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Prenatal Residential Proximity to Agricultural Pesticide Use and IQ in 7-Year-Old Children

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Abstract

Background: Residential proximity to agricultural pesticide use has been associated with neural tube defects and autism but more subtle outcomes like cognition have not been studied.

Objectives: Evaluate the relationship between prenatal residential proximity to agricultural use of potentially neurotoxic pesticides and neurodevelopment in 7-year old children.

Methods: Participants included mothers and children (n=283) living in the agricultural Salinas Valley of California enrolled in the Center for the Health Assessment of Mothers and Children Of Salinas (CHAMACOS) study. We estimated agricultural pesticide use within one km of maternal residences during pregnancy using a geographic information system, residential location, and California's comprehensive agricultural Pesticide Use Report data. We used regression models to evaluate prenatal residential proximity to agricultural use of five potentially neurotoxic pesticide groups (organophosphates, carbamates, pyrethroids, neonicotinoids, and manganese fungicides) and five individual organophosphates (acephate, chlorpyrifos, diazinon, malathion and oxydemeton-methyl) and cognition in 7-year old children. All models included prenatal urinary dialkyl phosphate metabolite concentrations.

Results: We observed a decrease of 2.2 points (95% Confidence Interval (CI): -3.9, -0.5) in Full-Scale intelligence quotient (IQ) and 2.9 points (95% CI: -4.4, -1.3) in verbal comprehension for each standard deviation increase in toxicity-weighted use of organophosphate pesticides. In separate models, we observed similar decrements in Full-Scale IQ with each standard deviation increase of use for two organophosphates (acephate and oxydemeton-methyl) and three neurotoxic pesticide groups (pyrethroids, neonicotinoids, and manganese fungicides).

Conclusions: This study identified potential relationships between maternal residential proximity to agricultural use of neurotoxic pesticides and poorer neurodevelopment in children.

Introduction

In 2007, 684 million pounds of pesticide active ingredients were used in agriculture in the U.S. (Grube et al. 2011). California, the state with the largest agricultural output, uses 25% of all U.S. agricultural pesticides, or 186 million pounds annually (CDPR 2014). Though recent studies have demonstrated widespread organophosphate (OP) pesticide exposures in the general U.S. population, including pregnant women and children (Whyatt et al. 2003; Berkowitz et al. 2003; Lu et al. 2001; Adgate et al. 2001; CDC 2009), exposures are often higher in agricultural populations (Lu et al. 2004; Fenske et al. 2002). Among pregnant women in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, all of whom lived in an agricultural region, and many of whom either worked in agriculture or lived with people who did, OP urinary metabolites or dialkylphosphate (DAPs) levels were ~40% higher than those in a representative sample of U.S. women of child-bearing age (Bradman et al. 2005). We observed adverse associations in the CHAMACOS study between prenatal maternal DAP concentrations and children's performance on the Bayley Scales of Infant Development at 2-years (Eskenazi et al. 2007), measures of attention at 5-years (Marks et al. 2010), and on the Wechsler Intelligence Scale for Children (WISC) at 7-years (Bouchard et al. 2011a). Several studies in other populations have similarly reported adverse associations of prenatal exposure to OP pesticides and child neurodevelopment (Engel et al. 2011; Rauh et al. 2011), but few studies have examined the effects of other potentially neurotoxic pesticides on child cognitive development.

Populations residing in agricultural areas may be exposed to a complex mixture of neurotoxic pesticides through pesticide drift and para-occupational exposures (Fenske et al. 2002). Some of these pesticide classes – including OPs as well as carbamates – share at least one mode of action,

depression of acetylcholinesterase (AChE), and there is *in vitro* evidence that there may be additive inhibitory effects from exposure to certain pesticide mixtures (Mwila et al. 2013). Furthermore, though biomarkers such as DAP metabolites are an important tool to assess exposures to pesticides, several challenges limit the utility of pesticide biomarkers in epidemiologic analyses. For example, many pesticides have a short half-life in the body and biomarkers reflect only very recent exposures, on the order of hours to days (Bradman et al. 2013). In addition, there are no biomarkers available for some pesticides, leaving only environmental concentrations or modeling to characterize exposure.

Since 1990, all agricultural pesticide applications in California have been compiled in the uniquely comprehensive Pesticide Use Reporting (PUR) database. In several studies, PUR data have been shown to correlate with pesticide levels in various media. For example, we have shown significant associations between nearby use of specific pesticides based on the PUR data and levels in house dust (Harnly et al. 2009; Gunier et al. 2011), and moderate to strong associations ($R^2 \sim 0.28 – 0.65$) between agricultural use of malathion, chlorpyrifos, and diazinon with community air samples (Harnly et al. 2005). In addition, several epidemiologic studies conducted in California have also shown that higher nearby agricultural pesticide use is associated with adverse health outcomes, including OP and fungicide use with Parkinson's disease (Costello et al. 2009; Wang et al. 2014), OP and pyrethroid use with autism (Shelton et al. 2014) and birth defects (Carmichael et al. 2014), organochlorine pesticide use with autism (Roberts et al. 2007), and carbamates (benomyl and methomyl) and the neonicotinoid imidacloprid with neural tube defects in children (Rull et al. 2006; Yang et al. 2014). To our knowledge, there have not been any previous studies evaluating residential proximity to reported agricultural pesticide use and more subtle neurodevelopmental outcomes like cognitive function

in children. In this study, we evaluate the relationship between prenatal residential proximity to agricultural use of a variety of neurotoxic pesticides and neurodevelopment using WISC assessed at 7-years of age because it provides a more specific and reliable measure of cognition than earlier neurodevelopmental assessments conducted in our cohort and may have greater implications for school performance.

Methods

Study population. We enrolled 601 pregnant women between October 1999 and October 2000 as part of the CHAMACOS study. Women were eligible if they were ≥ 18 years of age, <20 weeks gestational age, eligible for California's low-income health care program (MediCal), spoke English or Spanish, and were planning to deliver at the county hospital. We followed the women through the delivery of 537 live born children. We excluded twins (n=10) and children with medical conditions that could affect neurodevelopmental assessment (n=4, one child each with Downs syndrome, autism, deafness, and hydrocephalus). We included children who had a neurodevelopmental assessment at age 7 (n=330) and whose prenatal residential location was known for at least 75 days per trimester during two or more trimesters of pregnancy (n=283). We excluded two participants who did not have prenatal measurements of DAP metabolites. Our final study population for this analysis was 283. The mothers of children included in our analyses were more likely ($p<0.05$) than those not included to be married (85% vs. 77%), non-smokers during pregnancy (96% vs. 92%), and older at delivery (mean=26.9 vs. 24.9 years) than the mothers of children that were not included in these analyses; otherwise the two populations were similar demographically. Written informed consent was obtained from all women and oral assent from all children at age 7; all research was approved by the University of California, Berkeley, Committee for the Protection of Human Subjects prior to commencement of the study.

Cognitive assessment. We assessed cognitive abilities when the children were 7-years of age using the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) (Wechsler 2003). All assessments were completed by a single bilingual psychometrician, who was trained and supervised by a pediatric neuropsychologist. Scores for four domains were calculated based on the following subtests: Verbal Comprehension (composed of Vocabulary and Similarities subtests), Perceptual Reasoning (Block Design and Matrix Reasoning subtests), Working Memory (Digit Span and Letter-Number Sequencing subtests), and Processing Speed (Coding and Symbol Search subtests). We administered all subtests in the dominant language of the child, which was determined through administration of the oral vocabulary subtest of the Woodcock–Johnson/Woodcock–Munoz Tests of Cognitive Ability in both English and Spanish (Woodcock and Munoz-Sandoval 1990) at the beginning of the assessment. Among participants included in these analyses, 68% of children were tested in Spanish and 32% in English. The psychometrician was blinded to exposure status. We standardized WISC-IV scores against U.S. population–based norms for English- and Spanish-speaking children. We did not administer Letter-Number Sequencing or Symbol Search subtests for the first 3 months of assessments, therefore 27 participants lack scores for Processing Speed and Working Memory domains. A Full-Scale IQ was available for 255 children.

Maternal interviews and assessments. Bilingual interviewers conducted maternal interviews in Spanish or English twice during pregnancy (~13 and 26 weeks gestation), after delivery and when the children were 6 months and 1, 2, 3.5, 5 and 7-years of age. Interviews obtained demographic information including maternal age, education, country of birth, number of years lived in the United States, marital status, paternal education, and family income. We collected residential history information by asking participants if they had moved since the last

interview and, if so, the dates of all moves. We conducted home visits shortly after enrollment (~16 weeks gestation) and when the child was 6 months of age. For both visits, latitude and longitude coordinates of the participant's home were determined using a handheld global positioning system unit. Residential mobility during pregnancy was common in our cohort, with 53% of all participants moving at least once during pregnancy. For this analysis, we included women in the sample if their residential location was known for 75 days or more per trimester for at least two trimesters of pregnancy.

Mothers were administered the Peabody Picture Vocabulary Test (PPVT) for English speakers or the Test de Vocabulario en Imagenes Peabody (TVIP) for Spanish speakers at the six-month visit to assess verbal intelligence (Dunn and Dunn 1981). If maternal PPVT or TVIP scores were unavailable from the 6-month visit, we used scores from the re-administration of the test conducted at a 9-year visit (n=5) or assigned the mean score of the sample (n=2). A short version of the HOME (Home Observation for Measurement of the Environment) inventory was completed during the 7-year visit (Caldwell and Bradley 1984).

Geographic-based estimates of agricultural pesticide use. We estimated agricultural pesticide use near each woman's residence during pregnancy using California PUR data from 1999–2001 (CDPR 2015). We selected potentially neurotoxic pesticides with agricultural use in our study area (Monterey County, CA) during the prenatal period, including fifteen OPs and six carbamates (see Table S1), two manganese (Mn) based fungicides (maneb and mancozeb), eight pyrethroids (permethrin, cypermethrin, tau-fluvalinate, cyfluthrin, fenpropathrin, lambda-cyhalothrin, bifenthrin and esfenvalerate), and one neonicotinoid (imidacloprid). The PUR data include the amount (kg) of active ingredient applied, application date, and location, defined as a one-square mile section (1.6 km × 1.6 km) defined by the Public Land Survey System (PLSS).

We edited the PUR data to correct for likely outliers that had unusually high application rates, by replacing the amount of pesticide applied based on the median application rate for that pesticide and crop combination (Gunier et al. 2001). For each woman, we estimated the amount of all pesticides in each pesticide class used within a 1 km radius of the pregnancy residence using the latitude and longitude coordinates and a geographic information system. In all cases, the 1 km buffer around the home included more than one PLSS section; thus, we weighted the amount of pesticide applied in each section by the proportion of land area that was included in the buffer. 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). Detailed descriptions of the equations and methods that we used to calculate nearby pesticide use have been published previously (Gunier et al. 2011). We estimated pesticide use within 1 km of the maternal residence during each trimester of pregnancy for participants with residential location information available for two or more trimesters (n=283) and computed the average pesticide use during pregnancy by summing the trimester-specific values and dividing by the number of trimesters included. We also created individual variables for nearby use of the each of the five individual OP pesticides (acephate, chlorpyrifos, diazinon, malathion and oxydemeton-methyl) with the highest use in our study area during the prenatal period (Table S1).

Toxicity weighting and neurotoxic pesticide index. In addition to examining the simple sum of pesticide use in each class, we also used relative potency factors (RPF) to generate class specific toxicity-weighted sums to account for differences in neurotoxicity of individual pesticides in OP and carbamate classes. The RPF of a chemical is the ratio of the relevant toxicological dose of an index chemical to the relevant toxicological dose of the chemical of

interest. Currently, RPFs are available for OP and carbamate pesticides (USEPA 2006, 2007), but not for neonicotinoids, pyrethroids or Mn-fungicides. Thus, we were only able to create toxicity-weighted sum variables for OPs and carbamates. We calculated the toxicity-weighted use for each OP or carbamate pesticide, expressed as kg-equivalents of chlorpyrifos, by multiplying the kg of use within 1 km of the maternal residence during each trimester for each pesticide by the RPF of that pesticide, and summed to create the toxicity-weighted use for the fifteen OP and six carbamate pesticides. Additionally, since these pesticides share a common mechanism of toxicity (AChE inhibition), we also generated a toxicity-weighted sum for OPs and carbamates combined (Jensen et al. 2009; USEPA 2002). The RPFs, total kg and toxicity-weighted kg of use in the Salinas Valley in 2000 for each OP and carbamate pesticide are provided in Table S1.

Finally, we created a rank index of neurotoxic pesticide use that included the five pesticide classes of interest (i.e. OPs, carbamates, neonicotinoids, pyrethroids, Mn-fungicides) by generating a percentile rank of the participants from lowest to highest use for each neurotoxic pesticide class and then calculating the average percentile rank across the five classes. We also explored principal components analysis (PCA) as a method for combining pesticide use across the five different classes of neurotoxic pesticide.

Data analysis. We log10-transformed continuous pregnancy average and trimester-specific sums of pesticide use (kg/year + 1) to reduce heteroscedasticity and the influence of outliers, and improve the linear fit of the model. Scores for Full-Scale IQ and the four subdomains were normally distributed and were modeled as continuous outcomes.
We selected model covariates *a priori* based on factors associated with infant neurodevelopment in previous analyses [i.e., child's exact age at assessment, sex, maternal PPVT score (continuous)

and maternal education (< 6^{th} grade vs. ≥ 7^{th} grade)]. We considered the following variables as additional covariates in our models (Table 1): maternal country of birth, maternal age at delivery, marital status at enrollment, and maternal depression (≥16 on CES-D) at the child's 7-year visit. In addition, we considered covariates collected at each visit including housing density (number of persons per room), HOME score (continuous), household poverty level (<federal poverty level vs. ≥ federal poverty level), presence of father in the home (yes/no), maternal work status, location of assessment (field office or recreational vehicle), and season of assessment. We imputed missing values (<10% missing) at a visit point using data from the nearest available visit.

We retained covariates that were significant ($p < 0.2$) at any time point in the multivariate regression models, and used the same covariates in all models. We fit separate regression models for each pesticide class or individual pesticide and also models including multiple classes or pesticides.

We used generalized additive models (GAMs) with a three-degrees-of-freedom cubic spline function to test for non-linearity. None of the digression from linearity tests were significant ($p<0.05$), therefore we expressed neurotoxic pesticide use linearly (on the $\log_{10}$ scale) in regression models.

We controlled for DAP metabolites of OP insecticides measured in maternal urine sample (Bradman et al. 2005) collected during prenatal interviews at 13 weeks and 26 weeks gestation (n=283) in all models. Prenatal DAPs and agricultural use of OPs were not highly correlated in our cohort ($\rho=0.04$), therefore we believe that they provide complementary measures of exposure to OP pesticides. We averaged the two prenatal DAP measurements and used $\log_{10}$-transformed concentrations (nmol/L) in our analyses. In separate sensitivity analyses, we controlled for

exposure to other neurotoxicants, which we have previously found to be related to child IQ in our cohort (Eskenazi et al. 2013; Gaspar et al. 2015). Specifically, we considered $\log_{10}$-transformed lipid-adjusted concentrations (ng/g-lipid) measured in prenatal maternal blood samples of *p, p'*-dichlorodiphenyltrichloroethylene (DDT), *p, p'*-dichlorodiphenyldichloroethylene (DDE) (n=219) (Bradman et al. 2007) and polybrominated diphenyl ether flame retardants (PBDEs) (n=221) (Castorina et al. 2011). We used the sum of the four major congeners (BDE-47, -99, -100, and -153) to estimate PBDE exposure (Eskenazi et al. 2013).

In other sensitivity analyses, we excluded outliers identified with studentized residuals greater than three. To control for potential selection bias due to loss to follow-up, we ran regression models with weights determined as the inverse probability of inclusion in our analyses at each time point (Hogan et al. 2004). We determined probability of inclusion using multiple logistic regression models with baseline covariates as potential predictors.

Results

Most mothers were born in Mexico (88.0%), under 30 years of age at delivery (72.8%) and married or living as married (84.4%) at the time of enrollment (Table 1). Almost half of the mothers (46.6%) and most fathers (60.6%) had a 6th grade education or less, and most families (71.7%) were living below the poverty level at the time of the 7-year visit. Slightly more than half of the children were girls (53.7%) and most children completed their WISC-IV assessment in Spanish (68.2%).

The distributions of agricultural use of neurotoxic pesticides within 1 km of maternal residences during pregnancy are shown in Table 2. The most heavily used pesticide class was OPs, followed by Mn-fungicides. The geometric mean (GM) and geometric standard deviation

(GSD) for the cumulated use of 15 OP pesticides within 1 km of maternal residences during pregnancy was 75 (5) kg. For individual OP pesticides, the GM (GSD) ranged from 5 (6) kg for malathion to 23 (3) kg for diazinon and the use of these five individual OP pesticides was moderately (0.40 for malathion and diazinon) to highly (0.91 for acephate and oxydemeton-methyl) correlated. The GM (GSD) of the other neurotoxic pesticide groups ranged from 4 (2) kg for neonicotinoids to 54 (4) kg for Mn-fungicides. There was moderate to high correlation (0.68 – 0.90) between the use of the five different neurotoxic pesticide groups within one kilometer of the maternal residence during pregnancy (Table S2).

OP pesticides. In general, IQ scores decreased across all domains with increasing use of OP pesticides within 1 km of the maternal residence during pregnancy. Each SD increase in toxicity-weighted OP use during pregnancy was associated with an estimated 2.2 point (95% confidence interval (CI): -3.9, -0.5) (Table 3) decrease in Full-Scale IQ, which was very similar to but slightly greater than for the non-weighted use of all OP pesticides combined (-2.1 points; 95% CI: -3.8, -0.3) (Table 4). A SD increase in toxicity-weighted OP pesticide use during pregnancy was also associated with a 2.9 point decrease in Verbal Comprehension scores (95% CI: -4.4, -1.3) (Table 3). Results were similar in unadjusted and adjusted models. For the other WISC domains, there was a non-significant decrease of approximately 1.4 points per SD increase in toxicity-weighted OP pesticide use. The results were similar whether or not we included the maternal prenatal urinary DAP concentrations in the model. In fact, we observed independent and similar decreases in WISC scores for both a SD increase in prenatal urinary DAPs and a SD increase in toxicity-weighted OP pesticide use when both exposures were included in the same model (Table 3).

In separate adjusted models for individual OP pesticides (Table 4), there was a 2.3-point decrease in Full-Scale IQ for each SD increase in acephate (95% CI: -3.9, -0.6) or oxydemeton-methyl (95% CI: -4.0, -0.7). Although also negatively related, there was no significant relationship between use of chlorpyrifos, malathion, or diazinon and Full-Scale IQ. There was a significant negative relationship between agricultural use of acephate, chlorpyrifos, diazinon and oxydemeton-methyl and Verbal Comprehension. There was no relationship with Working Memory, Processing Speed or Perceptual Reasoning for any of the individual OPs evaluated. We also included the top 5 OP pesticides in the same model (Model 1, Table S3) and found no association ($p<0.05$) with Full-Scale IQ for any individual OP pesticide, but the strongest relationship was with oxydemeton-methyl (-4.2 points; 95% CI: -10.1, 1.8).

Other pesticide groups. We observed a nearly universal trend of lower IQ scores for all domains with greater use of individual OP pesticides and other potentially neurotoxic pesticide groups within 1 km of the maternal residence during pregnancy (Table 4). The combined toxicity-weighted use of OPs and carbamates was associated with decreased Full-Scale IQ, although the unweighted and toxicity-weighted use of carbamates alone was not significantly associated with Full-Scale IQ. In separate models with the other neurotoxic pesticide groups, the use of neonicotinoids, pyrethroids and Mn-fungicides were each significantly associated ($p<0.05$) with an approximately 2-point decrease in Full-Scale IQ, Perceptual Reasoning and Verbal Comprehension (Table 4).

In a single model which included all five neurotoxic pesticide groups, the strongest association with Full-Scale IQ was for toxicity-weighted OP pesticide use with a 2.8 point decrease (95% CI: -6.8, 1.3) for each SD increase (Model 2, Table S3). The associations with IQ scales were nearly identical for the first component from PCA, which explained about 80% of

the variance and weighted the five neurotoxic pesticide groups equally, and the average rank index with a 2.0 point decrease (95% CI: -3.7, -0.4) in Full-Scale IQ for each SD increase in estimated exposure (Table 4).

Sensitivity analyses. For all analyses, point estimates were similar but confidence intervals were wider when we restricted the study population to those mothers with residential location known for three trimesters of pregnancy (N = 150 with Full-Scale IQ) and associations were weaker when we included all mothers with residential location known for one trimester during pregnancy (N = 284 with Full-Scale IQ) (data not shown). Our results were very similar after excluding the relatively few outliers (1 – 3 participants per exposure/outcome) based on studentized residuals (data not shown). Associations between prenatal neurotoxic pesticide use and WISC scores at 7-years of age became slightly stronger when we used inverse probability weighting to adjust for potential selection bias (data not shown). Including other prenatal exposures that have been related to WISC scores at 7-years of age (DDT/DDE and PBDEs) reduced the number of participants (n=191 for Full-Scale IQ), but the results were very similar for toxicity-weighted OP pesticide use and the other neurotoxic pesticide groups for all WISC scales with and without inclusion of these other prenatal exposures in the models.

Discussion

We observed an inverse relationship between agricultural use of OP pesticides within one km of maternal residences during pregnancy and cognitive development in children at 7-years of age. For each standard deviation increase in agricultural use of total OPs (237 kg) or toxicity-weighted OPs, there was a two point decrease (15% of a standard deviation) in Full-Scale IQ. To put this in perspective, other authors have estimated that each one point decrease in IQ decreases worker productivity by approximately 2% (Grosse et al. 2002), reducing lifetime earnings by

$18,000 in 2005 dollars (Nedellec and Rabl 2016). The results were independent of prenatal urinary DAP concentrations in the model, and the effect estimates of nearby OP use and urinary DAPs were of similar magnitude. These independent associations suggest that our previous finding of a relationship between prenatal urinary DAPs and IQ (Bouchard et al. 2011a) did not completely account for exposure to OP pesticides during pregnancy and that using both urinary DAPs and PUR data seems to provide a more complete characterization of OP pesticide exposure. Urinary DAPs concentrations provide an estimate of exposure to some, but not all, of the OP pesticides we evaluated using PUR data (Castorina et al. 2010), and primarily reflect dietary exposures (McKone et al. 2007; Bradman et al. 2015). The two individual OP pesticides that had the strongest inverse relationship with Full-Scale IQ were acephate and oxydemeton-methyl, but agricultural use of these two pesticides was highly correlated ($r = 0.91$). It is important to note that although oxydemeton-methyl devolves to urinary DAPS, acephate does not, and that oxydemeton-methyl is the most toxic of all the OPs used in the Salinas Valley (about 11 times more toxic than acephate based on the RPF).

Agricultural use of other potentially neurotoxic pesticide classes was correlated with use of OPs and there were also significant inverse associations between Full-Scale IQ and nearby agricultural use of pyrethroid insecticides, Mn-based fungicides (mostly maneb), and a neonicotinoid insecticide (imidacloprid). The combined agricultural use of pesticides from five neurotoxic pesticide classes based on an average rank index and PCA produced similar results to those observed for toxicity-weighted OP pesticide use alone, making it difficult to determine if OP pesticide use alone is driving the relationship or if it is due to the combined use of neurotoxic pesticides that are highly correlated.

This is the first study to evaluate the relationship between cognitive abilities in children and reported agricultural use of neurotoxic pesticides near maternal residences during pregnancy. A recent study conducted in Spain that used residential proximity to agricultural fields as a proxy for pesticide exposure observed an inverse relationship between postnatal, but not prenatal, hectares of crops near the residence and Full-Scale IQ, Verbal Comprehension, and Processing Speed in children 6 – 11-years of age (Gonzalez-Alzaga et al. 2015). A study in California utilizing PUR data found that any agricultural use of OPs or pyrethroids within 1.5 km of maternal residences during the third trimester of pregnancy compared to no agricultural use of these pesticides was associated with an approximately two-fold increased risk of autism spectrum disorder (Shelton et al. 2014). Higher concentrations of pyrethroid metabolites in children's urine have been associated with increased risk of behavior problems in school-aged children (Oulhote and Bouchard 2013), and attention deficit/hyperactivity disorder in one study (Wagner-Schuman et al. 2015) but not another (Quiros-Alcala et al. 2014). Previous studies have observed inverse associations between children's cognition and levels of manganese in blood (Riojas-Rodriguez et al. 2010) and hair (Bouchard et al. 2011b; Menezes-Filho et al. 2011). Proximity to agricultural use of the neonicotinoid imidacloprid during pregnancy has been associated with greater risk of neural tube defects (Rull et al. 2006; Yang et al. 2014), but there are no previously published studies evaluating cognition in children.

The main strength of this study is the use of PUR data, which provides the amount of active ingredients and location for all agricultural pesticide applications, and represents a major improvement in exposure classification compared to using just crop locations as was done for the recent study in Spain. The PUR data allowed us to examine pesticides that do not have biomarkers and also to assess pesticide mixtures. We also had extensive information on potential

confounders and other chemical exposures available for the CHAMACOS cohort. However, there are some limitations of our study. We were only able to determine proximity to agricultural pesticide use at the maternal residence, not other locations where the mother may have spent time. Although we also used residential proximity to agricultural pesticide use as a proxy for pesticide exposure, previous studies have shown that PUR data is correlated with environmental pesticide concentrations (Harnly et al. 2009; Harnly et al. 2005), suggesting it is a meaningful indicator of pesticide exposure. We did not account for prenatal exposure information from other potential sources of pesticide exposure including home use, occupational take-home and dietary intake, but our models included prenatal urinary DAPs which we believe primarily reflect dietary and also residential exposures (McKone et al. 2007). In general, these limitations would likely lead to exposure misclassification and bias our results towards the null.

People living in agricultural communities are exposed to a complex mixture of many individual pesticide active ingredients and also potentially neurotoxic adjuvants included in the formulation. Better methods are needed for toxicity-weighting across neurotoxic pesticide classes. To improve pesticide exposure assessment based on PUR data, exposure models should be optimized using measured pesticide concentrations in air or house dust samples. In future analyses, we intend to utilize more complex statistical methods to address collinearity of exposures such as weighted quantile sum regression (Gennings et al. 2013), and hierarchical Bayesian models (Kalkbrenner et al. 2010; Rull et al. 2009). The results from this study need to be replicated in studies that include children living in both agricultural and non-agricultural communities to obtain more variability in exposure to pesticide mixtures.

Conclusions

We observed an inverse association between prenatal residential proximity to agricultural use of OPs and other neurotoxic pesticides and cognition in children at 7-years of age. The results of OPs based on PUR data remained significant when including prenatal urinary maternal DAP concentrations in the model, and the effect estimates of nearby OP use and urinary DAPs were of similar magnitude. The association also remained after adjustment for prenatal exposure to other neurotoxic chemicals. Agricultural use of individual pesticides and classes of neurotoxic pesticides were highly correlated, making it difficult to identify specific pesticides driving these associations.

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Table 1. CHAMACOS study cohort characteristics (n=283).

Cohort Characteristic	N (%)
Maternal Country of Birth	
Mexico	249 (88.0)
United States and other	34 (12.0)
Maternal Age at Delivery	
18 - 24	107 (37.8)
25 - 29	99 (35.0)
30 - 34	49 (17.3)
35 - 45	28 (9.9)
Maternal Education	
≤ 6th grade	132 (46.6)
7th grade or more	151 (53.4)
Marital Status at enrollment	
Married/Living as married	239 (84.4)
Not married	44 (15.6)
Paternal Education	
≤ 6th grade	129 (60.6)
7th grade or more	84 (39.4)
Family income at 7y visit	
< Poverty level	203 (71.7)
≥ Poverty level	80 (28.3)
Maternal depression at 7y visit	
Yes	78 (27.6)
No	205 (72.4)
Sex	
Girl	152 (53.7)
Boy	131 (46.3)
Language of WISC-IV tests	
Spanish	193 (68.2)
English	90 (31.8)

Table 2. Total neurotoxic pesticide use in Monterey County in 2000 and distributions of agricultural use within one kilometer of the maternal residence during pregnancy (n=283).

	kg use (2000)	kg use within 1 km of residence during pregnancy				
Neurotoxic Pesticides	Monterey	p25	p50	p75	Max.	GM (GSD)
Organophosphates (OPs)	244,696	31	93	218	1,615	75 (5)
Acephate	34,792	2	10	29	354	9 (4)
Chlorpyrifos	25,357	1	9	29	331	8 (5)
Diazinon	56,434	11	22	50	579	23 (3)
Malathion	37,161	0	2	19	422	5 (6)
Oxydemeton-methyl	28,767	2	11	24	260	9 (4)
OPs toxicity weighted	964,130	59	331	891	8,587	175 (9)
Carbamates	59,914	3	16	49	618	16 (5)
Carbamates toxicity weighted	335,611	14	66	291	9,222	66 (7)
Carbamates and OPs toxicity weighted	1,299,741	95	485	1,379	9,774	270 (9)
Neonicotinoids	7,103	1	3	6	34	4 (2)
Pyrethroids	16,386	1	4	15	79	6 (3)
Mn-fungicides	154,698	25	62	139	960	54 (4)

Table 3. Unadjusted and adjusted[a] associations between a standard deviation increase in toxicity-weighted OP pesticide use within one kilometer of maternal residence and urinary DAPs during pregnancy included in the same model and IQ scales at 7-years of age.

	Unadjusted Model				Adjusted Models					
Cognitive Test	Toxicity-Weighted OP pesticides (kg)				Toxicity-Weighted OP pesticides (kg)			Urinary DAPs (nmol/L)		
(WISC-IV Scale)	N	β	(95% CI)		β	(95% CI)		β	(95% CI)	
Working Memory	256	-0.9	(-2.7, 0.8)		-1.4	(-3.1, 0.4)		-1.7	(-3.3, -0.1)	*
Processing Speed	256	-0.9	(-2.6, 0.9)		-1.3	(-3.0, 0.5)		-1.2	(-2.8, 0.4)	
Verbal Comprehension	283	-3.5	(-5.5, -1.5)	**	-2.9	(-4.4, -1.3)	**	-2.8	(-4.3, -1.4)	**
Perceptual Reasoning	283	-1.2	(-3.1, 0.7)		-1.4	(-3.3, 0.5)		-1.7	(-3.5, 0.1)	
Full-Scale IQ	255	-2.1	(-4.0, -0.3)	*	-2.2	(-3.9, -0.5)	*	-2.4	(-4.0, -0.9)	**

[a] Adjusted for child's age at assessment, sex, language of assessment, maternal education, maternal intelligence, maternal country of birth, maternal depression at 7-year visit, HOME Score at 7-year visit and household poverty level at 7-year visit.
*p<0.05; **p<0.01.

Table 4. Adjusted association between a standard deviation increase in neurotoxic pesticide use within one kilometer of residence during pregnancy and IQ scales at 7-years of age from separate models for each exposure.

	Full-Scale IQ (n=255)		Working Memory (n=256)		Processing Speed (n=256)		Perceptual Reasoning (n=283)		Verbal Comprehension (n=283)	
Neurotoxic Pesticides	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)
Organophosphates	-2.1	(-3.8, -0.3)*	-1.3	(-3.1, 0.4)	-1.1	(-2.9, 0.7)	-1.8	(-3.7, 0.1)	-2.5	(-4.1, -1.0)**
Acephate	-2.3	(-3.9, -0.6)**	-1.4	(-3.1, 0.3)	-1.4	(-3.1, 0.2)	-1.8	(-3.6, 0.1)	-2.7	(-4.3, -1.2)**
Chlorpyrifos	-1.4	(-3.0, 0.2)	-1.3	(-3.0, 0.3)	-0.3	(-1.9, 1.4)	-0.8	(-2.6, 1.1)	-2.2	(-3.7, -0.7)**
Diazinon	-1.7	(-3.4, 0.1)	-1.3	(-3.0, 0.5)	-1.0	(-2.8, 0.7)	-1.7	(-3.6, 0.2)	-1.6	(-3.2, -0.1)*
Malathion	-0.8	(-2.5, 0.8)	-0.8	(-2.4, 0.9)	0.8	(-0.9, 2.5)	-1.2	(-3.0, 0.6)	-1.3	(-2.8, 0.2)
Oxydemeton-methyl	-2.3	(-4.0, -0.7)**	-1.5	(-3.2, 0.2)	-1.5	(-3.2, 0.2)	-1.5	(-3.4, 0.4)	-2.8	(-4.3, -1.3)**
Organophosphates toxicity weighted	-2.2	(-3.9, -0.5)**	-1.4	(-3.1, 0.4)	-1.3	(-3.0, 0.5)	-1.4	(-3.3, 0.5)	-2.9	(-4.4, -1.3)**
Carbamates	-1.2	(-2.8, 0.4)	-0.4	(-2.0, 1.2)	-0.2	(-1.8, 1.4)	-1.1	(-2.9, 0.7)	-2.4	(-3.9, -1.0)**
Carbamates toxicity weighted	-1.3	(-2.9, 0.3)	-0.6	(-2.2, 1.1)	-0.1	(-1.7, 1.5)	-1.0	(-2.8, 0.8)	-2.5	(-4.0, -1.0)**
Carbamates and Organophosphates toxicity weighted	-2.1	(-3.7, -0.4)*	-1.1	(-2.9, 0.6)	-1.0	(-2.7, 0.7)	-1.4	(-3.3, 0.5)	-2.9	(-4.5, -1.4)**
Neonicotinoids	-1.7	(-3.3, 0.0)*	-1.1	(-2.8, 0.5)	-0.8	(-2.4, 0.9)	-1.9	(-3.8, -0.1)*	-1.9	(-3.5, -0.3)*
Pyrethroids	-2.0	(-3.7, -0.3)*	-1.5	(-3.2, 0.2)	-1.1	(-2.8, 0.6)	-2.1	(-4.0, -0.2)*	-1.8	(-3.4, -0.3)*
Mn-fungicides	-2.0	(-3.7, -0.2)*	-1.2	(-2.9, 0.6)	-1.2	(-2.9, 0.6)	-1.7	(-3.6, 0.1)	-2.1	(-3.7, -0.6)**
Rank index of 5 neurotoxic groups	-2.0	(-3.7, -0.4)*	-1.3	(-3.0, 0.4)	-1.0	(-2.7, 0.7)	-1.9	(-3.8, -0.1)*	-2.4	(-4.0, -0.9)**

[a] Adjusted for child's age at assessment, sex, language of assessment, maternal education, maternal intelligence, maternal country of birth, maternal depression at 7-year visit, HOME score at 7-year visit, household poverty level at 7-year visit and prenatal urinary DAPs.

*p<0.05; **p<0.01