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Fetal exposure to chlordane and permethrin mixtures in relation to inflammatory cytokines and birth outcomes

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Abstract

We sought to characterize the relationships between cord serum concentrations of chlordane and permethrin pesticides, inflammatory cytokines, gestational age, and size at birth. Umbilical cord serum levels of *trans*-nonachlor, oxychlordane, *cis*- and *trans*-permethrin, piperonyl butoxide, and cytokines (TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, GMCSF), were quantified in 300 newborns at the Johns Hopkins Hospital in Baltimore, MD (2004–2005). Principal component analyses were used to quantitate chlordane and permethrin mixtures, and to identify independent cytokine components. Five cytokine components described 87% of the variance in cord serum cytokine levels; these (and predominant loadings) were: (1) all 9 cytokines; (2) acute phase (IL-1β, IL-6); (3) anti-inflammatory (IL-10) (4) TNF-α; and (5) IL-1β. Of these, the TNF-α component was significantly associated with a 2-day decrease in gestational age. Chlordane was associated with lower levels of the pro-inflammatory IL-1β [β: −0.11 (−0.20, −0.02)]. Permethrin was negatively associated with the anti-inflammatory cytokine IL-10 [β: −0.14 (−0.22, −0.05)]. Neither pesticides nor cytokines were significantly associated with birthweight, length or head circumference, and pesticides were not associated with gestational age. Our findings suggest that chlordane and permethrin concentrations in cord blood may be associated with levels of inflammatory cytokines in the fetus.

Keywords

umbilical cord blood; biomarkers; chlordane; permethrin; pesticides; principal component analysis; cytokines; fetal growth; gestational age

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Supporting Information

We have provided supplemental information on our study population, pesticide principal component analyses, and figures illustrating the linear relationships between pesticides and our outcomes of interest. This information is available free of charge via the Internet at http://pubs.acs.org.

Introduction

Insecticide exposure is widespread in the U.S. population (1). While most persistent insecticides were banned from use in the United States in the 1970s and early 1980s, chlordane was not banned until 1988 (2). It was widely used for termite control, and because of its persistence in the environment, indoor inhalation exposure may have continued for decades after its application (1). Most insecticides currently in use in the U.S. are non-persistent and have biological half-lives ranging from hours to weeks. One of the most common of these, permethrin, is a pyrethroid insecticide primarily used for residential and commercial insect control, food and feed crops, mosquito abatement programs, and on clothing (3). In commercial products permethrin is often combined with a synergist piperonyl butoxide (PBUT) that enhances its insecticidal properties.

In a prior study of newborn infants born at the Johns Hopkins Hospital (4), we found that exposures to permethrin and chlordane are ubiquitous and that chlordane- and permethrin-related compounds occur as mixtures in infant cord serum that can be identified using principal components analysis (PCA). However, little is known about potential developmental effects of chlordane and permethrin exposures. Chlordane levels in cord serum were associated with reductions in birthweight, length and head circumference in a small ($n = 41$) epidemiologic study conducted in Singapore in 2006 (5). For permethrin, an epidemiologic study in New York City did not find significant associations between concentrations of the pyrethroid metabolite 3-phenoxybenzoic acid in cord blood and birthweight, length, and head circumference (6).

Prior investigations demonstrated relationships between Th1 cytokine levels of TNF-α, IFN-γ, and IL-12 in cord serum and birth weight and gestational age (7). Both chlordane and permethrin exhibit immunotoxic properties. Several *in vitro* and animal studies have indicated that permethrin may alter immune status through down-regulation of signal transduction in T helper lymphocytes, inhibition of antibody production and macrophage function, apoptosis, and suppression of cellular immune response (8–11). Similarly, *in vitro* and animal studies have indicated that chlordane may alter immune status through reduced lysis of tumor cells by human natural killer (NK) cells, inhibition of chemotaxis, delay in macrophage induction, suppression of lymphocyte proliferation and cytokine release, or other sustained immune alterations that may be associated with autoimmune disease (12–16).

The aims of the current study were to follow up on our previous findings of neonatal exposures to permethrin and chlordane by conducting an epidemiologic investigation of *in utero* exposures to these pesticides and associations with cord blood cytokine levels, gestational age, and birthweight and size among a population of babies born in Baltimore, MD. Cytokine levels in cord blood provide a snapshot of the newborn's immune profile that could not otherwise be obtained from measuring levels in maternal serum.

Methods

Subjects

A cross-sectional study of newborn deliveries at the Johns Hopkins Hospital Labor and Delivery Suite in Baltimore was conducted. The Baltimore THREE (Tracking Health Related to Environmental Exposures) Study received approval from the Johns Hopkins Medicine Institutional Review Board. All study specimens collected would have otherwise been discarded. Medical records utilized for data collection were available to hospital and study personnel. Because all specimens and data collected from medical records were made anonymous, informed consent was not required and the study was determined to be HIPAA

exempt. All singleton live births delivered between November 26, 2004 and March 16, 2005 were eligible for study participation. Multiple births were excluded.

Details about the study population and data collection have been published previously (17). In brief, all singleton live births delivered during the study period were eligible for participation (n=591). Cord blood was collected from 341 of these births. The primary reasons for not collecting cord blood were either 1) lack of sufficient hospital staff to extract samples during a busy time of many births in the hospital or 2) lack of feasibility because newborn was too small and umbilical cord had insufficient volume of blood for collection. Additionally, 41 samples had insufficient cord blood volume for subsequent laboratory analyses, leaving a total of 300 samples available for laboratory analyses. Of these, 297 were successfully analyzed for chlordane pesticides, and 185 (62%), for permethrin pesticides.

Details about the cord blood collection and processing have been published previously (18–19). In brief, up to five 10-mL cord blood samples were collected by trained hospital personnel immediately after delivery. Once extracted, samples were placed in the Labor and Delivery Suite refrigerators until transported by study personnel to a laboratory at the Johns Hopkins Bloomberg School of Public Health for processing and storage at −80 degrees Celsius. Samples were collected continuously until there were 300 samples with sufficient volume of serum for chemical analysis. Frozen samples were transferred on dry ice to laboratories at the Centers for Disease Control and Prevention (CDC) in Atlanta and to the General Clinical Research Center at the Johns Hopkins Bayview Medical Center in Baltimore for pesticide and cytokine analyses, respectively. Lipid levels were also measured by the CDC.

Medical Records

Data on maternal and infant characteristics and health status were abstracted from clinical databases at the Johns Hopkins Hospital by two study personnel concurrently, using standardized forms and blinded to the environmental exposure status of the subjects. Maternal information was abstracted using the Electronic Patient Record by two study investigators at the Johns Hopkins Hospital; data from infant records was abstracted by two other study personnel using paper files. Information collected from medical records included demographic information, maternal medical history, and infant birth outcomes. A random ten percent sample of abstracted data was verified by two of the study investigators to ensure quality control.

Potential risk factors for pesticide exposure or dose that also may be associated with cytokine levels or infant size at birth were included in this study. Specifically, we examined the following maternal anthropometric and sociodemographic characteristics: age, race, body mass index (BMI), parity, education, and smoking. Maternal medical history on asthma, hypertension and diabetes during pregnancy was also included, as was information on infection during pregnancy and type of delivery. Age, race, education, and parity were based on self-report. BMI was calculated based on reported pre-pregnancy weight and height. Smoking was a dichotomous variable (yes/no) based on both clinical records and/or cotinine concentrations measured in cord blood. If clinical records indicated that the mother smoked during pregnancy and/or had a cotinine serum level above 10 ng/mL (1), we categorized her as a smoker.

Infant birth outcomes were birthweight, length, head circumference, ponderal index, and gestational age. Birthweight, measured in grams, was abstracted from maternal medical records and confirmed by infant medical records. Birth length and head circumference were recorded in centimeters in the infant medical records. Ponderal index, based on the ratio of

birthweight to length, was calculated using the formula: $birthweight/length^3 \times 100$. Gestational age was based on the best obstetric estimate in the maternal record.

Laboratory Analyses

We used an aliquot (1 mL) of the serum samples (n=297) from each participant to measure concentrations of a number of organochlorines including chlordane compounds (*trans*-nonachlor and oxychlordane). Subsequently, 185 serum samples had sufficient remaining volume (>200 μL) for analyses of non-persistent pesticides, including permethrin *cis*- and *trans*- isomers, and the permethrin synergist PBUT. Another aliquot (>50 μL) of serum samples (n=272) were analyzed for levels of nine cytokines: TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, and GMCSF. All laboratory methods included rigorous validation, inclusion of multiple QC samples in each analytical run, and blinded proficiency testing every 6 months.

Chlordane—As previously described (4), *trans*-nonachlor and oxychlordane were measured in cord serum using gas chromatography–isotope dilution high-resolution mass spectrometry (GC-IDHRMS) analysis (20). In brief, the method included automatic fortification of the samples with internal standards and solid phase extraction (SPE). Removal of co-extracted lipids was performed on a silica:silica/sulfuric acid column. Final analytical determinations of the target analytes were performed by GC-IDHRMS. Chlordane metabolites had limits of detection ranging from 2.3 to 3.3 pg/g serum.

Permethrin—As previously described (4), *cis*- and *trans*-permethrin isomers and PBUT were measured by the CDC using a modification of a previously published gas chromatography–high resolution mass spectrometry (GC-HRMS) method (21). Briefly, this method involves thawing samples to room temperature, spiking them with the labeled internal standard, denaturing serum proteins, separating samples using the OASIS SPE column, drying and eluting with methylene chloride, and preserving in toluene. Samples were analyzed for insecticide levels using GC-HRMS. Each insecticide chemical had an isotopically labeled internal standard for which each ion was monitored. The coefficients of variation were less than 20% at the low end of the method linear range. Levels of detection for *cis*-permethrin, *trans*-permethrin and PBUT were 0.03, 0.02 and 0.07 ng/mL, respectively.

Cytokines—We analyzed cytokines at the Bayview General Clinical Research Center using pro-inflammatory cytokine 9-plex kits (Meso Scale Discoveries (MSD)) (22). Concentrations of IFN-γ, TNF-α, GM-CSF, IL-1β, IL-2, IL-6, IL-8, IL-12p70, and IL-10 were assayed using 96-well multi-array plates. The immunoassay plates are pre-coated with cytokine-specific capture antibodies. All analyses were performed in duplicate for each serum sample and calibration solutions of known concentrations were included in each run. Samples were incubated in the multi-array plates, allowing cytokines to bind to their corresponding capture antibody. Cytokine concentrations were quantified using a cytokine-specific detection antibody labeled with MSD reagent. The coefficients of variation were less than 5% at the high end and less than 20% at the low end of the method linear range. Cytokines were detected using a SECTOR® Imager 6000 reader (MSD, Gaithersburg, MD). The distribution of cytokine concentrations were similar to those measured in cord serum from another population, as described previously (7).

Lipids—Serum lipid concentrations were analyzed by the CDC using Roche Diagnostics Corp. (Indianapolis, IN) test kits to quantify concentrations of total triglycerides (product no. 011002803-0600) and total cholesterol (product no. 011573303-0600). A Hitachi 912 Chemistry Analyzer (Hitachi, Tokyo, Japan) was used to determine concentrations.

Statistical Analysis

Descriptive statistics were used to describe cytokine concentrations in cord serum and birth outcomes. Because cytokine concentrations were right-skewed, they were natural log-transformed for analyses requiring assumptions of normality. There is strong evidence that each of the cytokines is linearly associated with the others. Thus, because of these strong overall associations, principal components analysis was needed to more precisely determine patterns of association among the cytokines. PCA was used to compute independent cytokine components using the covariate matrix to generate standardized, normally distributed principal components that are linear combinations of optimally-weighted observed data. Principal components with a minimum eigenvalue of 0.4 were retained. Cytokines with loading scores greater than ±0.40 were considered a significant element of a given component (23). Separate PCAs also were used to group chlordane chemicals and permethrin isomers + PBUT into a single chlordane and permethrin component, respectively, herein referred to as mixtures. (See Table S2, Supporting Information.) Pesticide chemical concentrations below the limit of detection were imputed using the formula LOD/√2, as described previously (4).

We modeled chlordane using unadjusted serum levels and controlled for lipid levels in regression analyses. Linear regression was used to test for associations between levels of chlordane or permethrin mixtures and 1) cytokine components, 2) gestational age, and 3) size parameters at birth. We estimated the mean change in cytokine component levels per unit change in levels of permethrin or chlordane mixtures using multiple linear regression, controlling for selected *a priori* covariates in our study which were determinants of cytokine levels and which were associated with insecticide levels at birth: gestational age, parity, smoking status of the mother, infant gender, and maternal medical conditions at the time of delivery including hypertension and intrapartum fever. We also examined other medical conditions (i.e., asthma, diabetes, infection during pregnancy, and type of delivery) as potential confounders, but they were not associated with pesticide levels, and thus were not included in the final models. Additionally, smoking status was evaluated as an effect modifier. Linear regression models were also used to estimate the mean change in gestational age, birthweight, length, head circumference and ponderal index per unit change in permethrin or chlordane levels, controlling for covariates which are known determinants of changes in fetal growth and development including maternal age, race, pre-pregnancy BMI, smoking status, and whether the mother had hypertension at the time of delivery. For models of birthweight, length, head circumference, and ponderal index, we included gestational age as a covariate.

For all models, pesticide mixtures were also evaluated as categorical variables in three categories (low, medium, high), determined based on tertiles. Tests for trend were conducted by modeling the categorical exposure variables as continuous, such that *p*-trends were calculated based on the median value for each category. Statistical analyses were performed using Stata SE 11.0 statistical software (StataCorp, College Station, TX).

Results

Geometric mean cord serum concentrations and ranges for chlordane and permethrin-related chemicals were reported in detail previously (4). In brief, *trans*-nonachlor, oxychlordane, *trans*-permethrin, *cis*-permethrin, and PBUT were detected in 93, 84, 52, 41, and 36 percent of samples measured, respectively, with geometric mean (and maximum) concentrations 9.4 (185.5), 6.5 (80.7), 36.3 (276.6), 33.6 (98.9), and 61.3 (705.0) pg/mL, respectively.

Table 1 describes characteristics of the study population and shows cytokine distributions were skewed to the right. The mean gestational age was 272 days and mean birthweight was

3,191 grams in this study. Table 2 describes the five cytokine components resulting from PCA of all nine cytokines. These five components describe 87% of the total variance. All cytokines positively and roughly equally load onto the first component, labeled the “global cytokine component”, which accounts for 53% of the variance in cytokine levels. The remaining four cytokine components show more specific patterns of cytokine expression. Component 2, which we call the “acute phase cytokine component” primarily is composed of IL-1β and IL-6, the primary cytokines of an acute phase response (24), and describes 13% of the variance in cytokine levels. Component 3, the “anti-inflammatory cytokine component”, is positively loaded with IL-10 and negatively loaded with IL-12p70, characteristic of an anti-inflammatory or immunoregulatory response involved with allergic immunity (24), and describes 8% of the variance. The fourth and fifth components, the “TNF-α component” and “IL-1β component,” describe 7% and 6% of the variance, respectively, and have patterns consistent with pro-inflammatory responses (24).

Table 3 shows effect estimates from adjusted linear regression models of the five cytokine component levels on chlordane and permethrin mixture levels. Permethrin levels were significantly (p-value <0.05) negatively associated with an anti-inflammatory response (component 3) and positively associated with an IL-1β response (component 5). These associations were significant only among the most highly exposed group compared with the lowest exposure group. The association between permethrin and anti-inflammatory response was also consistent with a curvilinear relationship, confirmed by the inclusion of a quadratic term (p-value <0.05) in the model (data not shown). Chlordane levels were significantly negatively associated with an IL-1β response and non-significantly positively associated with an anti-inflammatory response. The observation of an association between chlordane and an IL-1β response was significant for both the moderately exposed and highly exposed groups compared with the lowest exposure group. The associations between pesticide mixtures and cytokine components did not change after excluding women who had intrapartum fever or infection (data not shown).

Table 4 shows results of linear regression models of chlordane and permethrin on birth outcomes. A one-unit change in chlordane levels was associated with a 21-gram decrease in birthweight (adjusting for lipids and gestational age) which was not statistically significant. Chlordane exposure was associated with an 85-gram decrease in birthweight in the most highly exposed group compared with the lowest exposure group, but the association was not statistically significant. A one-unit change in permethrin levels was associated with a non-significant 1-day decrease in gestational age (95% CI: −2 to 0.2 days), while the most highly exposed group was associated with a 2-day decrease compared with the lowest exposure group (Table 4). While permethrin levels were not associated with any of the birth outcomes evaluated in this study, the most highly exposed group had non-significant decreases in all five birth outcomes evaluated. The linear predictions of birth outcomes and cytokine component levels in relation to pesticide mixtures are presented in Figures S1 and S2, Supporting Information. Smoking status was not an effect modifier of the association between chlordane or permethrin levels and any of the study end points (data not shown).

We also evaluated relationships between birth outcomes and cytokine component levels using multivariate linear regression models (data not shown). A one-unit increase in TNF-α was associated with a 2-day decrease in gestational age (95% CI: −4.2 to −0.1), consistent with previous findings that higher TNF-α levels were associated with shorter gestational age (7). None of the cytokine components were significantly associated with changes in birthweight, length or head circumference in regression models; however, higher levels of cytokines were non-significantly associated with increases in all three measures of size at birth.

Discussion

The results of this study suggest that chlordane and permethrin concentrations may be associated with expression of specific serum cytokines in cord blood. Our data also suggest that insecticide levels may be negatively associated with birthweight and size parameters. Larger studies will be required to determine whether such relationships truly exist.

Our findings suggest that chlordane exposure may be associated with lower levels of pro-inflammatory IL-1β expression, which is involved with tumor suppression (25). IL-1β is produced in a variety of cells including monocytes, macrophages, and B cells (26). Its major signaling functions include maturation and proliferation of B cells, activation of NK cells and inflammation (26). When IL-1β is suppressed, the ability of NK cells to lyse tumor cells may be inhibited. Previous *in vitro* and animal studies have found that chlordane concentrations, even at low levels of exposure, are associated with immunosuppression of NK cells and other immune cells involved with tumor suppression (14, 27–28). Additionally, epidemiological studies have found a link between chlordane exposure and increased risk of leukemia and Non-Hodgkin's lymphoma (NHL) (29–31).

Our findings suggest that permethrin exposure may be associated with lower levels of anti-inflammatory IL-10 expression, which is part of an underlying immune mechanism characteristic of allergic diseases (32–33). The anti-inflammatory cytokine component in our study primarily consists of positive loadings of IL-10 and, to a lesser degree, negative loadings of IL-12p70. IL-10 is an anti-inflammatory and immunoregulatory cytokine produced by a variety of immune cells including T cells, B cells, monocytes and mast cells. Its major functions include immunosuppression of cytokines associated with cellular immunity and allergic inflammation (24). Diminished IL-10 expression has been found to be associated with asthma and allergic rhinitis (34). Toxicity studies suggest that permethrin exposure may be associated with immunosuppressive effects related to allergic immunity (8, 35). Additionally, an epidemiologic case-control study found that self-reported permethrin use among farm women was associated with an increased risk of atopic and non-atopic asthma (OR=1.7 and 2.2, respectively; $p < 0.05$) (36). Our data showed that increasing cord serum levels of permethrin were not only associated with decreasing levels of the anti-inflammatory cytokine component, but also significantly associated with decreasing levels of IL-10 *per se* (data not shown). These findings are consistent with the literature suggesting that permethrin exposure may be associated with allergy or asthma.

IL-12 is another important cytokine in allergic immunity. IL-12 plays an indirect but critical role in counter-regulating allergic inflammation mediated by IgE by inducing IFN-γ (24). Allergic diseases such as asthma and allergic rhinitis are characterized by the inappropriate production of IgE in response to allergens (24). Two key cytokines involved with the regulation of IgE synthesis are IL-4 and IFN-γ, which inhibit allergic responses by inhibiting the mechanism involved with the production of IgE. IL-12 may play a key role in allergic immunity because it is an inducer of IFN-γ (24). Interestingly, our data suggest that permethrin levels were positively associated with levels of IL-12p70. Additionally, levels of IFN-γ and IL-12p70 were slightly higher in mothers with a medical history of asthma compared with mothers without asthma, but the difference was not statistically significant (data not shown). Levels of the anti-inflammatory cytokine component were lower in women with a history of asthma compared with non-asthmatics, but again the difference was not significant. It is possible that mothers in our study with a medical history of asthma may not have been having active asthma at the time when the study was conducted. We did not have information on whether mothers were under treatment for asthma or whether the disease was quiescent.

While our data suggest a negative association between levels of permethrin and levels of anti-inflammatory cytokine component, which may be associated with allergy and atopic disease, we are unable to assess whether the immune response is a direct effect of permethrin exposure, or whether permethrin exposure is a marker for another underlying cause of atopic immunity. Permethrin use could be an indication of an environment that has low levels of allergens such as dust mites or cockroaches (37–38). Or, perhaps more likely, it could have an irritant effect that would exacerbate allergic responses.

Finally, we identified a global cytokine component that seems to reflect overall cytokine synthesis, implying that individuals may generally have higher or lower levels of both the inflammatory and anti-inflammatory cytokines that we measured. Our study could not reveal the basis for a global differential expression of these cytokines; however, the global component was not associated with pesticide exposures.

Our cross-sectional study design poses several limitations. Because permethrin is a non-persistent chemical and levels were measured only at the time of birth, we are unable to characterize exposure during potentially critical windows earlier in gestation. We also may be underestimating the average permethrin exposures and related risks because all of the women in our study gave birth between November and March, excluding the spring and summer months when pesticide use may be higher (39). Because chlordane is a persistent chemical that was banned decades ago, a major source of exposure is through residues in and around homes and levels of chlordane in body fat are likely to reflect exposure integrated over a period of months to years. Serum levels are likely to reflect both current exposure and long term exposure via equilibration of levels from adipose to serum. Thus, unless the mother had just recently experienced an increase in exposure, we can assume that concentrations were relatively constant throughout pregnancy.

Another potential limitation of our study is the interpretability of changes in cytokine levels and whether the birth process itself could induce changes in cytokines. The immune system is very complex; cytokines are produced by a variety of different types of immune cells and are pleiotropic, having a variety of functions. Changes in levels of a single cytokine could be an indication of any one of many different underlying immune mechanisms. One advantage to our study was that, rather than examining associations of individual cytokines, we developed independent cytokine components representing different immune mechanisms. For example, while IL-10 levels did not differ between mothers with asthma and mothers without asthma, levels of the anti-inflammatory cytokine component were lower among asthmatic mothers compared with non-asthmatic mothers – consistent with the immune profile of allergic diseases as described earlier. However, because our study was cross-sectional, we were unable to determine whether differences in cytokine levels at birth translate into health effects later in life. The limited variability in our measures of fetal growth and development also may have limited our ability to detect relationships between pesticides and these birth outcomes.

Although our findings suggest that permethrin and chlordane levels in cord blood are associated with changes in cytokine concentrations at birth, we should interpret these findings with caution. First, our study provides no evidence that such changes would be associated with birth outcomes. Second, in terms of concern about later outcomes, our study is the first study to examine associations between fetal exposures to pesticide chemicals and immune activity in cord blood. Because we evaluated a variety of exposure markers and a variety of immune markers, it is possible that our findings may be false-positives. In future studies trying to replicate these findings it will be important to determine whether such changes have later health consequences for the infant. While biologically plausible,

longitudinal research is needed to determine whether changes in cytokines levels at birth have short- or long-term health consequences later in life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMI	Body mass index
GC-HRMS	Gas chromatography high resolution mass spectrometry
GM	Geometric mean
GM-CSF	Granulocyte macrophage colony-stimulating factor
IFN	Interferon
IL	Interleukin
LOD	Limit of detection
PBUT	Piperonyl butoxide
PCA	Principal component analysis
SPE	Solid phase extraction
THREE	Tracking Health Related to Environmental Exposures
TNF	Tumor necrosis factor

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Table 1

Characteristics of the study population (n=341) by whether in the current study and distribution of cytokine concentrations in umbilical cord serum (n=272), THREE Study, 2004–2005

	In study (n=297)[a]	Not in study (n=44)[b]	$P_{heterogeneity}$
Characteristic	**N (%)**	**N (%)**	
Maternal age			
<18	25 (8.4)	4 (9.1)	0.960
18–35	248 (83.5)	36 (81.8)	
>35	24 (8.1)	4 (9.1)	
Maternal race			
African-American	210 (70.7)	25 (56.8)	0.152
Asian	23 (7.7)	4 (9.1)	
Caucasian	64 (21.6)	15 (34.1)	
Parity			
0	123 (41.4)	28 (63.6)	0.006
1+	174 (58.6)	16 (36.4)	
Smoked cigarettes during pregnancy			
No	241 (81.4)	39 (90.7)	0.134
Yes	55 (18.6)	4 (9.3)	
Infant gender			
Female	132 (44.4)	18 (40.9)	0.659
Male	165 (55.6)	26 (59.1)	
Hypertension during pregnancy			
No	266 (89.6)	36 (81.8)	0.132
Yes	31 (10.4)	8 (18.2)	
Intrapartum fever			
No	274 (93.2)	42 (95.4)	0.571
Yes	20 (6.8)	2 (4.6)	
	Mean (SD)	**Mean (SD)**	$P_{t\text{-test (two-sided)}}$
Maternal pre-pregnancy body mass index (kg/m^2)	26.4 (6.8)	27.5 (8.8)	0.380
Gestational age (days)	271.8 (13.8)	268.8 (18.5)	0.208
Birthweight (grams)	3191 (594)	2962 (662)	0.019

Characteristic	In study (n=297)[a] N (%)	Not in study (n=44)[b] N (%)	$P_{heterogeneity}$
Birth length (cm)	49.8 (3.1)	49.1 (3.6)	0.099
Head circumference (cm)	33.4 (1.9)	33.1 (2.6)	0.249
Ponderal index (grams/cm^3)	2.57 (0.49)	2.47 (0.26)	0.202

	Geometric	Percentile				
Cytokine	Mean, pg/mL	10th	25th	50th	75th	90th
IL-1β[c]	0.45	0.15	0.29	0.47	0.68	0.99
IL-2	1.76	0.94	1.33	1.81	2.39	3.12
IL-6	4.94	1.88	2.59	3.67	6.50	17.38
IL-8	8.08	3.77	5.00	6.97	10.64	17.18
IL-10[c]	1.41	0.62	0.94	1.48	2.20	2.78
IL-12p70	1.87	1.15	1.43	1.88	2.41	2.97
IFN-γ	1.95	1.17	1.44	1.85	2.58	3.47
TNF-α	4.61	3.35	3.95	4.52	5.40	6.28
GM-CSF	0.80	0.40	0.55	0.75	1.12	1.59

[a] Individuals for whom pesticides in cord serum were analyzed

[b] Individuals for whom cord serum had insufficient volume for analyses (n=41) or pesticides were unsuccessfully analyzed (n=3)

[c] For IL-1β and IL-10, data on two subjects were missing.

Table 2

Principal component loadings for nine cytokines measured in cord serum (*n*=272).

	Principal Components				
Cytokines	**1. Global**	**2. Acute Phase**	**3. Anti-inflammatory**	**4. TNF-α**	**5. IL-1β**
IL-1β	0.31	0.43	−0.01	−0.08	0.75
IL-2	0.37	−0.01	−0.39	−0.07	0.03
IL-6	0.31	0.50	0.26	0.01	−0.12
IL-8	0.32	0.42	−0.10	0.34	−0.53
IL-10	0.30	−0.21	0.70	−0.30	−0.19
IL-12p70	0.34	−0.19	−0.49	−0.18	−0.23
IFN-γ	0.36	−0.38	−0.09	−0.23	0.11
TNF-α	0.28	−0.38	0.14	0.83	0.20
GM-CSF	0.39	−0.12	0.12	−0.14	0.01
Eigenvalue	4.79	1.16	0.73	0.61	0.49
% Variance explained	53	13	8	7	6
% Cumulative variance	53	66	74	81	87

Table 3

Cytokine component levels in relation to chlordane and permethrin mixtures in cord serum assessed as continuous and categorical (tertile) variables.[a]

		Cytokine components[b]				
		1. Global	2. Acute phase	3. Anti-inflammatory	4. TNF-α	5. IL-1β
	Median (Range)	β[c] (95% CI)	β[c] (95% CI)	β[c] (95% CI)	β[c] (95% CI)	β[c] (95% CI)
Chlordane						
(continuous variable)	1.72 (<LOD, 5.33)	0.12 (−0.17, 0.40)	0.02 (−0.12, 0.16)	0.08 (−0.03, 0.19)	−0.01 (−0.12, 0.09)	*−0.11 (−0.20, −0.02)*
Low	0.61 (<LOD, 1.21)	reference	reference	reference	reference	reference
Medium	1.71 (1.22, 2.11)	0.50 (−0.15, 1.15)	0.14 (−0.17, 0.46)	*0.30 (0.05, 0.55)*	−0.05 (−0.28, 0.19)	*−0.32 (−0.53, −0.11)*
High	2.54 (2.12, 5.33)	0.41 (−0.27, 1.10)	−0.01 (−0.34, 0.32)	0.23 (−0.04, 0.49)	−0.09 (−0.34, 0.15)	*−0.25 (−0.46, −0.03)*
p-trend[d]		0.199	0.950	0.066	0.457	*0.015*
Permethrin						
(continuous variable)	5.22 (<LOD, 8.54)	0.14 (−0.05, 0.32)	0.02 (−0.09, 0.12)	*−0.14 (−0.22, −0.05)*	0.05 (−0.04, 0.14)	*0.09 (0.01, 0.17)*
Low	3.47 (<LOD, 4.40)	reference	reference	reference	reference	reference
Medium	5.19 (4.41, 6.04)	0.92 (0.19, 1.65)	−0.08 (−0.49, 0.34)	−0.03 (−0.39, 0.33)	−0.07 (−0.42, 0.27)	0.11 (−0.19, 0.42)
High	6.12 (6.12, 8.54)	0.34 (−0.24, 0.92)	−0.04 (−0.37, 0.29)	*−0.28 (−0.56, 0.00)*	0.16 (−0.12, 0.44)	*0.31 (0.07, 0.56)*
p-trend[d]		0.297	0.813	*0.049*	0.240	*0.011*

[a]Models adjusted for parity, smoking status, newborn gender, hypertension, intrapartum fever, and gestational age. Chlordane models additionally were adjusted for total serum lipid concentration.

[b]Cytokine components are based on log-transformed cytokine variables.

[c]β represents the mean change in levels of cytokine components per unit increase in pesticide component levels.

[d]*P*-trend based on median value for each group.

Italics denote statistical significance (*p*-value < 0.05).

Table 4

Birth outcomes in relation to serum chlordane and permethrin concentrations assessed as continuous variables and as tertiles.[a]

		Birth outcomes				
		Gestational age (days)	**Birthweight (grams)**	**Birth length (cm)**	**Head circumference (cm)**	**Ponderal index (g/cm^3)**
	Median (Range)	**β[b] (95% CI)**	**β[b] (95% CI)**	**β[b] (95% CI)**	**β[b] (95% CI)**	**β[b] (95% CI)**
Chlordane[c]						
(continuous variable)	1.72 (<LOD, 5.33)	−0.12 (−1.70, 1.47)	−21.3 (−77.9, 35.3)	0.18 (−0.13, 0.48)	0.00 (−0.19, 0.19)	−0.02 (−0.08, 0.03)
Low	0.61 (<LOD, 1.21)	reference	reference	reference	reference	reference
Medium	1.71 (1.22, 2.11)	1.63 (−2.11, 5.36)	7.1 (−126.4, 140.6)	0.57 (−0.14, 1.28)	−0.13 (−0.57, 0.31)	−0.09 (−0.23, 0.05)
High	2.54 (2.12, 5.33)	1.12 (−2.87, 5.11)	−85.1 (−227.1, 56.8)	0.54 (−0.22, 1.31)	−0.04 (−0.51, 0.43)	−0.06 (−0.21, 0.08)
p-trend[d]		0.519	0.283	0.134	0.803	0.329
Permethrin						
(continuous variable)	5.22 (<LOD, 8.54)	−0.94 (−2.10, 0.22)	−2.2 (−48.8, 44.4)	−0.01 (−0.23, 0.22)	−0.04 (−0.20, 0.12)	0.00 (−0.03, 0.03)
Low	3.47 (<LOD, 4.40)	reference	reference	reference	reference	reference
Medium	5.19 (4.41, 6.04)	0.26 (−4.18, 4.70)	67.4 (−106.5, 241.4)	0.11 (−0.74, 0.96)	−0.02 (−0.63, 0.58)	0.05 (−0.06, 0.16)
High	6.12 (6.12, 8.54)	−2.06 (−5.78, 1.66)	−26.3 (−173.6, 121.0)	−0.16 (−0.88, 0.56)	−0.15 (−0.65, 0.34)	−0.01 (−0.10, 0.08)
p-trend[d]		0.270	0.720	0.653	0.543	0.894

[a] Models adjusted for maternal age, race, BMI, parity, smoking, gender, hypertension, and additionally for gestational age for birth outcomes other than gestational age.

[b] β represents the mean change in birth outcome measure (i.e. in days, grams, centimeters, etc.) per unit increase in pesticide component levels.

[c] Chlordane models additionally adjusted for total serum lipid concentration.

[d] *P*-trend based on median value for each group.