American Journal of Epidemiology

Vol. 184, No. 5
DOI: 10.1093/aje/kwv447
Advance Access publication:
August 18, 2016

Original Contribution

An Observational Study to Evaluate Associations Between Low-Level Gestational Exposure to Organophosphate Pesticides and Cognition During Early Childhood

Stephanie Donauer*, Mekibib Altaye, Yingying Xu, Heidi Sucharew, Paul Succop, Antonia M. Calafat, Jane C. Khoury, Bruce Lanphear, and Kimberly Yolton

* Correspondence to Dr. Stephanie Donauer, Division of General and Community Pediatrics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3026 (e-mail: stephanie.donauer@cchmc.org).

Initially submitted July 2, 2015; accepted for publication December 18, 2015.

Prenatal exposure to organophosphate pesticides, which is ubiquitous, may be detrimental to neurological development. We examined 327 mother/infant pairs in Cincinnati, Ohio, between 2003 and 2006 to determine associations between prenatal exposure to organophosphate pesticides and neurodevelopment. Twice during pregnancy urinary concentrations of 6 common dialkylphosphates, nonspecific metabolites of organophosphate pesticides, were measured. Aggregate concentrations of diethylphosphates, dimethylphosphates, and total dialkylphosphates were calculated. Bayley Scales of Infant Development, Second Edition-Mental and Psychomotor Developmental indices were administered at ages 1, 2, and 3 years, the Clinical Evaluation of Language Fundamentals-Preschool, Second Edition, at age 4, and the Wechsler Preschool and Primary Scale of Intelligence, Third Edition, at age 5. Mothers with higher urinary total dialkylphosphate concentrations reported higher levels of socioeconomic status and increased fresh fruit and vegetable intake. We found no associations between prenatal exposure to organophosphate pesticides and cognition at 1–5 years of age. In our cohort, exposure to organophosphate pesticides during pregnancy was not associated with cognition during early childhood. It is possible that a higher socioeconomic status and healthier diet may protect the fetus from potential adverse associations with gestational organophosphate pesticide exposure, or that dietary exposure to the metabolites is innocuous and not an ideal measure of exposure to the parent compound.

cognition; gestation; organophosphate pesticides; pregnancy; prenatal exposures

Abbreviations: Bayley-II, Bayley Scales of Infant Development, Second Edition; DAP, dialkylphosphate; DE, diethylphosphate; DM, dimethylphosphate; IQ, intelligence quotient; MDI, Mental Developmental Index.

Exposure to organophosphate pesticides during pregnancy may be detrimental to a child's neurological development, and several mechanisms of deleterious action have been proposed (1–3). Organophosphate pesticides are one of the primary insecticides used in agriculture, and exposure to organophosphate pesticides during pregnancy has been associated with several deficits throughout childhood. These include delays in cognitive abilities (4–8), impairments in motor skills (8), and child behaviors characteristic of attentional disorders (4, 9). Infants exposed prenatally to a common agricultural organophosphate pesticide (chlorpyrifos) have been shown to demonstrate abnormal structural changes in areas of the brain related to attention and receptive language, social cognition, reward, emotion, and inhibitory control (10). Although postnatal exposures to organophosphate pesticides have also been examined, when taken together, studies have demonstrated more frequent and/or stronger associations between prenatal exposures and adverse outcomes than with postnatal exposures (4, 6, 9). However, to date, studies examining these associations have focused primarily on cohorts that were likely exposed to higher-than-national-average levels of organophosphate pesticides, either through heavy use of pesticides for inner-city pest control or as members of farming communities (4–10).

Because residential use of chlorpyrifos in the United States was banned in 2001, the primary route of human exposure to organophosphate pesticides is through the ingestion of food treated with pesticides during agricultural processing. Although the ban of home usage has reduced human exposure, some studies suggest that widespread gestational exposure to organophosphate pesticides continues despite the ban (11, 12). The purpose of the current study was to examine the association of prenatal exposure to organophosphate pesticides with early childhood cognition in a sample of children whose mothers' exposures during pregnancy are similar to reported US levels. This study was initiated after the ban of residential use of chlorpyrifos in 2001, whereas previously published studies included mostly subjects exposed prior to the residential ban (4–10).

METHODS

Study population

The study comprises women and children enrolled in the Health Outcomes and Measures of the Environment Study (HOME Study), an ongoing prospective pregnancy and birth cohort study designed to examine the associations of low-level prenatal and early childhood exposure to a variety of environmental toxicants with child health and development. Between February 2003 and January 2006, 468 healthy pregnant women in the Cincinnati, Ohio, area were enrolled; 398 remained in the study and delivered live infants. Detailed eligibility criteria and enrollment methods have been described elsewhere (13, 14). Institutional review boards of all involved research institutions, hospitals, and laboratories approved the study protocol.

We excluded 9 sets of twins because some studies have demonstrated that this may be a significant factor associated with intelligence quotient (IQ) scores (15, 16). Of the remaining 389 singletons, the sample was further restricted to 382 children whose mothers provided urine samples at gestational weeks 16 and 26. This was done to avoid overlapping time periods that could obscure estimates of time-specific exposures. Finally, the sample was restricted to the 327 children who had at least 1 developmental examination performed at age 1, 2, 3, 4, or 5 years.

Exposure measurements

Spot urine samples were collected from the mothers at 2 time points plus or minus 4 weeks: 16 (standard deviation, 1.8) weeks' and 26 (standard deviation, 1.9) weeks' gestation. Samples were analyzed at the Centers for Disease Control and Prevention in 2010. In each of the urine samples, 6 dialkylphosphates that are nonspecific metabolites of organophosphate pesticides were measured. Specifically, dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylphosphate, diethylthiophosphate, and diethyldithiophosphate were measured using a modified version of the method described by Bravo et al. (17). Metabolite concentrations on a molar basis were summed to obtain aggregated concentrations of diethylphosphates (total DE = diethylphosphate + diethylthiophosphate + diethyldithiophosphate); dimethylphosphates (total DM = dimethylphosphate + dimethylthiophosphate + dimethyldithiophosphate); and dialkylphosphates (total DAP = diethylphosphate + diethylthiophosphate + diethyldithiophosphate + dimethylphosphate + dimethylthiophosphate + dimethyldithiophosphate). Each analytical batch included quality control materials and reagent blanks to ensure the accuracy and reliability of the data. The concentrations of the quality control materials had to meet standard statistical probability rules (18) or the samples included in the batch were reanalyzed. The coefficients of variation were between 7% and 17% at concentrations ranging from around 3–15 ng/mL, depending on the analyte. These methods have been described in further detail elsewhere (19).

Child cognitive assessments

Neurodevelopmental outcomes were assessed annually at ages 1–5 years by 2 trained examiners. These examiners were trained and certified by a trained expert and observed and recertified biannually. At 1, 2, and 3 years, we assessed mental and motor development using the Bayley Scales of Infant Development, Second Edition (Bayley-II). Cognitive and language development was assessed by using the Mental Developmental Index (MDI), while gross and fine motor development was assessed by using the Psychomotor Developmental Index. At 4 years, language skills were assessed by using the Clinical Evaluation of Language Fundamentals-Preschool, Second Edition. This test measured the children's core language skills and also provided individual assessments of their receptive language, expressive language, language content, and language structure skills. At 5 years, intelligence was assessed by using the Wechsler Preschool and Primary Scale of Intelligence, Third Edition. The Wechsler Preschool and Primary Scale of Intelligence, Third Edition, provided measurement of the children's verbal IQ, performance IQ, processing speed quotient, and full-scale IQ.

Measured covariates

For all analyses, we examined the following additional covariates for their potential contributions to both organophosphate pesticide exposure and developmental and cognitive outcomes: child's sex and birth weight, mother's race, parity, age at delivery, education, marital status, intelligence quotient, and reported alcohol and marijuana use during pregnancy (obtained from a maternal questionnaire). We also examined household income, maternal whole blood lead and serum cotinine during pregnancy, and the quality of the home environment measured by using the Home Observation for Measurement of the Environment score (Home score) (20). The child's sex, maternal race, maternal IQ, and household income were determined a priori to be retained in all models as a standard set of covariates.

Statistical analysis

All analyses were conducted by using SAS, version 9.3, software (SAS Institute, Inc., Cary, North Carolina) (21). If a metabolite concentration was below the limit of detection, the following procedure was used: For concentrations reported as a positive value, that value was used in analyses; for concentrations reported as zero or if no numerical value was reported, the concentration was imputed by using methods described elsewhere (19). The mean of creatinine basis concentration total DE, total DM, and total DAP at 16 and 26 weeks was used as the primary exposure variable.

The Bayley-II MDI and Bayley-II Psychomotor Developmental Index data were collected at 1, 2, and 3 years of age. When the association between exposure and outcome was <0.20, we ran a multivariable model in which we included all potential covariates. A regression model with backwards stepwise selection was used, in which the main exposure variable and a standard set of covariates (child's sex, maternal race, maternal IQ, and household income) were retained in the model regardless of significance. Other covariates were removed from the model if they were found to be insignificant ($P \geq 0.05$, and their removal did not modify the

Table 1. Characteristics of the Mothers Enrolled in a Cincinnati-Based Pregnancy Cohort (The HOME Study) Between 2003 and 2006

Maternal Characteristic	Full Sample (*n* = 327)				Participants Not Included (*n* = 62)			
	No.	%	Geometric Mean	95% CI	No.	%	Geometric Mean	95% CI
Age at delivery, years[a]	30 (5.7)				26 (5.6)			
Race								
Caucasian, non-Hispanic	221	68			16	28		
African American, non-Hispanic	85	26			36	65		
Other	21	6			3	5		
Marital status								
Married	229	70			19	33		
Not married, living with someone	41	13			15	26		
Not married, living alone	57	17			23	40		
Household income, $[b]	55,000 (27,000, 85,000)				22,000 (12000, 56,000)			
Home score at 12 months only[a]	39 (5.1)				38 (3.0)			
Employed	269	82			41	72		
Education								
High school or less or GED	65	20			30	53		
Some college or college graduate	189	58			19	33		
Graduate or professional school	73	22			8	14		
IQ, score[a]	107 (14.4)				97 (12)			
Moderate-to-severe depression								
At baseline and 4 weeks	80	24			26	45		
Postnatal (1, 2, and 3 years)								
Ever	66	20						
Alcohol use during pregnancy								
Never drank alcohol during pregnancy	176	54			40	70		
Drank <1 alcoholic drink/month	106	32			9	16		
Drank ≥1 alcoholic drink/month	45	14			8	14		
Marijuana use during pregnancy	16	5			14	22		
Blood lead, μg/dL			0.80	0.77, 0.84			0.88	0.80, 0.97
Serum cotinine, ng/mL			0.084	0.064, 0.111			0.95	0.43, 2.09
Total DE, nmol/g creatinine			9.3	7.8, 11.0			9.3	6.2, 13.9
Total DM, nmol/g creatinine			45.9	39.3, 53.6			39.1	27.5, 55.5
Total DAP, nmol/g creatinine			73.7	64.5, 84.3			60.7	44.8, 82.0

Abbreviations: CI, confidence interval; DAP, dialkylphosphate; DE, diethylphosphate; DM, dimethylphosphate; GED, general equivalency diploma; Home score, Home Observation for Measurement of the Environment score; HOME Study, Health Outcomes and Measures of the Environment Study; IQ, intelligence quotient; SD, standard deviation.

[a] Values are expressed as the mean (SD).

[b] Values are expressed as the median (25th, 75th percentiles).

regression coefficient of the organophosphate pesticides by >10%.

For the Clinical Evaluation of Language Fundamentals-Preschool, Second Edition, at 4 years, we considered standard scores for overall core language as well as component indices for receptive, expressive, content, and structure of language. For the Wechsler Preschool and Primary Scale of Intelligence, Third Edition, at age 5, we examined full-scale IQ, performance IQ, processing speed quotient, and verbal IQ scores. We conducted a bivariate association analysis between each of these outcome measures and exposure variables (total DE, total DM, total DAP). When the association between exposure and outcome was <0.20, we followed the same procedures for multivariable analysis as described for the Bayley-II outcome.

RESULTS

A total of 327 mother-child pairs were included. Table 1 displays the maternal characteristics of the study sample. Mothers who were excluded because they did not provide both 16-week and 26-week urine samples ($n = 7$) or because their children did not have at least 1 developmental examination performed at age 1, 2, 3, 4, or 5 years ($n = 55$) were demographically different from mothers who were included. (Demographic information for these mothers is also included in Table 1.) Mothers who were not included were younger, were less likely to be non-Hispanic Caucasian, and had lower levels of education and different prenatal exposure patterns from mothers who were included.

Table 2. Characteristics of the Children Enrolled in a Cincinnati-Based Pregnancy Cohort (The HOME Study) Between 2003 and 2006

Child's Characteristic	No.	%	Mean (SD)	Geometric Mean	95% CI
Male	147	45			
Birth weight, g			3,398 (625)		
Gestational age, weeks			39 (1.7)		
Birth order					
First child	141	43			
Second child	105	32			
Third child or later	81	25			
Blood lead, μg/dL					
1 year				1.69	1.57, 1.82
2 years				1.70	1.56, 1.84
3 years				1.26	1.15, 1.38
Mean (1, 2, and 3 years)				1.67	1.55, 1.79
Maximum (1, 2, and 3 years)				2.01	1.87, 2.17
Bayley-II scores					
1-year MDI ($n = 321$)			93.41 (9.88)		
1-year PDI ($n = 320$)			91.35 (13.63)		
2-year MDI ($n = 268$)			89.43 (14.47)		
2-year PDI ($n = 268$)			91.06 (13.92)		
3-year MDI ($n = 244$)			93.31 (13.83)		
3-year PDI ($n = 242$)			96.96 (14.80)		
Clinical Evaluation of Language Fundamentals-Preschool, Second Edition					
Core language ($n = 182$)			99.14 (16.35)		
Receptive ($n = 181$)			98.40 (15.85)		
Expressive ($n = 180$)			100.19 (14.96)		
Wechsler Preschool and Primary Scale of Intelligence, Third Edition					
Verbal IQ ($n = 196$)			101.39 (15.40)		
Performance IQ ($n = 197$)			102.07 (16.07)		
Processing speed quotient ($n = 187$)			98.86 (15.18)		
Full-scale IQ ($n = 197$)			105.73 (15.50)		

Abbreviations: Bayley-II, Bayley Scale of Infant Development, Second Edition; CI, confidence interval; HOME Study, Health Outcomes and Measures of the Environment Study; IQ, intelligence quotient; MDI, Mental Developmental Index; PDI, Psychomotor Developmental Index; SD, standard deviation.

Table 2 summarizes characteristics of the children. Average blood lead levels at ages 1, 2, and 3 years were below the current reference range of 5.0 µg/dL (1.69 µg/dL at age 1, 1.70 µg/dL at age 2, and 1.26 µg/dL at age 3). The mean scores for each of the neurobehavioral outcomes were within normal limits (22–24).

Table 3 displays the creatinine basis concentration DAP metabolite concentrations during pregnancy and the median levels of total DE, total DM, and total DAP (refer to Appendix Table 1 for additional metabolite concentrations). Median values of the mean exposure categories for gestational weeks 16 and 26 in this study were 21.1, 64.3, and 96.7 nmol/L for total DE, total DM, and total DAP, respectively. Mothers who were non-African American, had a college education or higher, or who were married had higher urinary concentrations of organophosphate pesticide metabolites compared with mothers who were African American, received no more than a high school education, or were unmarried.

Associations between maternal gestational levels of total DM and Bayley-II MDI are summarized in Table 4. At the 2-year visit, total DM and total DE were positively associated with Bayley-II MDI at the $P < 0.20$ level ($\beta = 0.003$ and $\beta = 0.003$, respectively). However, as displayed in Table 4, these relationships were attenuated after controlling for relevant covariates ($\beta = 0.002$ and $\beta = 0.002$, respectively).

At 4 years, there were no significant relationships between the mean exposure variables and any of the child language index measures at the $P < 0.20$ level, as measured by the Clinical Evaluation of Language Fundamentals-Preschool, Second Edition (results not shown). Therefore, additional multivariable analyses were not performed.

As shown in Table 5, at 5 years, total DAP and total DM were positively associated with full-scale IQ at the $P < 0.20$ level in unadjusted analyses ($\beta = 0.004$ and $\beta = 0.004$, respectively). However, these relationships were attenuated after controlling for relevant covariates ($\beta = 0.001$ and $\beta = 0.002$ for total DAP and total DM, respectively). Finally, total DAP, total DM, and total DE were positively associated with verbal IQ at the $P < 0.20$ level ($\beta = 0.005$, $\beta = 0.005$, and $\beta = 0.048$, respectively), but these relationships were attenuated after controlling for relevant covariates ($\beta = 0.003$, $\beta = 0.003$, and $\beta = -0.027$), as shown in Table 5.

DISCUSSION

In this study, children of women exposed to organophosphate pesticides during pregnancy at levels that resemble average US levels as determined by the National Health and Nutrition Examination Survey (NHANES) (25) were assessed for cognitive, motor, language, and intelligence outcomes

Table 3. Creatinine-Corrected Dialkyl Phosphate Metabolite Urinary Concentrations, by Maternal and Child Characteristics, in Mothers Enrolled in a Cincinnati-Based Pregnancy Cohort (The HOME Study) Between 2003 and 2006

	No.	Total Diethylphosphates		Total Dimethylphosphates		Total Dialkylphosphates	
		Median[a]	25th, 75th Percentiles	Median[a]	25th, 75th Percentiles	Median[a]	25th, 75th Percentiles
Overall	327	21.1	8.2, 43.4	64.3	25.6, 190.9	96.7	44.6, 255.1
Child's sex							
Female (referent)	180	21.0	7.5, 46.3	65.4	25.5, 159.2	95.4	43.9, 256.5
Male	147	21.1	9.5, 42.9	64.3	25.6, 220.2	100.5	48.1, 256.5
Maternal race							
Non-African American (referent)	242	23.9	11.8, 51.1	73.6	32.6, 214.7	109.8	56.8, 268.8
African American	85	13.0[b]	4.0, 36.3	42.4[b]	15.7, 141.9	64.1[b]	24.6, 171.8
Education							
High school graduate or less (referent)	65	12.0	3.0, 32.7	42.7	13.0, 100.4	68.6	18.9, 124.0
Some college	84	16.6	5.3, 35.0	51.6	19.0, 112.1	74.3	37.3, 140.0
College graduate	105	23.7[b]	10.7, 65.7	90.1[b]	36.3, 270.0	118.1[b]	61.4, 338.4
Graduate/professional degree	73	29.2[b]	18.1, 53.9	96.1[b]	32.6, 360.6	127.6[b]	63.7, 421.4
Marital status							
Married (referent)	229	24.5	11.8, 53.2	76.6	32.6, 232.8	111.1	54.7, 289.7
Not married, living with partner	41	15.0[b]	3.0, 30.5	37.3[b]	11.3, 98.8	60.5[b]	31.1, 125.8
Not married, living alone	57	15.8[b]	5.3, 35.1	53.4[b]	15.7, 141.9	74.3[b]	25.7, 171.8
Maternal employment							
Not employed (referent)	58	15.1	4.0, 33.2	42.8	18.0, 128.9	59.8	25.7, 165.3
Employed	269	23.7[b]	9.6, 44.9	72.2[b]	28.2, 214.7	108.7[b]	40.9, 267.7

Abbreviation: HOME Study, Health Outcomes and Measures of the Environment Study.

[a] Median in nmol/g creatinine.

[b] Significant difference compared with referent using log-transformed data due to distribution of data ($P < 0.05$).

Table 4. Associations Between Maternal Gestational Levels of Creatinine-Basis Mean Dimethylphosphate and Dialkylphosphate Metabolite Urinary Concentrations and the Bayley-II Motor Developmental Index and the Bayley-II Psychomotor Developmental Index Among Children Aged 1, 2, and 3 Years Enrolled in a Cincinnati-Based Pregnancy Cohort (The HOME Study) Between 2003 and 2006

Organophosphate and Age of Child	Estimate (Standard Error)	*P* Value
Bayley-II Mental Developmental Index		
Total DAP		
12 Months[a]	−0.002 (0.011)	0.85
24 Months[a]	0.007 (0.017)	0.68
36 Months[a]	0.024 (0.025)	0.33
Total DM[a]		
12 Months[a]	−0.002 (0.001)	0.25
24 Months[a]	0.003 (0.002)	0.16
24 Months[b]	0.002 (0.002)	0.28
36 Months[a]	0.002 (0.002)	0.29
Total DE[a]		
12 Months[a]	−0.002 (0.001)	0.25
24 Months[a]	0.003 (0.002)	0.16
24 Months[b]	0.002 (0.002)	0.37
36 Months[a]	0.002 (0.002)	0.27
Bayley-II Psychomotor Developmental Index		
Total DAP		
12 Months[a]	−0.014 (0.016)	0.37
24 Months[a]	0.006 (0.017)	0.71
36 Months[a]	−0.006 (0.026)	0.83
Total DM		
12 Months[a]	0.000 (0.002)	0.99
24 Months[a]	0.001 (0.002)	0.59
36 Months[a]	0.000 (0.002)	0.82
Total DE		
12 Months[a]	0.000 (0.002)	0.93
24 Months[a]	0.001 (0.002)	0.57
36 Months[a]	0.000 (0.002)	0.84

Abbreviations: Bayley-II, Bayley Scales of Infant Development, Second Edition; DAP, dialkylphosphate; DE, diethylphosphate; DM, dimethylphosphate; HOME Study, Health Outcomes and Measures of the Environment Study.

[a] Unadjusted model.

[b] Model adjusted for household income, maternal intelligence quotient, maternal race, child's sex, and maternal lead level.

Table 5. Associations Between Maternal Gestational Levels of Creatinine-Basis Mean Dialkylphosphate Metabolite Urinary Concentrations and Wechsler Preschool and Primary Scale of Intelligence, Third Edition, Among Children Enrolled at Age 5 Years in a Cincinnati-Based Pregnancy Cohort (The HOME Study) Between 2003 and 2006

Organophosphate	Wechsler Preschool and Primary Scale of Intelligence, Third Edition	
	Estimate (Standard Error)	*P* Value
Full-Scale IQ		
Total DAP[a]	0.004 (0.002)	0.111
Total DAP[b]	0.001 (0.002)	0.494
Total DM[a]	0.004 (0.003)	0.124
Total DM[b]	0.002 (0.002)	0.441
Total DE[a]	0.031 (0.029)	0.289
Performance IQ		
Total DAP[a]	0.003 (0.003)	0.277
Total DM[a]	0.003 (0.003)	0.265
Total DE[a]	−0.001 (0.030)	0.979
Verbal IQ		
Total DAP[a]	0.005 (0.002)	0.034
Total DAP[c]	0.003 (0.002)	0.179
Total DM[a]	0.005 (0.002)	0.043
Total DM[c]	0.003 (0.002)	0.144
Total DE[a]	0.048 (0.029)	0.099
Total DE[c]	−0.027 (0.024)	0.259

Abbreviations: DAP, dialkylphosphate; DE, diethylphosphate; DM, dimethylphosphate; HOME Study, Health Outcomes and Measures of the Environment Study; IQ, intelligence quotient.

[a] Unadjusted model.

[b] Adjusted for household income, maternal race, child's sex, maternal intelligence quotient; and maternal education.

[c] Adjusted for household income, maternal race, child sex, maternal intelligence quotient; Home inventory score, maternal age at birth, alcohol consumption during pregnancy, and parity.

from 1 to 5 years of age. In unadjusted analyses, several significant positive associations were found between urinary concentrations of metabolites of organophosphate pesticides, but these associations became nonsignificant in multivariable analyses that included adjustment for covariates of exposure and outcome.

The positive associations found in the unadjusted analyses in this cohort are likely a result of confounding by socioeconomic status and dietary intake of fruits and vegetables, and not due to beneficial properties of pesticides. This is verified by the lack of association observed after adjustment for confounders. The mothers in this cohort who had higher urinary DAP concentrations were more likely to be married, better educated, more likely to be employed, and less likely to be African American than mothers with lower urinary DAP concentrations. In this same cohort, Yolton et al. (19) found that mothers who had higher concentrations of organophosphate pesticide metabolites also reported increased daily fruit and vegetable uptake, and they were more likely to consume organic food products. It is possible that mothers with higher socioeconomic status during pregnancy may consume a more nutrient-rich diet. It has been shown that maternal nutritional status in pregnancy can influence fetal brain development, and it is possible that a similar mechanistic model is occurring within our cohort and potentially influencing our results (26). It is worth noting that other studies have found significant positive associations that were contrary to their a priori hypotheses with regard to organophosphate pesticides and neurodevelopment in infants and young children (6, 27).

It is possible that we may be observing evidence of a threshold at which these organophosphate pesticides have no negative associations with cognition and neurodevelopment in children. Dose responses have generally been observed in other studies assessing the association between exposure to organophosphate pesticides and neurodevelopmental constructs (3), and it is therefore possible that the exposure in our cohort was too low to detect an association with our outcomes. Because the urinary concentrations in our cohort closely mirrored those reported nationally for women (25), our findings are reassuring to those concerned about pesticide exposure that may result from average intake of fresh produce consumption.

Our findings are in agreement with those of Lu et al. (28) who concluded that prenatal pesticide exposure, as measured once during pregnancy, did not play a significant role in the performance of the cognitive development evaluation in children from 4 to 10 years of age. Our findings are also in agreement with those of Cartier et al. (29), who recently found no evidence that prenatal organophosphate pesticide exposure adversely affected cognitive function in children aged 6 years. Similar to our study, the study by Cartier et al. also found paradoxical results and concluded that their population was well educated and therefore may have had fewer risk factors and more compensatory stimulation. The evidence here is also in partial agreement with that of Engel et al. (5), who found no relationship between organophosphate pesticides and the Bayley-II Psychomotor Developmental Index at either 12 or 24 months. Interestingly, however, our findings are not in accordance with those of the same study by Engel et al., who found that exposure to prenatal organophosphate pesticides is negatively associated with cognitive development as measured by the Bayley-II MDI and Wechsler psychometric intelligence examinations, with associations beginning at 12 months and continuing through early childhood. However, higher median urinary concentrations of total DE, total DM, and total DAP were reported in Engel et al.'s cohort (20.2, 44.8, and 81.3 nmol/L, respectively) compared with our cohort (9, 38, and 60 nmol/L). It should also be noted that in Engel et al.'s study, higher concentrations of total DAP were positively associated with Bayley-II MDI scores at 12 months among subjects who identified as non-Hispanic white. Our findings also differ from those of Eskenazi et al. (6), who reported that maternal urinary concentrations of DAP metabolites were related to poorer mental development and maternally reported symptoms consistent with pervasive developmental disorder in children 2 years of age. However, in the CHAMACOS Study, the majority of subjects were from a longitudinal birth cohort of primarily farm-worker families, and they had mean concentrations of total DE, total DM, and total DAP (18.1, 81.5, and 114.9 nmol/L, respectively) higher than those measured in our cohort (9.3, 45.9, and 73.7 nmol/L). At 16 weeks, the geometric mean of dimethylthiophosphate for our study was comparable to the US National Health and Nutrition Examination Survey, 2003–2004, general population data (25) (2.16 vs. 2.06 µg/L, respectively), but was slightly lower at 26 weeks (1.44 vs. 2.06 µg/L, respectively).

This study does have limitations. The first limitation relates to the use of spot urine samples obtained at only 2 points during pregnancy to categorize exposure; given the short half-life of DAP metabolites and the likely episodic nature of the exposure (e.g., diet), the risk of exposure misclassification exists. However, our study is improved over other studies that collected only 1 sample during pregnancy. Further, because the misclassification is likely to be nondifferential, any errors are likely to skew our results toward the null. Differences existed between mothers who were either included or excluded, both demographically and with regard to total DAP at 16-weeks' gestation. It is reassuring, however, that the 26-week total DAP and mean total DAP were similar between the 2 groups. Because children of the 62 excluded mothers were missing most assessment data, we were unable to perform additional analyses to further investigate whether or how this exclusion may have influenced our main results. Additional limitations are the narrow range and relatively low concentrations of DAP in our cohort, which may have hindered our ability to detect cognitive differences related to prenatal exposure. However, it should be noted that the urinary concentrations of these metabolites are similar to those reported nationally and may thus increase our generalizability and relevance to a large percentage of the population. A final limitation is that we measured urinary DAP, and, particularly for dietary exposures (30), these may reflect exposure to nontoxic preformed metabolites as well as the parent organophosphate pesticides (which are toxic). Therefore, it is impossible to determine whether exposure was to the toxic organophosphate pesticides or to the nontoxic DAP environmental metabolites.

Given that urinary metabolite concentrations on a molar basis of organophosphate pesticide insecticides were higher in children born to mothers who were, in general, better educated and consumed more fruits and vegetables daily during their pregnancy, it is possible that these factors may act as confounders. However, we found no significant adverse associations between urinary concentrations of DAP metabolites of organophosphate pesticide insecticides measured in pregnant women and neurodevelopmental outcomes measured annually in their children at ages 1 through 5 years. This is especially reassuring as urinary concentrations of these metabolites in our cohort are similar to those of adult females from the US general population.

ACKNOWLEDGMENTS

Author affiliations: Division of General and Community Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio (Stephanie Donauer, Yingying Xu, Kimberly Yolton); Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio (Mekibib Altaye, Heidi Sucharew, Jane C. Khoury); Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio (Paul Succop); National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia (Antonia M. Calafat); and Faculty of Health Science, Simon Fraser University, Burnaby, British Columbia, Canada (Bruce Lanphear).

This work was partially supported by grants from the National Institute of Environmental Health Sciences (R01 ES015517, T32 HP10027).

This work was presented by Dr. Stephanie Donauer at the 2014 Prenatal Programming and Toxicity Conference, October 27, 2014, Boston, Massachusetts.

The contents of this paper are solely the responsibility of the authors and do not represent the official views of the Centers for Disease Control and Prevention.

Conflict of interest: none declared.

REFERENCES

1. Barr DB, Bradman A, Freeman N, et al. Studying the relation between pesticide exposure and human development. In: Bellinger DC, ed. *Human Developmental Neurotoxicology*. London, England: Taylor & Francis; 2006:253–286.
2. Lee I, Eriksson P, Fredriksson A, et al. Developmental neurotoxic effects of two pesticides: behavior and biomolecular studies on chlorpyrifos and carbaryl. *Toxicol Appl Pharmacol*. 2015;288(3):429–438.
3. Muñoz-Quezada MT, Lucero BA, Barr DB, et al. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. *Neurotoxicology*. 2013;39:158–168.
4. Bouchard MF, Chevrier J, Harley KG, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect*. 2011;119(8):1189–1195.
5. Engel SM, Wetmur J, Chen J, et al. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect*. 2011;119(8):1182–1188.
6. Eskenazi B, Marks AR, Bradman A, et al. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect*. 2007;115(5):792–798.
7. Rauh V, Arunajadai S, Horton M, et al. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect*. 2011;119(8):1196–1201.
8. Rauh VA, Garfinkel R, Perera FP, et al. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics*. 2006;118(6):e1845–e1859.
9. Marks AR, Harley K, Bradman A, et al. Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ Health Perspect*. 2010;118(12):1768–1774.
10. Rauh VA, Perera FP, Horton MK, et al. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proc Natl Acad Sci U S A*. 2012;109(20):7871–7876.
11. Stout DM 2nd, Bradham KD, Egeghy PP, et al. American Healthy Homes Survey: a national study of residential pesticides measured from floor wipes. *Environ Sci Technol*. 2009;43(12):4294–4300.
12. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. *Environ Health Perspect*. 2011;119(6):878–885.
13. Yolton K, Khoury J, Xu Y, et al. Low-level prenatal exposure to nicotine and infant neurobehavior. *Neurotoxicol Teratol*. 2009;31(6):356–363.
14. Geraghty SR, Khoury JC, Morrow AL, et al. Reporting individual test results of environmental chemicals in breastmilk: potential for premature weaning. *Breastfeed Med*. 2008;3(4):207–213.
15. Raznahan A, Greenstein D, Lee NR, et al. Prenatal growth in humans and postnatal brain maturation into late adolescence. *Proc Natl Acad Sci U S A*. 2012;109(28):11366–11371.
16. Ronalds GA, De Stavola BL, Leon DA. The cognitive cost of being a twin: evidence from comparisons within families in the Aberdeen children of the 1950s cohort study. *BMJ*. 2005;331(7528):1306.
17. Bravo R, Caltabiano LM, Weerasekera G, et al. Measurement of dialkyl phosphate metabolites of organophosphorus pesticides in human urine using lyophilization with gas chromatography-tandem mass spectrometry and isotope dilution quantification. *J Expo Anal Environ Epidemiol*. 2004;14(3):249–259.
18. Caudill SP, Schleicher RL, Pirkle JL. Multi-rule quality control for the age-related eye disease study. *Stat Med*. 2008;27(20):4094–4106.
19. Yolton K, Xu Y, Sucharew H, et al. Impact of low-level gestational exposure to organophosphate pesticides on neurobehavior in early infancy: a prospective study. *Environ Health*. 2013;12(1):79.
20. Caldwell BM, Bradley RH. *Home Observation for Measurement of the Environment: Administration Manual*. Tempe, AZ: Family & Human Dynamics Research Institute, Arizona State University; 2003.
21. SAS Institute, Inc. *SAS 9.3 In-Database Products: User's Guide*. 4th ed. Cary, NC: SAS Institute, Inc; 2012.
22. Bayley N. *Bayley Scales of Infant Development Manual*. 2nd ed. San Antonio, TX: The Psychological Corporation; 1993.
23. Wiig E, Secord W, Semel E. *Clinical Evaluation of Language Fundamentals—Preschool 2*. 2nd ed. San Antonio, TX: Psychological Corporation; 2004.
24. Wechsler D. *Wechsler Preschool and Primary Scale of Intelligence*. 3rd ed. San Antonio, TX: The Psychological Corporation; 2002.
25. Centers for Disease Control and Prevention. *Fourth National Report on Human Exposure to Environmental Chemicals*. Atlanta, GA: Centers for Disease Control and Prevention; 2009.
26. Anjos T, Altmäe S, Emmett P, et al. Nutrition and neurodevelopment in children: focus on NUTRIMENTHE project. *Eur J Nutr*. 2013;52(8):1825–1842.
27. Young JG, Eskenazi B, Gladstone EA, et al. Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology*. 2005;26(2):199–209.
28. Lu C, Essig C, Root C, et al. Assessing the association between pesticide exposure and cognitive development in rural Costa Rican children living in organic and conventional coffee farms. *Int J Adolesc Med Health*. 2009;21(4):609–621.
29. Cartier C, Warembourg C, Le Maner-Idrissi G, et al. Organophosphate insecticide metabolites in prenatal and childhood urine samples and intelligence scores at 6 years of age: results from the Mother-Child PELAGIE Cohort (France). *Environ Health Perspect*. 2016;124(5):674–680.
30. Lu C, Bravo R, Caltabiano LM, et al. The presence of dialkylphosphates in fresh fruit juices: implication for organophosphorus pesticide exposure and risk assessments. *J Toxicol Environ Health A*. 2005;68(3):209–227.

(Appendix follows)

Appendix Table 1. Individual Organophosphate Metabolites[a]

Organophosphate	Geometric Mean	95% CI	% >Limit of Detection
Diethyldithiophosphate	0.02	0.019, 0.03	30.6
Diethylphosphate	0.50	0.39, 0.64	78.6
Diethylthiophosphate	0.11	0.10, 0.14	58.7
Dimethyldithiophosphate	0.08	0.06, 0.09	46.5
Dimethylphosphate	1.45	1.16, 1.81	80.4
Dimethylthiophosphate	2.11	1.77, 5.53	97.9
Total diethylphosphates	9.3	7.8, 11.0	91
Total dimethylphosphates	45.9	39.3, 53.6	99
Total dialkylphosphates	73.7	64.5, 84.3	100

Abbreviation: CI, confidence interval.

[a] Diethyldithiophosphate, diethylphosphate, diethylthiophosphate, dimethyldithiophosphate, dimethylphosphate, and dimethylthiophosphate were measured in units of μg/g creatinine; total diethylphosphates, total dimethylphosphates, and total dialkylphosphates were measured in units of nmol/g creatinine.