, 6.8) and black participants ($n = 42$) ($\beta = 5.1$; 95% CI: 0.8, 9.4), but, notably, not among Hispanics ($n = 87$) ($\beta = 0.2$; 95% CI: −2.8, 3.2); sample sizes were small in these subgroups as reflected by wide confidence intervals. Although we observed weak associations of DEs with ASD-related traits in CHAMACOS, associations with DMs were stronger and more consistent with total DAPs, which was expected given their strong correlations. Differences in these associations across cohorts may reflect different exposure patterns among an urban vs. agricultural cohort (DM concentrations were considerably higher in CHAMACOS than in the New York City cohort) (Harley et al. 2016).

The Childhood Autism Risks from Genetics and Environment study (CHARGE), a population-based case–control study in California, used PUR data to estimate prenatal pesticide exposure and found that residential proximity to agricultural OP use within 1.25 km (yes vs. no) during the third trimester was associated with twice the odds of ASD (OR = 2.0; 95% CI: 1.1, 3.6) (Shelton et al. 2014). This finding is consistent with an older case–control study of ASD in California that used PUR data and also found associations of OP pesticide use and risk for ASD (coefficient of ASD odds for the highest nonzero quartile of gestational exposure to OPs using a 0.25-km buffer radius vs. no exposure = 0.46, p-value = 0.04) (Roberts et al. 2007). Positive findings from these two studies are in contrast with the essentially null associations that we report for residential proximity to agricultural use of OPs (kg within 1 km) in relation to ASD traits in CHAMACOS. Inconsistency could arise from different outcome assessment (i.e., ASD traits in CHAMACOS vs. ASD clinical diagnosis in these other studies) or differences in exposure patterns. For example, the majority of CHARGE participants (70%) had no pesticide use within the buffer zone, whereas in CHAMACOS, nearly all participants lived in close proximity to agricultural fields and therefore had quantifiable exposure. Thus, whereas CHAMACOS participants, on average, had higher exposure to pesticides, there were considerably fewer unexposed individuals, which may have limited exposure variability and thus the ability to detect associations across the range of OP exposure.

Within CHAMACOS, we found inconsistent associations for residential proximity to OP use and DAPs with ASD-related traits. Although we saw fairly robust associations of DAPs with poorer parent- and teacher-reported social behavior, as measured with the SRS-2 and BASC-2, associations for residential proximity to OP use was essentially null for total and individual OPs and all outcome measures. Prenatal DAPs and residential proximity to OP use were not correlated in CHAMACOS (Spearman correlation coefficient = 0.02) and are probably markers of different sources and time periods of OP exposure. For example, although PUR data reflect proximity to agricultural pesticide use over the entire pregnancy (Gunier et al. 2016), urinary DAPs reflect some of these ambient exposures, but, to a larger degree,

Table 3. Adjusted change in 14-year SRS-2 total T-score, and the two SRS-2 DSM-V compatible SCI and RRB T-scores, per 10-fold increase in creatinine-adjusted prenatal urinary DAPs, DEs, DMs and OP pesticide use within 1 km of residence during pregnancy using pesticide use reporting (PUR) data in the CHAMACOS study population, enrolled 1999–2000 in Salinas Valley, California.

		SRS-2 Total T-score[a]	SCI T-score[a]	RRB T-score[a]
OP pesticide exposure	*n*	β (95% CI)	β (95% CI)	β (95% CI)
Urinary metabolites				
DAPs	246	2.7 (0.9, 4.5)	2.8 (0.9, 4.6)	2.2 (0.3, 4.0)
DEs	246	1.0 (−0.8, 2.7)	0.8 (−1.0, 2.6)	1.5 (−0.2, 3.2)
DMs	247	2.4 (0.8, 4.0)	2.5 (0.8, 4.2)	1.7 (0.1, 3.3)
PUR data (kg within 1 km)				
Chlorpyrifos	235	−0.6 (−1.8, 0.7)	−0.7 (−2.0, 0.6)	−0.1 (−1.4, 1.1)
Diazinon	235	−0.4 (−2.0, 1.1)	−0.5 (−2.1, 1.1)	−0.3 (−1.8, 1.3)
Malathion	235	0.5 (−0.7, 1.8)	0.4 (−0.9, 1.6)	0.9 (−0.3, 2.1)
Oxydemeton-methyl	235	−0.3 (−1.7, 1.0)	−0.5 (−1.9, 0.9)	0.2 (−1.1, 1.5)
Total OP pesticides	235	−0.2 (−1.4, 1.0)	−0.3 (−1.6, 0.9)	0.3 (−1.0, 1.5)
Toxicity-weighted OP pesticides[b]	235	−0.2 (−1.0, 0.7)	−0.3 (−1.1, 0.6)	0.1 (−0.8, 0.9)

OP, organophosphate; DAPs, dialkyl phosphates; Des, diethyl phosphates; DMs, dimethyl phosphates; SRS-2, Social Responsiveness Scale, Version 2; SCI, Social Communication and Interaction; RRB, Restricted and Repetitive Behaviors; CI, confidence interval.
Adjusted for maternal age, education, country of birth, years in the United States, language of questionnaire, parity, marital status, depression, child's age at assessment, sex and quality of the home environment. Maternal depression score was missing for one participant, leaving 235 observations in models of associations with PUR data, 247 for total DMs, and 246 for total DEs and DAPs.
[a]T-score standardized mean = 50 (SD = 10); higher SRS score indicates more ASD-related traits.
[b]OP toxicity-weighted use in kg-equivalents of chlorpyrifos.

Table 4. Adjusted change in BASC-2 social skills and affect recognition scores per 10-fold increase of creatinine-adjusted prenatal urinary DAPs, DEs and DMs and OP pesticide use within 1 km of residence during pregnancy using pesticide use reporting (PUR) data in the CHAMACOS study population, enrolled 1999–2000 in Salinas Valley, California.

	BASC-2 Social Skills T-score[a] Teacher 7y		BASC-2 Social Skills T-score[a] Parent report 7,10½,14 y[b]		Affect Recognition[a] ENI 9 y		Affect Recognition[a] NEPSY-II 12y	
OP pesticide exposure	*n*	β (95% CI)	*n*	β (95% CI)	*n*	β (95% CI)	*n*	β (95% CI)
Urinary metabolites								
DAPs	282	−2.9 (−5.4, −0.4)	354	−3.1 (−4.9, −1.2)	324	−0.1 (−0.3, 0.2)	323	0.4 (−0.5, 1.3)
DEs	282	0.0 (−2.2, 2.3)	354	−0.5 (−2.3, 1.4)	324	−0.1 (−0.4, 0.2)	323	0.6 (−0.2, 1.4)
DMs	283	−3.0 (−5.2, −0.8)	354	−2.8 (−4.4, −1.2)	325	−0.1 (−0.5, 0.3)	323	0.2 (−0.6, 1.0)
PUR data (kg within 1 km)								
Chlorpyrifos	270	0.3 (−1.4, 2.0)	354	−0.5 (−1.7, 0.7)	310	−0.1 (−0.3, 0.1)	307	0.5 (−0.1, 1.1)
Diazinon	270	−0.8 (−2.9, 1.3)	354	−0.2 (−1.9, 1.5)	310	−0.1 (−0.4, 0.1)	307	0.9 (0.2, 1.6)
Malathion	270	−1.7 (−5.4, 2.0)	354	−0.7 (−1.9, 0.5)	310	−0.1 (−0.2, 0.1)	307	0.1 (−0.5, 0.6)
Oxydemeton-methyl	270	−0.3 (−2.1, 1.5)	354	−0.1 (−1.6, 1.3)	310	−0.1 (−0.3, 0.1)	307	0.6 (−0.1, 1.2)
Total OP pesticides	270	−0.8 (−2.5, 0.8)	354	−0.4 (−1.8, 0.9)	310	−0.1 (−0.3, 0.1)	307	0.5 (−0.1, 1.1)
Toxicity-weighted OP pesticides[c]	270	−0.3 (−1.5, 0.8)	354	−0.1 (−1.0, 0.9)	310	−0.1 (−0.2, 0.1)	307	0.4 (0.0, 0.8)

OP, organophosphate; DAPs, dialkyl phosphates; Des, diethyl phosphates; DMs, dimethyl phosphates; ENI, Evaluación Neuropsicológica Infantil Facial Expression Recognition Test; BASC-2, Behavioral Assessment Scale for Children, 2nd Edition; CI, confidence interval.

Adjusted for maternal age, education, country of birth, years in the United States, language of questionnaire, parity, marital status, depression, child's age at assessment, sex and quality of the home environment. Maternal depression score was missing for one participant.

[a]Lower scores consistent with more ASD-related traits; BASC-2 T-score standardized mean = 50 (SD = 10); ENI mean = 6.6 (SD = 1.2);NEPSY-II mean = 26.6 (SD = 3.6).

[b]Repeated measures analysis (Generalized Estimating Equations) of parent report at age 7, 10½, and 14 years.

[c]OP toxicity-weighted use in kg-equivalents of chlorpyrifos.

dietary exposures during the 48 hours prior to sample collection (Gunier et al. 2016; McKone et al. 2007).

Although a proxy for personal pesticide exposure, PUR data do not account for environmental fate and transport of the pesticides, exposure away from the home or via diet, or personal pesticide use. This would have resulted in measurement error that biased estimates towards the null and may have explained the mostly null associations of residential proximity and ASD-related traits we see in this study. However, previous studies have shown that PUR data are well correlated with environmental pesticide concentrations (Harnly et al. 2005, 2009). In addition, recent studies report that outdoor air samples from homes located more than 0.25 km from fruit orchards had significantly lower concentrations of two OP pesticides than did samples from homes within 0.25 km of orchards (Gibbs et al. 2017) and average pesticide concentrations in house dust were 64% lower in homes located 0.25 km compared to 0.02 km from treated fields (Deziel et al. 2017).

In addition, urinary DAP measures have some notable limitations. OPs are rapidly metabolized in the body, and urinary DAPs reflect relatively recent exposure to OPs (Bradman et al. 2005). There is therefore a fair degree of exposure measurement error that results when using this measure to represent exposure during the entire pregnancy. We may have offset this error somewhat by collecting urine at two time points in early and later pregnancy and averaging these measures. DAPs are also nonspecific metabolites, and we therefore cannot make inferences about exposure to any one specific OP pesticide. They also do not reflect exposure to several OPs that do not devolve to DAPs. Finally, DAPs may overestimate true OP exposure because they can reflect exposure to preformed DAPs in the environment and food, as well as parent OP compounds (Lu et al. 2005; Quirós-Alcalá et al. 2012).

OP pesticide levels in the United States, even in this population living in close proximity to agriculture, are likely to be too low to induce acetylcholinesterase inhibition, one of the main pathways of OP neurotoxicity. Animal studies have reported other pathways by which OPs may be exerting effects on the developing brain that could underlie associations with ASD-related traits, including adverse effects on axonal dendritic and synaptic development (Howard et al. 2005; Qiao et al. 2004; Slotkin and Seidler 2007; Yang et al. 2011), and increased oxidative stress in the brain (De Felice et al. 2016). Disruption of gamma-aminobutyric acid (GABA) signaling pathways, which have been related to ASD-related deficits (Blatt et al. 2001; Fatemi et al. 2012), have also been linked with OP exposure in animal studies (Mohammadi et al. 2008; Noriega-Ortega et al. 2011). Other mechanisms of OP neurotoxicity that have been linked with autism pathophysiology include neuroinflammation and disruption of thyroid hormones (Banks and Lein 2012; Shelton et al. 2012; Slotkin et al. 2013). A small neuroimaging study ($n = 40$) found associations of low-level prenatal chlorpyrifos exposure with structural neuroanatomical differences in children age 5–11 using magnetic resonance imaging, including reduced cortical thickness in prefrontal and parietal regions and enlargement in the superior temporal gyrus and medial superior frontal gyrus (Rauh et al. 2012). These are all brain regions that underlie traits related to ASD, suggesting an anatomical basis for effects of OP-exposure ASD (Patriquin et al. 2016). Human (Furlong et al. 2014; Marks et al. 2010) and rat studies (Levin et al. 2001, 2010) have reported stronger OP-related associations with ASD-related traits among males. Overall, we did not observe a consistent pattern of sex differences for OP pesticides exposure and ASD-related traits in CHAMACOS.

To our knowledge, this study is the first to investigate associations of OP-pesticide exposure with tests of facial-expression recognition, which assesses the ability to recognize the mental state of others using only their facial expression, an important aspect of theory of mind and social responsiveness. We did not see any consistent associations of DAPs or residential proximity to agricultural OP use with scores on tests administered to the child at age 9 or 12 y. Although performance on these tests has been shown to be poorer among individuals with ASD (Berggren et al. 2016), it did not appear to be sensitive to prenatal OP exposure in CHAMACOS. In fact, when we examined prenatal DAPs in relation to a question on the SRS-2 about facial-expression recognition ("Is able to understand the meaning of other people's tone of voice and facial expressions"), we found null associations (data not shown), despite finding positive associations with the Total SRS-2 T-score. This finding suggests that prenatal DAPs, though not associated with the ability to recognize mental states in others, may be related to other aspects of ASD.

There was substantial attrition of the CHAMACOS cohort from birth ($n = 534$) until 14 y ($n = 247$ with measured DAPs and an SRS-2 score; $n = 330$ with a parent-reported BASC-2 score). As we report (Table 1), characteristics for these groups were similar, with some minor exceptions; adjusting for these covariates in our multivariable models did not change effect estimates, suggesting that any resulting selection bias was likely minimal.

ASD-related traits, such as poor social cognition and poor theory of mind, are common in other neurodevelopmental disorders, such as ADHD and schizophrenia. We therefore interpret these associations with caution when inferring etiologic risk factors for ASD. However, overlap of multiple traits calls into question whether these disorders are in fact distinct; it is therefore possible that there are also etiologic overlaps for these disorders (Sagiv et al. 2015a).

Although we measured postnatal OP exposure in CHAMACOS, we do not present associations with ASD-related traits in this paper. Previous published reports in CHAMACOS, which have found associations of prenatal DAPs with poorer neurodevelopment, have reported considerably weaker or null associations of postnatal DAPs with these endpoints (Bouchard et al. 2011; Marks et al. 2010). Nevertheless, brain development, which continues until early adulthood, is also vulnerable to toxic insults; we will therefore examine associations of postnatal OP exposure, measured with DAPs and residential proximity to agriculture OP pesticide use, with ASD-related traits in future work. Finally, associations found in this unique cohort of primarily Mexican-American children may not be generalizable to other populations with different ethnic distributions.

Our study had some notable strengths, including a prospective design with exposure estimated during the prenatal period, when rapid development of the central nervous system can heighten vulnerability to environmental insults. CHAMACOS also collected data on traits related to ASD using diverse study instruments (parent report, teacher report, objective neuropsychological testing) at multiple time points (in this analysis we include data from ages 7, 9, 10½, 12, and 14 y), allowing for a longitudinal examination of associations with traits related to ASD. In addition, examination of quantitative traits offers a statistically powerful approach to studying modifiable environmental exposures with neurodevelopmental disorders, such as ASD (Sagiv et al. 2015a).

Conclusions

We found that urinary OP metabolites but not residential proximity to agriculture OP pesticide use during pregnancy were associated with more ASD-related traits in childhood and adolescence. These findings contribute to evidence showing associations of modifiable environmental risk factors with ASD-related traits.

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