Association of *in Utero* Organophosphate Pesticide Exposure and Fetal Growth and Length of Gestation in an Agricultural Population

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Although pesticide use is widespread, little is known about potential adverse health effects of *in utero* exposure. We investigated the effects of organophosphate pesticide exposure during pregnancy on fetal growth and gestational duration in a cohort of low-income, Latina women living in an agricultural community in the Salinas Valley, California. We measured nonspecific metabolites of organophosphate pesticides (dimethyl and diethyl phosphates) and metabolites specific to malathion (malathion dicarboxylic acid), chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphoro-thioate], and parathion (4-nitrophenol) in maternal urine collected twice during pregnancy. We also measured levels of cholinesterase in whole blood and butyryl cholinesterase in plasma in maternal and umbilical cord blood. We failed to demonstrate an adverse relationship between fetal growth and any measure of *in utero* organophosphate pesticide exposure. In fact, we found increases in body length and head circumference associated with some exposure measures. However, we did find decreases in gestational duration associated with two measures of *in utero* pesticide exposure: urinary dimethyl phosphate metabolites [$\beta_{adjusted}$ = –0.41 weeks per $\log_{10}$ unit increase; 95% confidence interval (CI), $^{-}$0.75–$^{-}$0.02; p = 0.02], which reflect exposure to dimethyl organophosphate compounds such as malathion, and umbilical cord cholinesterase ($\beta_{adjusted}$ = 0.34 weeks per unit increase; 95% CI, 0.13–0.55; p = 0.001). Shortened gestational duration was most clearly related to increasing exposure levels in the latter part of pregnancy. These associations with gestational age may be biologically plausible given that organophosphate pesticides depress cholinesterase and acetylcholine stimulates contraction of the uterus. However, despite these observed associations, the rate of preterm delivery in this population (6.4%) was lower than in a U.S. reference population. *Key words:* birth outcomes, birth weight, cholinesterase, dialkyl phosphates, fetal growth, gestational age, organophosphates, pesticides, urinary metabolites. *Environ Health Perspect* 112:1116–1124 (2004). doi:10.1289/ehp.6789 available via *http://dx.doi.org/* [Online 11 March 2004]

More than one billion pounds of pesticides are used each year in the United States, with more than 700 million pounds used annually in agriculture (Donaldson et al. 2002). Recent studies have demonstrated widespread pesticide exposures for the U.S. population, including pregnant women and children (Adgate et al. 2001; Berkowitz et al. 2003; Bradman et al. 1997, 2003; Hill et al. 1995; Loewenherz et al. 1997; Lu et al. 2001; National Center for Environmental Health 2003; Whyatt et al. 2002), with several studies suggesting that resident farm families and farm workers have higher exposures than do other populations (Curl et al. 2002; Fenske et al. 2002; Lu et al. 2000; McCauley et al. 2001; O'Rourke et al. 2000; Simcox et al. 1999).

In 1993, a National Academy of Sciences report (National Research Council 1993) stated that current tolerances for pesticide levels in food may not adequately protect fetuses and children and that the U.S. Environmental Protection Agency (EPA) needs to consider both dietary and nondietary sources of exposures in setting pesticide tolerances. The National Academy of Sciences called for research to fill the gaps of information on exposures and health consequences of pesticide exposures to the fetus and child.

Exposure of rodent dams during pregnancy to certain organophosphate pesticides, such as chlorpyrifos (Chanda et al. 1995; Muto et al. 1992), quinalphos (Srivastava et al. 1992), and dimethoate (Srivastava and Raizada 1996), has been associated with decrements in fetal growth in some studies. Other studies of the same pesticides (Institoris et al. 1995) and other organophosphates (Clemens et al. 1990; Institoris et al. 1995; Spyker and Avery 1977) have shown no association with fetal growth. To our knowledge, no animal studies have examined the relationship of length of gestation and organophosphate pesticide exposure.

The few studies that have examined the association of prenatal pesticide exposure and fetal growth or gestational duration in humans have also shown conflicting results (Fenster and Coye 1990; Grether et al. 1987; Kristensen et al. 1997; Perera et al. 2003; Restrepo et al. 1990; Savitz et al. 1989; Thomas et al. 1992; Willis et al. 1993; Xiang et al. 2000). Only three of these studies used biomarkers to measure pesticide exposure. Perera et al. (2003) found that, in residents of upper Manhattan, New York, increasing levels of the organophosphate pesticide chlorpyrifos in umbilical cord blood were associated with decreased birth weight and birth length but not with head circumference. Gestational duration was not examined. Berkowitz et al. (2004) reported that, in residents of east Harlem, levels of the urinary metabolite of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCPy), were not associated with decreased birth weight, birth length, or head circumference or shortened gestation. However, head circumference was diminished in the children of women with low expression of paraoxonase 1 (PON1), an esterase involved in the detoxification of organophosphates. Willis et al. (1993) estimated pesticide exposure using plasma cholinesterase levels but failed to find an association with birth weight or preterm delivery. An additional eight studies have estimated prenatal pesticide exposure based either on the mother's occupation or location of mother's residence in relation to areas where pesticides were sprayed. Of these, five studies (Dabrowski et al. 2003; Kristensen et al. 1997; Restrepo et al. 1990; Savitz et al. 1989; Xiang et al. 2000) found that potential exposure of women to pesticides during pregnancy was associated with an increased risk of low birth weight, small for gestational age (SGA), preterm delivery, or shortened gestation, whereas three studies (Fenster and Coye 1990; Grether et al. 1987; Thomas et al. 1992) found no association. In general, most studies have been hampered by their inability to accurately classify pesticide exposure.

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The purpose of the present analysis is to determine whether organophosphate pesticide exposure, as assessed by biologic markers, is associated with poorer fetal growth and shortened length of gestation in a cohort of pregnant women living in an agricultural community in the Salinas Valley of California (Eskenazi et al. 2003). The Salinas Valley is located southeast of San Francisco and runs approximately 60 miles within Monterey County. This area is often referred to as the "nation's salad bowl," growing primarily lettuce, broccoli, other cole crops, strawberries, artichokes, and grapes. Approximately 500,000 lb of organophosphate pesticides are applied annually in the Salinas Valley (California EPA 2002).

Materials and Methods

Participants and recruitment. The CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) project, a component of the Center for Children's Environmental Health Research at the University of California, Berkeley, is a longitudinal birth cohort study of the effects of pesticides and other environmental exposures on the health of pregnant women and their children living in the Salinas Valley. Pregnant women entering prenatal care at Natividad Medical Center, a county hospital located in the town of Salinas, or at one of five centers of Clinica de Salud del Valle de Salinas (located in Castroville, Salinas, Soledad, and Greenfield) were screened for eligibility over 1 year between October 1999 and October 2000. Clinica de Salud del Valle de Salinas is a network of community clinics located throughout the Salinas Valley and serving a low-income population, many of whom are farm workers.

Eligible women were ≥ 18 years of age, < 20 weeks gestation at enrollment, English or Spanish speaking, Medi-Cal eligible, and planning to deliver at the Natividad Medical Center. Of 1,130 eligible women, 601 (53.2%) agreed to participate in this multiyear study. Women who declined to participate were similar to study subjects in age and parity but were more likely to be English speaking and born in the United States and less likely to be living with agricultural field workers. After losses due to miscarriage, moving, or dropping from the study before delivery, birth weight information was available for 538 women. We excluded from these analyses women with gestational or preexisting diabetes (n = 26), hypertension (n = 15), twin births (n = 5), or stillbirths (n = 3). We also excluded one woman for whom birth weight information was out of range (< 500 g). Eleven infants diagnosed with congenital anomalies at birth [*International Classification of Diseases, 9th Revision* (ICD-9; 1989) codes 740–759] were included in the final sample because their exclusion did not materially affect the results. The final sample size was 488. Written informed consent was obtained from all participants, and the study was approved by the institutional review boards.

Interview and medical record abstraction. Women were interviewed twice during pregnancy and again shortly after delivery. The baseline interview occurred at a mean of 13 weeks gestation (range, 4–29 weeks), and the second interview occurred at a mean of 26 weeks gestation (range, 18–39 weeks). Interviews were conducted in English or Spanish by bilingual, bicultural interviewers.

Demographic information obtained during the baseline interview included maternal age, family income, the number of people supported by this income, country of birth, and number of years lived in the United States. Information on alcohol, tobacco, drug and caffeine use, and agricultural work was obtained at each interview. Information about previous pregnancies and any medical conditions, medications, or pregnancy complications was obtained by interview and confirmed by medical records. Medical records from prenatal visits and delivery were abstracted by a registered nurse.

Pesticide exposure measurement. Exposure to organophosphate pesticides was assessed in three ways: *a*) by measuring organophosphate dialkyl phosphate metabolites in maternal urine during pregnancy; *b*) by measuring seven different pesticide-specific metabolites in maternal urine during pregnancy; and *c*) by measuring cholinesterase (ChE) in whole blood and butyryl cholinesterase (BChE) in plasma collected from mothers during pregnancy and at delivery and from the umbilical cord.

For dialkyl phosphate metabolites, spot urine samples were collected from the pregnant women at the time of the two pregnancy interviews. Urine specimens were aliquoted and stored at –80°C until shipment to the Centers for Disease Control and Prevention (CDC; Atlanta, GA) for analysis of dialkyl phosphate and pesticide-specific metabolite levels.

Six dialkyl phosphate metabolites were measured in the urine samples using gas chromatography and mass spectrometry and quantified using isotope dilution calibration (Bravo et al. 2002). The dialkyl phosphates measured were dimethylphosphate, dimethyldithiophosphate, dimethylthiophosphate, diethylphosphate, diethyldithiophosphate, and diethylthiophosphate. Approximately 80% of the organophosphate pesticides used in the Salinas Valley devolve to one or more of these metabolites, which are excreted in urine. The most commonly used pesticides in this region that devolve to dialkyl phosphates are presented in Table 1.

Quality control (QC) procedures were conducted on laboratory and field samples. Laboratory QC was established by repeat analysis of two in-house urine pools enriched with known amounts of pesticide residues whose target values and confidence limits were previously determined. An analytical run was considered "out-of control" if the QC value failed to meet the requirements of the Westgard QC multirules (Westgard 2002). Data were not reported from runs considered "out-of-control." Mean recoveries for laboratory QC samples ranged from 98 to 105%, and the coefficients of variation (CV) ranged from 11 to 15%. Field QC was conducted by blindly inserting QC samples among the study samples in the field. These QC materials were thawed in the field, aliquoted into regular sample vials, and shipped with the samples on dry ice to CDC. The concentrations of the field QC materials, which were blinded to CDC analysts, generally agreed well with the spike concentrations (recovery and CV ranged from 94 to 103% and from 4.3 to 8.7%, respectively), indicating little contamination and/or degradation during the sampling procedures and further establishing the validity of the analytic measurements (Bravo et al., in press).

Because dialkyl phosphates originate from more than one organophosphate pesticide, quantities of the six metabolites were converted to molar concentration (nanomoles per liter) and summed to obtain the total concentrations of dialkyl phosphate metabolites for each woman. This provided an estimate of total organophosphate exposure for each individual at each of the two measurement times. The three dimethyl phosphate metabolites and three diethyl phosphate metabolites were also summed to obtain total concentrations of dimethyl and diethyl phosphate metabolites. For eight women, the level for one of the six metabolites was not readable because of analytic interference. Because metabolites within each group (i.e., diethyl or dimethyl phosphates) were highly correlated, the missing values were imputed using regression analysis to predict the missing metabolite level based on the other metabolites levels for that woman at that time point. Metabolite levels below the limit of detection (LOD) were given the value of the LOD divided by the square root of two (Hornung and Reed 1990).

Creatinine concentrations in urine were determined using a commercially available diagnostic enzyme method (Vitros CREA slides; Ortho Clinical Diagnostics, Raritan, NJ). Urine samples with creatinine levels < 10 mg/dL were considered too dilute for accurate analysis, and one measurement for one woman was excluded because of low creatinine levels.

Total dialkyl phosphate and dimethyl phosphate metabolite levels were available for 485 women, and diethyl phosphate metabolite levels were available for 486 women.

For pesticide-specific metabolites, the same spot urine samples were analyzed using analytic and QC procedures similar to those used for the dialkyl phosphate metabolites (Olsson et al. 2003). The metabolites measured were malathion dicarboxylic acid (MDA; derived from malathion); 4-nitrophenol (PNP; derived from methyl parathion, parathion, and other nonpesticide chemicals); TCPy (from chlorpyrifos and chlorpyrifos methyl); 2-diethylamino-4-hydroxy-6-methylpyrimidine (DEAMPY; from pirimiphos methyl); 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY; from diazinon); 3-chloro-4-methyl-7-hydroxycoumarin (CMHC; from coumaphos and coumaphos methyl); and 5-chloro-1-isopropyl-3-hydroxytriazole (CIT; from isazophos and isazophos methyl). With the exception of PNP, all of these metabolites derive from parent organophosphate pesticide compounds alone (Table 1). As with dialkyl phosphates, metabolite levels below the LOD were given the value of the LOD divided by the square root of two.

Specific metabolite levels were available for 482 women for six metabolites and, because of technical problems, for 382 women for the metabolite MDA.

For cholinesterase, because organophosphate pesticides at high doses are known to depress acetylcholinesterase, we measured ChE (or whole blood ChE) and BChE (or plasma ChE) in maternal and umbilical cord blood. Blood was collected from mothers at the time of the second pregnancy interview and in the hospital before delivery. Umbilical cord blood was collected by delivery room staff.

Blood samples were analyzed for ChE and BChE using a modification of the Wilson et al. (2002) microtiter-plate–adapted assay based on the original Ellman procedure (Ellman et al. 1961). Blood samples were stabilized immediately at the time of collection by diluting them 1:10 with 0.1 M $NaPO_4$ buffer (pH 8.0) containing 1% Triton X-100. Processed samples were stored at –80°C before being shipped on dry ice to the University of Washington, Seattle, for analysis. At the time of assay, the samples were thawed, mixed thoroughly, and diluted 1:25 with 0.1 M $NaPO_4$ buffer (pH 8.0) without Triton X-100 for a final 1:250 dilution of sample. One hundred microliters of 1:250 diluted samples were distributed to the wells of a microtiter plate. The reaction was initiated with 100 µL of a 2× assay mix of either ChE or BChE substrate, resulting in 1 mM final substrate. Assays were followed continuously at ambient temperature (23°C) at 412 nm for 10 min in a SpectraMax PLUS Microplate Reader (Molecular Devices Corp, Sunnyvale, CA). The initial linear rates of hydrolysis were obtained in units of optical density per minute. The path length for each well of the plate was measured immediately after the reaction, and rates were converted to rates of change in A412 per minute, and then to units of enzyme activity per milliliter. Each assay was run in triplicate. If the rate values varied by more than 5%, the sample was reanalyzed. A standard ChE sample supplied by the laboratory of B. Wilson (Departments of Environmental Toxicology and Avian Sciences, University of California, Davis, CA) was used to assure interlaboratory standardization.

ChE and BChE analysis was performed on all samples available and stabilized for this analysis, for a total of 292 women during pregnancy, 357 women at delivery, and 340 umbilical cord bloods.

Definition of outcomes. Infant birth weight, crown–heel length, and head circumference were obtained from hospital delivery logs and medical records. Infant ponderal index, a measure of proportionality of growth, was calculated as (birth weight in grams × 100)/(length in centimeters)^3. Gestational age was obtained from medical records and was based on ultrasound procedures for 25% of women. Because ultrasound estimates of gestational age may mask intrauterine growth retardation, we also estimated gestational age based on the woman's self-reported date of last menstrual period. Results were similar using both methods, and the medical record gestational age is reported, except where noted.

Low birth weight was defined as < 2,500 g. Preterm delivery was defined as birth at less than 37 completed weeks of gestation. An SGA birth was defined as birth weight < 10th percentile for gestational age according to ethnicity (Mexican American or non-Hispanic white), parity, and infant sex from national data (Overpeck et al. 1999).

Data analysis. Linear regression models were used to test for associations between exposure measurements and length of gestation, birth weight, length, head circumference, and ponderal index. Logistic regression was used to test for associations between exposure measurements and low birth weight, preterm delivery, and SGA births.

ChE and BChE were analyzed as continuous variables. Dialkyl phosphate metabolites were analyzed as continuous variables on a $\log_{10}$ scale. The pesticide-specific metabolite levels were analyzed as categorical variables because of the large proportion of women

Table 1. Number of women with measurements and percentage detectable, median values, and ranges (average of two measurements) of various organophosphate exposure measures during pregnancy: CHAMACOS study, Salinas Valley, California, 2000–2001.

Marker of exposure	Parent compounds or class	No.	LOD	Percent > LOD[a]	Median (range)[b]	Sample measured
Dialkyl phosphate metabolites (nmol/L)						
Dimethyl phosphates	Malathion, oxydemeton-methyl, dimethoate, naled, methidathion[c]	486	0.08–1.2	99.8	101 (5–6,587)	Urine
Diethyl phosphates	Diazinon, chlorpyrifos, disulfoton	485	0.05–0.8	99.8	22 (2–680)	Urine
Total dialkyl phosphates	All of above	485	0.05–1.2	99.8	136 (10–6,854)	Urine
Pesticide-specific metabolites (µg/L)						
MDA	Malathion	382	0.29	30.1	0.2 (0.2–28.9)	Urine
TCPy	Chlorpyrifos, chlorpyrifos methyl	482	0.26	76.3	3.3 (0.2–56.1)	Urine
PNP	Methyl parathion, parathion, EPN[d]	482	0.14	54.4	0.5 (0.1–34.7)	Urine
DEAMPY	Pirimiphos methyl	482	0.21–0.22	5.0	0.2 (0.1–14.9)	Urine
IMPY	Diazinon	482	0.69	2.4	0.5 (0.5–7.1)	Urine
CMHC	Coumaphos, coumaphos methyl	482	0.18	0.7	0.1 (0.1–0.3)	Urine
CIT	Isazophos, isazophos methyl	482	1.50	10.9	1.1 (1.1–36.0)	Urine
Cholinesterase (µmol/min/mL)						
ChE	Organophosphates and *n*-methyl carbamates	340	NA	NA	3.8 (1.7–6.1)	Whole blood (cord)
		357	NA	NA	5.1 (0.7–10.2)	Whole blood (maternal delivery)
		292	NA	NA	5.7 (2.2–10.9)	Whole blood (maternal pregnancy)
BChE	Organophosphates and *n*-methyl carbamates	340	NA	NA	1.2 (0.6–2.7)	Plasma (cord)
		357	NA	NA	1.4 (0.6–3.9)	Plasma (maternal delivery)
		292	NA	NA	1.4 (0.3–2.7)	Plasma (maternal pregnancy)

NA, Not applicable.
[a]Percentage of women with metabolite levels above the LOD for at least one measurement during pregnancy. [b]Urinary metabolites: average of two pregnancy measurements, not adjusted for creatinine. [c]Only parent compounds with annual use in Salinas Valley > 10,000 lb are listed. [d]PNP may also derive from nonpesticide chemicals used in industrial processes.

with nondetectable levels. For each pesticide-specific metabolite, women were assigned to one of three groups: no detectable levels (referent group), detectable levels below the median of the detectable levels, and detectable levels above the median. Associations with all urinary metabolites (dialkyl phosphates and pesticide-specific metabolites) were analyzed both adjusted and unadjusted for creatinine.

For analysis, the two pregnancy measurements of each urinary metabolite were averaged for each woman. This method was justified because there was no evidence of trend over time in metabolite levels and because there was a large within person variability in the metabolite measures, which was reduced by using the average. In addition, average metabolite level can be viewed as an approximate measure of cumulative pesticide dose over pregnancy. For specific metabolites, if a woman was below the LOD for both measurements, she was classified as below LOD for the average. If she was above the LOD at either or both measurements, her measurements were averaged and classified in relation to the median.

To identify "critical windows" of fetal development when exposure may have a greater impact, we analyzed the associations of outcomes and metabolite levels measured during moving 6-week windows of pregnancy (e.g., 5–10 weeks, 6–11 weeks, 7–12 weeks) using a series of multiple regression analyses. Six weeks was chosen because this time frame ensured a sample size of at least 100 women in each interval. When a woman had two measurements within a single interval, one measurement was randomly selected.

For analyses using gestational age as an outcome, metabolite levels were dropped for 19 women whose measurements occurred after 30 weeks gestation (the gestational duration of the earliest birth) to prevent a "survival" bias associated with a late metabolite measurement.

All models of birth weight, length, head circumference, and ponderal index were adjusted for gestational age and gestational age squared. We selected potential confounders for the multivariate models based on associations reported in the literature, and we included in the models those that changed the coefficient of exposure by 10% or more. The models included continuous variables for maternal age, pregnancy weight gain, and week of initiating prenatal care and categorical variables for parity, infant sex, mother's country of birth, body mass index (BMI), and family income. Poverty level was calculated by dividing household income by the number of people supported by that income and comparing it with federal poverty thresholds (U.S. Census Bureau 2000). Smoking, alcohol, and illicit drug use were not included in the models because very few women reported use and controlling for these variables did not alter the results. The more commonly reported exposure to environmental tobacco smoke and caffeinated beverages also did not alter the relationship of pesticide metabolites and birth outcome and were not included. Additional analyses were conducted including history of low birth weight and history of preterm delivery, but these covariates were dropped from the final analyses because they did not affect the results. All analyses were conducted using Stata, version 8.0 (Stata Corporation, College Station, TX).

Results

Table 2 describes the sociodemographic characteristics of the population. The women averaged 25 years of age (SD = 5); two-thirds were multiparous, 80% were married, 79% had not graduated from high school, 58% were overweight or obese, 88% preferred to speak Spanish, and 84% were born in Mexico, with more than half residing in the United States for < 5 years (data not shown). Almost all of the women were living within 200% of the poverty level. Very few women reported smoking (6%), drug use (2%), or alcohol consumption (1%) during pregnancy. Approximately 28% of the women had worked in the fields during the pregnancy, and another 14% had worked at other jobs in agriculture, including packing shed, nursery, and greenhouse work. Overall, 85% of the women had agricultural workers living in their homes during their pregnancy (data not shown).

Table 1 shows the percentage of women with detectable levels of each urinary metabolite during pregnancy as well as the median values and ranges. The median dimethyl, diethyl, and total dialkyl phosphate metabolite levels for the study population were 101 nmol/L, 22 nmol/L, and 136 nmol/L, respectively. Only one woman had no detectable levels of dialkyl phosphate metabolites in urine during pregnancy. The percentages of women with detectable levels of MDA, TCPy, and PNP during pregnancy were 30, 77, and 54%, respectively. The median level for MDA was 0.2 μg/L, for TCPy was 3.3 μg/L, and for PNP was 0.5 μg/L. Because only a small percentage of women had levels above the LOD for the pesticide-specific metabolites DEAMPY, IMPY, CMHC, and CIT, we did not analyze their associations with birth outcomes.

The mean levels of ChE were 5.2 μmol/min/mL in maternal blood during pregnancy, 5.7 μmol/min/mL in maternal blood immediately before delivery, but somewhat lower at 3.8 μmol/min/mL in umbilical cord blood. For BChE, the mean levels were similar for all three. Maternal dialkyl phosphate metabolite levels and ChE levels collected concurrently at the second pregnancy interview were not correlated (Pearson $r = 0.02$, $p = 0.71$), and a small positive rather than the expected negative correlation was seen between average dialkyl phosphate metabolite levels during pregnancy and predelivery maternal blood (Pearson $r = 0.11$, $p = 0.04$) and umbilical cord ChE levels (Pearson $r = 0.13$, $p = 0.02$).

The mean (± SD) duration of gestation was 38.9 ± 1.7 weeks; mean birth weight was 3,449 ± 516 g; mean body (crown–heel) length was 50.2 ± 2.7 cm; mean head circumference was 34.1 ± 1.5 cm; and mean ponderal index was 2.7 ± 0.3 g/m^3. A total of 3.7% ($n = 18$) of children were born of low birth weight; 4.8%

Table 2. Demographic characteristics of CHAMACOS study population, Salinas Valley, California, 2000–2001 ($n = 488$).

Characteristics	No. (%)
Age (years)	
18–24	237 (48.6)
25–29	151 (30.9)
30–34	71 (14.5)
≥ 35	29 (5.9)
Parity	
0	162 (32.5)
≥ 1	336 (67.5)
Education	
< 6th grade	205 (42.0)
7th–12th grade	180 (36.9)
Completed high school	103 (21.1)
Marital status	
Married/living as married	391 (80.1)
Single	97 (19.9)
Preferred language	
Spanish	429 (87.9)
English	30 (6.1)
Both	24 (4.9)
Other	5 (1.0)
Country of birth	
Mexico	409 (83.8)
United States	68 (13.9)
Other	11 (2.3)
Family income	
≤ Poverty level	282 (61.3)
Within 200% of poverty level	161 (35.0)
> 200% of poverty level	17 (3.7)
Body mass index (kg/m^2)	
Underweight (< 18.5)	176 (37.6)
Normal (18.5–24.9)	194 (41.5)
Overweight (25–29.9)	2 (0.4)
Obese (> 30)	96 (20.5)
Pregnancy weight gain (lbs)	
< 25	159 (32.6)
25–35	173 (35.5)
> 35	156 (32.0)
Smoked during pregnancy	
Yes	30 (6.1)
No	458 (93.9)
History of preterm/low-birth-weight delivery	
Not applicable	162 (37.6)
Neither	217 (50.3)
Preterm only	14 (3.2)
Low birth weight only	17 (3.9)
Both	21 (4.9)
Work status during pregnancy	
Not working	170 (35.9)
Working in fields	133 (28.1)
Other agricultural work	65 (13.7)
Other work (not agricultural)	105 (22.2)

(n = 23) were SGA births, and 6.6% (n = 32) were preterm.

Table 3 presents the adjusted regression results for dialkyl phosphate metabolite levels and pesticide-specific metabolite levels with measures of fetal growth and length of gestation. After adjusting for covariates, a 10-fold increase (i.e., one log-unit increase) in average dialkyl phosphate metabolite concentration was associated with an increase in infant's body length of 0.52 cm (p = 0.06), and in head circumference of 0.32 cm (p = 0.03). Figure 1A–B indicates that these positive associations of dialkyl phosphate metabolite levels are related to exposure throughout the gestation period, although rarely significantly at any point for body length. Similar increases in body length and head circumference were seen when dimethyl and diethyl phosphate metabolites were examined separately, although these increases were not statistically significant (Table 3).

As shown in Table 3, a 10-fold increase in average dimethyl but not diethyl phosphate metabolites was associated with a decrease of 3 days in gestational duration (p = 0.02). Figure 1C shows that only after 22 weeks of gestation do increasing levels of dimethyl phosphate metabolites have a significant adverse association with gestational duration.

Dimethyl, diethyl, and total dialkyl phosphate metabolite levels were not associated with birth weight or infant ponderal index and were also unrelated to risk of preterm delivery, low birth weight, and SGA births. The findings of increased head circumference and decreased gestational duration persisted when metabolite levels were controlled for creatinine, but the finding of increased body length did not (data not shown).

No adverse associations were found between MDA or TCPy and parameters of fetal growth or gestational age. Increased head circumference was seen in infants of women with PNP levels above the median (β = 0.29 cm, p = 0.06) when compared with women with no detectable levels. Women with levels of PNP below the median also showed increased length (β = 0.60 cm, p = 0.03) compared with women with no detectable levels, but this effect was not observed in women with levels above the median. Borderline significant associations were seen between PNP and decreasing gestational age (β = –0.37 weeks, p = 0.06) and ponderal index (β = –0.08 g/cm^3, p = 0.06), but only in the group below the median. However, associations seen with PNP should be viewed with caution because PNP may derive from compounds other than parathion (Table 1).

The associations between ChE levels in umbilical cord blood and parameters of fetal growth and length of gestation are shown in Table 4. Lower levels of ChE in umbilical cord blood were associated with significantly shorter length of gestation, averaging 0.34 weeks (p = 0.001) for each unit decrease in ChE (in micromoles per minute per milliliter; range of ChE in cord blood is 4.4 units). Decreasing levels of ChE in umbilical cord blood were also associated with increased risk of preterm delivery [adjusted odds ratio (OR) = 2.3; 95% confidence interval (CI), 1.1–4.8; p = 0.02] and low birth weight (adjusted OR = 4.3; 95% CI, 1.1–17.5; p = 0.04); however, 6 of the 11 low birth weight infants were also preterm (data not shown). Lower levels of ChE in maternal blood at delivery were associated with decreased gestational duration, but only when the estimate based on last menstrual period was used (β = 1.1 days, p = 0.04; data not shown). Lower ChE levels in maternal blood collected earlier in pregnancy were not associated with gestational duration but were somewhat associated with an increased risk of preterm delivery (OR = 1.6; 95% CI, 1.0–2.5; p = 0.06; data not shown). Neither maternal nor umbilical cord ChE levels were associated with any other parameters of fetal growth. BChE levels in maternal and umbilical cord blood were not associated with any birth outcome.

Discussion

We found clear decreases in gestational duration associated with two measures of *in utero* pesticide exposure: levels of metabolites of dimethyl phosphate pesticide compounds and whole blood ChE. Shortened gestational duration was most clearly related to increasing exposure levels in the latter part of pregnancy. However, the results of this study failed to demonstrate an adverse relationship between fetal growth and *in utero* organophosphate pesticide exposure as assessed by multiple measures of exposure, including plasma and whole blood cholinesterase, urinary dialkyl phosphate metabolites, and pesticide-specific metabolites of parathion, malathion, and chlorpyrifos. In fact, we found increases in length and head circumference associated with some of these measures.

Our results are consistent with those of Berkowitz et al. (2004), who found no adverse relationship between any measures of fetal growth or length of gestation and maternal urinary levels of TCPy, the metabolite of the diethyl organophosphate pesticide chlorpyrifos.

Table 3. Association of average urinary metabolites of organophosphate pesticides measured at two points during pregnancy[a] with length of gestation and fetal growth: CHAMACOS study, Salinas Valley, California, 2000–2001.

		Length of gestation (weeks)[c]		Birth weight (g)[d]		Length (cm)[d]		Head circumference (cm)[d]		Ponderal index (g/cm^3)[d]	
Metabolite	No.[b]	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value
Dialkyl phosphate metabolites (nmol/L, $\log_{10}$ scale)											
Dimethyl phosphates	485	–0.41 (−0.75–−0.07)	0.02**	41 (−40–122)	0.32	0.42 (−0.07–0.91)	0.09*	0.25 (−0.02–0.52)	0.07*	–0.03 (−0.10–0.04)	0.45
Diethyl phosphates	486	–0.16 (−0.53–0.22)	0.41	52 (−40–144)	0.26	0.40 (−0.15–0.94)	0.16	0.28 (−0.02–0.59)	0.07*	–0.01 (−0.09–0.07)	0.74
Total dialkyl phosphates	485	–0.20 (−0.55–0.15)	0.27	42 (−46–131)	0.35	0.52 (−0.01–1.05)	0.06*	0.32 (0.03–0.62)	0.03**	–0.04 (−0.12–0.04)	0.28
Pesticide-specific metabolites (μg/L)											
MDA											
No detectable levels	233	Referent		Referent		Referent		Referent		Referent	
Detectable levels < median	74	–0.13 (−0.55–0.30)	0.55	–45 (−154–63)	0.41	–0.53 (−1.18–0.11)	0.11	–0.16 (−0.52–0.19)	0.37	0.05 (−0.05–0.14)	0.33
Detectable levels ≥ median	75	–0.21 (−0.62–0.20)	0.32	56 (−49–161)	0.29	0.14 (−0.48–0.76)	0.66	0.11 (−0.24–0.46)	0.53	0.02 (−0.07–0.12)	0.60
TCPy											
No detectable levels	41	Referent		Referent		Referent		Referent		Referent	
Detectable levels < median	220	–0.17 (−0.74–0.40)	0.55	–6 (−138–126)	0.93	0.09 (−0.70–0.87)	0.83	0.06 (−0.37–0.49)	0.78	–0.01 (−0.12–0.11)	0.89
Detectable levels ≥ median	221	–0.06 (−0.63–0.51)	0.84	27 (−106–159)	0.69	0.44 (−0.35–1.22)	0.27	0.04 (−0.39–0.47)	0.85	–0.04 (−0.16–0.08)	0.50
PNP											
No detectable levels	124	Referent		Referent		Referent		Referent		Referent	
Detectable levels < median	179	–0.37 (−0.76–0.02)	0.06*	34 (−57–125)	0.46	0.60 (0.06–1.13)	0.03**	0.18 (−0.12–0.48)	0.23	–0.08 (−0.16–0.0)	0.06
Detectable levels ≥ median	179	0.18 (−0.21–0.57)	0.36	49 (−42–140)	0.29	0.41 (−0.13–94)	0.14	0.29 (−0.01–0.58)	0.06*	–0.03 (−0.11–0.05)	0.48

[a]Urinary metabolite levels are not adjusted for urinary creatinine concentration. [b]Numbers vary slightly for different outcomes due to missing data. [c]Models adjusted for timing of urine collection, timing of entry into prenatal care, maternal age, parity, country of birth, and poverty level. [d]Models adjusted for timing of urine collection, timing of entry into prenatal care, maternal age, parity, infant sex, country of birth, weight gain, BMI, poverty level, gestational age, and (gestational age)^2.
*p < 0.10; **p < 0.05.

Our results are also consistent with those of Willis et al. (1993), who reported no association of either fetal growth or length of gestation and maternal plasma BChE levels. However, our results differ from those of Perera et al. (2003), who reported decreased birth weight and length in association with blood measurements of the parent compound chlorpyrifos in pregnant residents of New York City.

Disparities among study results may be due, in part, to differences in exposure measurements. For example, in our study and in the study by Willis et al. (1993), there was no association between fetal outcome measures and plasma BChE levels, but we did observe clear associations with shortened gestation using whole blood ChE as the measure of exposure. Whole blood ChE reflects exposure to organophosphate and *n*-methyl carbamate pesticides over a few months, whereas plasma BChE reflects more immediate exposure (Karlsen et al. 1981; Lessenger and Reese 1999; Sanz et al. 1991; U.S. EPA 2000; Yuknavage et al. 1997). In our study, as in the study by Willis et al. (1993), we used the absolute level of cholinesterase as an indicator of exposure, rather than the change from a preexposure baseline level, as is typically used in occupational monitoring of pesticide poisoning. There is some evidence that absolute ChE measurements can be effective in establishing effects of organophosphate pesticide exposures. For example, this approach was used to show a significant inhibition of ChE in children exposed to rain water runoff from a large crop-dusting airport (McConnell et al. 1999).

Both the present study and the study by Berkowitz et al. (2004) found no association of the metabolite TCPy and fetal growth or length of gestation, although our methods of chemical analysis may have differed. Berkowitz et al. (2004) reported a detection frequency of 42% versus our report of 77%. Their lower detection frequency is not surprising given that our LOD was nearly 50 times lower. However, the median TCPy level in these New York City residents was more than twice (7.5 vs. 3.3 μg/L) that of our residents who lived in an agricultural community where > 50,000 lb of chlorpyrifos were applied annually to agricultural fields. The median level in both studies was considerably higher than the median (1.7 μg/L) from a stratified random sample of the U.S. population participating in the National Health and Nutrition Examination Survey (NHANES; National Center for Environmental Health 2003) and analyzed by the same CDC laboratory as samples in our study. In addition, the present study and both New York City studies (Berkowitz et al. 2004; Perera et al. 2003) straddled the time frame of the U.S. EPA ban on chlorpyrifos use in the home, beginning 1 January 2001 (U.S. EPA 2000). Although chlorpyrifos exposure from

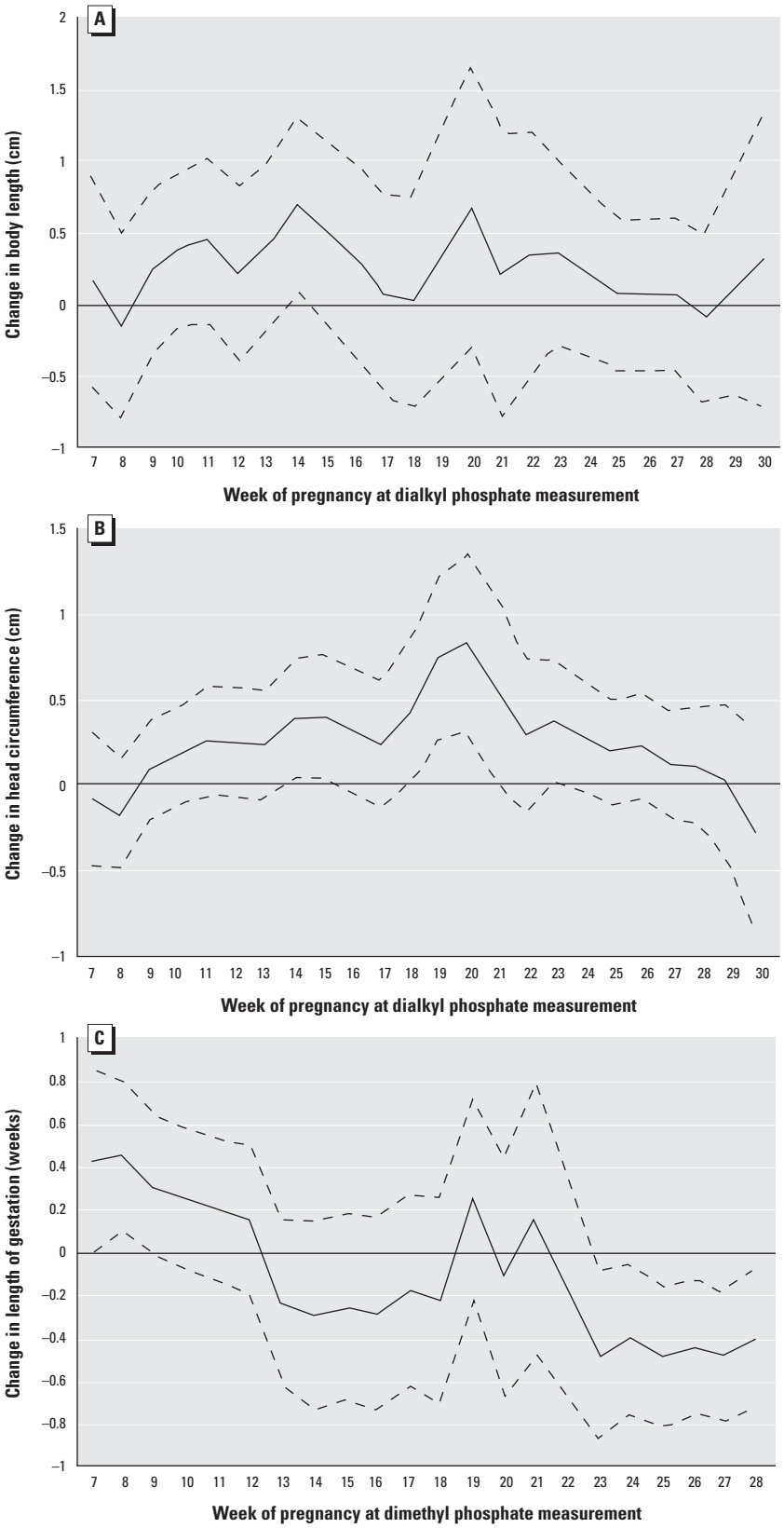

Figure 1. Adjusted regression coefficients (solid lines) and 95% CIs (dashed lines) for the association of urinary dialkyl phosphate metabolites (log_{10} scale) and (*A*) crown–heel length, (*B*) head circumference, and (*C*) the association of dimethyl phosphates and length of gestation according to timing of exposure during pregnancy. Regression coefficients were adjusted to control for timing of urine collection, week of entry to prenatal care, maternal age, parity, infant sex, maternal BMI, maternal weight gain, country of birth, and poverty level.

home use is likely to have had an important contribution to exposure in New York City, very few of the home pesticides found in the homes of CHAMACOS participants contained chlorpyrifos. Possible sources of chlorpyrifos exposure to our population include diet, residues in the home from past home pesticide use, or agricultural use, which was largely unaffecated by the U.S. EPA regulation.

Measurements of pesticides in blood, as in the study by Perera et al. (2003), is a direct measure of exposure to the parent compound and may more accurately reflect the dose to the target organ than measurements of metabolites in urine (Barr et al. 1999; Needham et al. 1995).

Although blood measurements may be preferable in certain cases, estimating organophosphate pesticide exposure with urinary levels of dialkyl phosphate metabolites has an important advantage beyond the ease of specimen collection. The dialkyl phosphate metabolites reflect exposure to about 80% of the organophosphate pesticides used in the Salinas Valley (California EPA 2000), although a number of highly used organophosphate pesticides (e.g., acephate) do not devolve into these urinary metabolites. Although dialkyl phosphate metabolites measurements do not allow differentiation between exposures that result from more or from less toxic pesticides [e.g., oxydemeton-methyl is orders of magnitude more acutely neurotoxic than is malathion; both may devolve to dimethyl metabolites (Olsson et al. 2003; Wessels et al. 2003)], they are an excellent nonspecific but integrated measure of exposure to a class of pesticides. We have partially addressed this limitation by complementing measurements of dialkyl phosphate metabolites with available analyses of pesticide-specific organophosphate metabolites. However, currently, there are no analytical methods for measurement of specific exposure to many important organophosphate pesticides (e.g., oxydemeton-methyl) in urine or in blood, and even some of the ones we can measure (e.g., PNP) may derive from other sources in addition to the pesticide of interest. Thus, measurement of dialkyl phosphate metabolites may be the only biologic measure currently available to characterize and integrate exposure to multiple organophosphate pesticides that may originate from different sources.

Current biomarkers used to assess pesticide exposure, whether in blood or urine, can result in exposure misclassification. Organophosphate pesticides and their metabolites have a short residence time in the body [Abu-Qare et al. 2001; Garfitt et al. 2002; Griffin et al. 1999; World Health Organization (WHO) 1996]. Exposures may be transient and highly variable. In fact, we found that the within-person standard deviation for the two urinary dialkyl phosphate metabolite measurements was approximately three times larger than the between-person standard deviation. Thus, measurements conducted on one [as in the studies of Berkowitz et al. (2004) and Perera et al. (2003)] or two (as in the present study) blood or urine specimens during pregnancy may not accurately reflect exposures over the entire pregnancy. Furthermore, the interrelationship of these different exposure measurements has not been well studied and may differ in pregnancy.

Understanding the mechanism of pesticide exposure and shortened gestational duration will require further examination of the interrelationship of different exposure measurements. For example, we found no correlation between concurrent measurements of dialkyl phosphate metabolites in urine and ChE in blood, and we found an unexpected small positive relationship between ChE and average dialkyl phosphate levels over pregnancy. The absence of a negative correlation between dialkyl phosphate metabolites and ChE is perhaps partially caused by substantial measurement error in both measures. The fact that decreases in gestational age were seen with both dimethyl phosphates and cord ChE may reflect a true association of organophosphate exposure and length of gestation, even though measurement error prevents these two markers of exposure from being negatively correlated with each other. An additional explanation for the lack of expected correlation between ChE and dialkyl phosphate metabolites is that dialkyl phosphate metabolites are specific to organophosphate compounds, whereas ChE levels may reflect exposure to both organophosphate and *n*-methyl carbamate pesticides. *n*-Methyl carbamate use in Monterey County in the year this study was conducted exceeded 100,000 lb (California EPA 2002) and may be a major contributor to ChE levels in this population. We note, however, that at least among the women who delivered prematurely, we found the anticipated negative correlation between dialkyl phosphate metabolites and ChE levels ($r = -0.45$, $p = 0.05$). Thus, population correlations may be masking associations in high-risk groups.

Despite difficulties in exposure assessment, the associations between pesticide exposure measures and gestational duration are quite compelling. The relation of ChE and shortened gestation may be biologically plausible. Cholinergic nerves play a significant role in the control of the uterine musculature and myometrium. Acetylcholine stimulates contraction of the uterus and dilates its arterial supply (Papka et al. 1999). Thus, an inhibition of acetyl ChE could produce an accumulation of acetylcholine in the neuronal junctions and hence the overstimulation of cholinergic fibers resulting in premature initiation of labor. Our results suggest that exposure to dimethyl organophosphate pesticides in the latter part of pregnancy may be particularly suspect. This is further supported by the observation that the urinary MDA levels (parent compound is malathion, a dimethyl organophosphate) during the latter part of pregnancy were also associated with significant increased risks for preterm delivery (OR = 5.2, 95% CI, 1.2–22.1, $p = 0.03$ for levels below the median; OR = 3.5, 95% CI, 0.9–13.3 $p = 0.07$ for levels above the median), although the numbers were small (data not shown). Nevertheless, the CHAMACOS cohort has a substantially lower rate of preterm delivery (6.4%) than that reported for Mexican-born women in the United States (10%) (Singh and Yu 1996). This suggests that although our findings are biologically intriguing, the potential effects of pesticides have had little clinical impact at the population level.

Table 4. Association of ChE and BChE in maternal blood during pregnancy and at delivery and in umbilical cord blood with length of gestation and fetal growth: CHAMACOS study, Salinas Valley, California, 2000–2001.

		Length of gestation (weeks)[b]		Birth weight (g)[c]		Length (cm)[c]		Head circumference (cm)[c]		Ponderal index (g/cm^3)[c]	
	No.[a]	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value
ChE (μmol/min/mL)											
Maternal blood, pregnancy	340	0.01 (−0.15–0.17)	0.87	8 (−35–52)	0.71	0.05 (−0.20–0.29)	0.72	0.06 (−0.09–0.21)	0.45	0.00 (−0.03–0.03)	0.90
Maternal blood, delivery	357	0.09 (−0.04–0.23)	0.16	6 (−30–43)	0.73	0.05 (−0.17–0.27)	0.67	−0.07 (−0.19–0.05)	0.27	0.00 (−0.03–0.03)	0.95
Cord blood	292	0.34 (0.13–0.55)	0.001**	12 (−46–70)	0.68	−0.01 (−0.36–0.34)	0.95	−0.04 (−0.23–0.14)	0.65	0.02 (−0.03–0.07)	0.43
BChE (μmol/min/mL)											
Maternal blood, pregnancy	340	−0.2 (−0.64–0.27)	0.42	56 (−67–179)	0.37	0.07 (−0.63–0.78)	0.83	0.12 (−0.31–0.56)	0.58	0.03 (−0.06–0.12)	0.51
Maternal blood, delivery	357	−0.1 (−0.48–0.36)	0.78	−90 (−206–25)	0.13	0.05 (−0.65–0.75)	0.89	−0.07 (−0.45–0.31)	0.73	−0.07 (−0.16–0.03)	0.16
Cord blood	292	−0.2 (−0.78–0.32)	0.41	111 (−35–257)	0.14	0.23 (−0.65–1.12)	0.6	−0.03 (−0.50–0.45)	0.91	0.05 (−0.07–0.17)	0.45

[a]Numbers vary slightly for different outcomes due to missing data. [b]Models adjusted for timing of urine collection, timing of entry into prenatal care, maternal age, parity, country of birth, and poverty level. [c]Models adjusted for timing of urine collection, timing of entry into prenatal care, maternal age, parity, infant sex, country of birth, weight gain, BMI, poverty level, gestational age, and (gestational age)^2. *$p < 0.10$; **$p < 0.05$.

We have no ready explanation for an increase in some measures of fetal growth (i.e., head circumference and crown–heel length) in relation to pesticide exposure measures. These results were apparent only after controlling for gestational age. Berkowitz et al. (2004) reported a decrease in head circumference only in those infants whose mothers had low expression of PON1. Future investigations will determine whether PON1 status (genotype and phenotype) modifies the association of birth outcomes and pesticide exposure in the CHAMACOS cohort.

In summary, we have found no adverse association of *in utero* organophosphate pesticide exposure and measures of fetal growth, but a fairly consistent adverse association with gestational duration. The strengths of this study include the use of multiple exposure biomarkers in a large population of women living in an agricultural community. However, as in all studies of the effects of pesticide exposure, we are limited in our ability to accurately characterize exposure to multiple pesticides and multiple classes of pesticides over the course of pregnancy. Nevertheless, given the importance of premature delivery on the viability and health of the fetus, these findings warrant further evaluation of the risks associated with pesticide exposure, especially as new measures of exposure are developed. Furthermore, additional research should determine whether certain subpopulations are more biologically susceptible to the potential hazards of organophosphate exposure.

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