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Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants

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ABSTRACT

Previous research has shown that prenatal exposure to pesticides may be associated with decreased fetal growth. The specific pesticides investigated and results reported across studies have been inconsistent, and there is a mounting need for the consideration of mixtures rather than individual agents in studies of health outcomes in relation to environmental exposures. There are also many individual pesticides that have not been investigated in human health studies to date. We conducted a pilot study in rural Zhejiang province, China, measuring 20 non-persistent pesticides (10 insecticides, 6 herbicides, 3 fungicides, and 1 repellant) in umbilical cord blood of 112 full term (>37 weeks) infants. The pesticides detected with the greatest frequency were diethyltoluamide (DEET) (73%), a repellant, and vinclozolin (49%), a fungicide. The samples had detectable concentrations for a mean of 4.6 pesticides (SD = 1.9) with a maximum of 10. Adjusting for potential confounders, newborn birth weight was inversely associated with the number of pesticides detected in cord blood ($p = 0.04$); birth weight decreased by a mean of 37.1 g (95% CI, −72.5 to −1.8) for each detected pesticide. When assessing relationships by pesticide type, detection of fungicides was also associated with decreased birth weight (adjusted $\beta = -116$ g [95% CI, −212 to −19.2]). For individual pesticides analyzed as dichotomous (detect vs. non-detect) variables, only vinclozolin (adjusted $\beta = -174$ g [95% CI, −312 to −36.3]) and acetochlor (adjusted $\beta = -165$ g [95% CI, −325 to −5.7]) were significantly associated with reduced birth weight. No significant associations were seen between birth weight and individual pesticides assessed as continuous or 3-level ordinal variables. Our findings from this pilot investigation suggest that exposure to fungicides may adversely impact fetal growth. Exposure to mixtures of multiple pesticides is also of concern and should be explored in addition to individual pesticides. Additional research is needed to establish causality and to understand the function and impact of fungicides and pesticide mixtures on fetal development.

1. Introduction

Pesticide use is common worldwide. According to the most recent estimates available, pesticide use in the United States exceeded 1.1 billion pounds in 2007, accounting for an estimated 22% of the 5.2 billion pounds used worldwide (EPA, 2011). In the US, the agricultural sector accounts for nearly 80% of conventional use non-persistent pesticides, including herbicides, insecticides, and fungicides. China is the world's largest user and producer of pesticides (Yang, 2007), using more than 2.6 billion pounds of pesticides annually (Li, 2006). The highest use occurs in China's wealthy, developed areas near the southeast coast (Hamburger, 2002). The current study was conducted in rural Zhejiang, an eastern coastal province, which has one of the highest pesticide use rates in the country at double the national average rate (Huang et al., 2001). According to a survey study, there is a common belief among Zhejiang farmers that the amount recommended for use is insufficient, which results in the over-application of pesticides and potentially increased human exposure (Huang et al., 2001).

Pesticide exposure can occur through multiple sources, pathways, and routes, including residential use, consumption of contaminated food, runoff into drinking water, occupational exposure from pesticide application or production, and agricultural workers "take-home" exposure to spouses and children. There is growing concern regarding developmental effects of commonly used pesticides. Pregnant women and their developing fetuses are especially vulnerable to environmental exposures to pesticides (Stillerman et al., 2008), particularly during critical developmental windows. Studies show that pesticides detected in

Abbreviations: OP, organophosphate; GC–MS, gas chromatography–mass spectrometry; LOD, limit of detection; ND, non-detect; Hb, hemoglobin; DEET, diethyltoluamide; EDP, endocrine disrupting pesticide.

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maternal serum were commonly detected in cord serum, indicating that a portion of the maternal dose was transferred to the fetus (Barr et al., 2010; Whyatt et al., 2003).

Ecological and more individually specific estimates of pesticide exposure have been explored in association with birth weight in previous studies, mostly focusing on organophosphate (OP) insecticide exposure. Results have been inconsistent. Some studies report that exposure to OPs and other pesticides is associated with reduced fetal growth (Eskenazi et al., 2004; Whyatt et al., 2004, 2005), while others do not (Chevrier et al., 2011; Villanueva et al., 2005; Wang et al., 2011). To our knowledge only three studies, all conducted in the US, have assessed the relationship between birth outcomes such as birth weight and contemporary use (i.e. non-persistent) pesticide concentrations measured in cord blood (Barr et al., 2010; Neta et al., 2011; Whyatt et al., 2005). Our aim was to quantify fetal exposure to 20 non-persistent pesticides in cord blood, compare concentrations with previous studies of pesticides in cord blood samples from the U.S., and assess the cross-sectional relationship between cord blood pesticides and birth weight as part of a prospective cohort study in rural Zhejiang province.

2. Methods

2.1. Study participants and measure of maternal and fetal characteristics

The study involved a subset of pregnant women participating in an ongoing study of early iron deficiency and neurodevelopment. Women at 36 weeks gestation who reported a healthy, uncomplicated, singleton pregnancy were recruited from Fuyang Maternal and Children's hospital and invited to participate in the study. Consecutive participants in the ongoing study with due dates between April and June 2009 were included in this pilot study of pesticide exposure. Institutional review board approval was obtained from the ethics committees of Children's Hospital Zhejiang University and the University of Michigan. Approximately 99.6% of eligible women agreed to participate. Exclusionary criteria included chronic diseases (such as chronic heart disease, hepatitis), complicated pregnancies (such as maternal diabetes), and hereditary or metabolic diseases. Maternal demographics were gathered from patient charts during two prenatal visits, the first at <16 weeks gestation and the latest at the 3rd trimester (about 36 weeks gestation). Additional maternal and infant characteristics were obtained during the delivery hospitalization.

The sample was composed of 116 pregnant women who gave birth to full-term infants (>37 weeks gestation) and for whom cord blood samples were analyzed for 20 pesticides. Of these, two women were missing all demographic information. Additionally, one woman was missing data for maternal hemoglobin at delivery. Consequently, we had complete data for 113 participants. After fitting our final multivariate linear regression model, one observation was highly influential with a studentized residual value greater than 3 and was removed from bivariate and multivariate analyses. Thus, our final study population for assessing relationships between pesticides and birth weight was 112.

2.2. Pesticide analysis

In the delivery room, a 30 ml umbilical cord blood sample was collected and separated, and serum was stored at −80 C. Pesticide analysis took place at the Institute of Toxicology at Nanjing Medical University. In the laboratory, cord serum samples underwent solid-phase extraction and highly selective analysis using isotope dilution gas chromatography–mass spectrometry (GC–MS). We investigated 20 representative pesticides selected based on pesticide usage data, availability of analytical standards (≥98.0%, Dr. E, Germany), method compatibility, and preliminary pilot data. The pesticides are listed here by type: organophosphate insecticides (chlorpyrifos, diazinon, fonofos, malathion, parathion-ethyl, parathion-methyl, profenofos, terbufos), carbamate insecticides (carbofuranphenol, propoxur), herbicides (acetochlor, alachlor, atrazine, linuron, metolachlor, trifluralin), fungicides (dicloran, metalaxyl, vinclozolin), and repellant (diethyltoluamide). Concentrations of the pesticides in cord serum were analyzed by Thermo Trace GC and DSQ Mass Spectrometer (Thermo, USA). The samples were first deposited by saturated $(NH_4)_2SO_4$, and then solid phase extraction was performed with a C18 column (200 mg, 3 ml, Dikma, USA). The treated samples were determined by a selected ion monitor mass method, one quantitative ion and one qualitative ion were selected for each compound. 1 μl of each treated sample was injected into the GC with a splitless inlet method, with an injector temperature of 250 °C. A DB-5 UI column (Agilent, USA), and helium at a flow rate of 1 ml/min were used for separation. The program was started at 60 °C, held for 2 min, then programmed at 10 °C min^{-1} up to 150 °C, held for 10 min, then programmed at 5 °C min^{-1} up to 200 °C, held for 5 min, and finally programmed at 20 °C min^{-1} up to 280 °C and held for 20 min. The fetal bovine serum simulation system was selected as a blank to prepare the spiked cord serum of standard solution which contained a serial concentration between 0.01 and 50 μg/l ($r>0.99$). The reported limit of detection (LOD) for most of the selected pesticides was 0.05 μg/l but a few were as high as 0.50 μg/l. Quality control samples were analyzed in parallel with unknown samples in each analytical series.

2.3. Statistical analysis

All statistical analyses were conducted using SAS 9.2 (Cary, North Carolina). In preliminary analyses we determined means or percentages for maternal and newborn characteristics such as the number of pesticides detected in cord blood, maternal age, BMI, gestational age, newborn birth weight and newborn sex. Distributions of pesticide concentrations in cord blood were tabulated. Percentile values that fell below the LOD were represented as non-detect (ND). Geometric mean concentrations were calculated for pesticides detected in >45% of samples. For these calculations, and analyses in which individual pesticide concentrations were included in a model as a continuous variable, levels below the LOD were assigned a value of the LOD divided by the square root of 2.0 (Hornung and Reed, 1990).

We conducted 3 sets of analyses to assess the relationship between pesticide exposure and birth weight. Because most humans are exposed to multiple environmental contaminants, including pesticides that may impact health additively or synergistically (Rider et al., 2010), we first explored the relationship between newborn birth weight and the number of pesticides detected in cord blood. In our first approach we formed an index of all measured pesticides since a particular pesticide may have multiple modes of action, and because multiple mechanisms potentially related to pesticide exposure may impact fetal growth and birth weight. A binary exposure variable was created for each pesticide such that concentrations less than the LOD were given a value of 0, otherwise they were give a value of 1. For each subject, the sum of these 20 binary variables represents the number of detected pesticides and was our primary predictor variable. Multivariable regression models were adjusted a priori for the following covariates: gestational age (weeks), maternal age (years), maternal BMI at early pregnancy (kg/m^2), and maternal Hb at delivery (g/dl). Newborn sex and newborn serum ferritin were also considered but did not act as confounders statistically (were not associated with both pesticide exposure and birth weight) and were not included in the final models. In regression diagnostics observations with a studentized residual greater than 3 or less than −3 were considered influential. One observation had a residual value of 3.4 and was not included in final models.

We also assessed the association between type of pesticide and birth weight. For this analysis, pesticides were grouped by type as mentioned in the pesticide analysis methods (insecticides [OPs and/or carbamates], herbicides, fungicides, and repellant). Within each pesticide type, the number of pesticides detected in cord blood was our primary exposure variable.

To assess the relationship between individual pesticides and birth weight, all pesticides detected in at least 4 subjects were analyzed separately as both dichotomous (detect/non-detect) and as 3-level ordinal variables in linear regression models to assess trend. To do so, newborns were categorized into 3 exposure groups based on cord blood pesticide concentration levels. The lowest exposure group included subjects with samples below the LOD (non-detect group) and the remaining subjects were ranked into two (medium and high) exposure groups of equal size. The creation of ordinal variables was only done for pesticides with detection rates greater than or equal to 18%, which assured that there were at least 10 subjects in the medium and high groups.

3. Results

3.1. Study participants

Maternal and newborn characteristics are presented in Table 1. There were no study participants who reported smoking or alcohol use while pregnant. Maternal age ranged from 21 to 39 years with mean (SD) of 27.3 (3.6) years. The mean (SD) maternal BMI at early pregnancy was 21.4 (3.2) and most (72.8%) had a healthy BMI (18.5 to <25 kg/m^2). All births were full term (>37 weeks), the mean (SD) gestational length was 39.4 (0.93) weeks. According to common practice in China, most deliveries were by cesarean section (77.2%). The mean (SD) newborn birth weight was 3405 (418.9) grams with a range from 2600 to 4500 g. Of the 20 pesticides measured, the mean (SD) number detected in cord blood per study participant was 4.6 (1.9) with a maximum of 10. Almost all study participants (98.3%) had at least one pesticide detected in cord blood.

3.2. Pesticide exposure

Table 2 summarizes the concentrations for 20 pesticides measured in cord blood. Two pesticides were detected in >45% of samples. These were the repellant, diethyltoluamide (DEET) (73%) and the fungicide, vinclozolin (48%). The geometric mean concentrations were 0.12 ng/ml (Geometric SD 4.2) for DEET and 0.18 ng/ml (GSD 3.3) for vinclozolin. Metolachlor and trifluralin were only detected in two subjects each, and metalaxyl and parathion-ethyl were detected in 3.

Table 1
Characteristics of study population (n = 113).

Characteristics	Values[a]
Maternal age (years)	27.3 ± 3.6
Body mass index (kg/m^2)	
Early pregnancy	21.4 ± 3.2
Late pregnancy	27.0 ± 3.0
Delivery	27.5 ± 3.0
Height (cm)	158.7 ± 4.7
Weight at early pregnancy (kg)	53.9 ± 8.5
High blood pressure during pregnancy or delivery (%)	10.6
Maternal hemoglobin (g/dl)	10.8 ± 1.7
Early pregnancy	10.9 ± 1.4
Late pregnancy	12.4 ± 1.2
Delivery	11.7 ± 0.9
Delivery type (% cesarean section)	77.2
Gestation age of newborn (weeks)	39.4 ± 0.9
Newborn sex (% female)	53.6
Newborn birth weight (g)	3405 ± 418
Pesticides detected in cord blood (of 20 possible)	4.6 ± 2.0

[a] Values represent mean ± SD or percent.

Table 2
Distribution of pesticide concentrations in umbilical cord blood (ng/ml) at delivery, Zhejiang Province, China 2009 (n = 116).

Pesticide			Selected percentiles				
	LOD[a]	No. > LOD (%)	50th	75th	90th	95th	Max
Organophosphates							
Chlorpyrifos	0.05	27 (23.3)	ND	ND	0.17	0.17	0.26
Diazinon	0.05	17 (14.7)	ND	ND	0.27	0.62	3.45
Fonofos	0.05	19 (16.4)	ND	ND	0.30	0.73	1.29
Malathion	0.50	30 (25.9)	ND	1.96	3.13	4.79	9.49
Parathion-ethyl	0.05	3 (2.6)	ND	ND	ND	ND	14.1
Parathion-methyl	0.05	33 (28.5)	ND	0.32	1.43	2.07	2.69
Profenofos	0.50	29 (25.0)	ND	ND	0.68	0.72	0.96
Terbufos	0.05	36 (31.0)	ND	0.16	0.27	0.37	0.49
Carbamates							
Carbofuranphenol	0.05	40 (34.5)	ND	14.4	24.8	36.0	78.7
Propoxur	0.05	12 (10.3)	ND	ND	0.19	0.57	1.95
Herbicides							
Acetochlor	0.50	30 (25.9)	ND	0.59	0.96	2.32	8.85
Alachlor	0.05	25 (21.6)	ND	ND	2.21	3.45	5.26
Atrazine	0.25	42 (36.2)	ND	0.64	1.47	1.68	2.99
Linuron	0.50	13 (11.2)	ND	ND	1.10	1.31	2.62
Metolachlor	0.05	2 (1.7)	ND	ND	ND	ND	2.83
Trifluralin	0.05	2 (1.7)	ND	ND	ND	ND	0.059
Fungicides							
Dicloran	0.05	33 (28.5)	ND	0.56	4.73	7.87	13.9
Metalaxyl	0.05	3 (2.6)	ND	ND	ND	ND	18.6
Vinclozolin	0.05	57 (49.1)	ND	0.49	0.94	1.31	1.85
Repellant							
Diethyltoluamide	0.05	85 (73.3)	0.22	0.46	0.77	1.07	2.71

ND = non-detect.
[a] LOD = limit of detection.

3.3. Association between pesticide exposure and birth weight

The crude relationship between the number of pesticides detected in cord blood and decreasing birth weight is represented in Fig. 1. The spearman correlation coefficient for this association was −0.16 ($p = 0.09$). Table 3 shows the results from multivariable linear regression. We found that, on average, birth weight decreased by 37.1 g (95% CI, −72.5 to −1.8) for each detected pesticide. Birth weight also increased significantly with increasing gestational age (weeks) and early pregnancy BMI (kg/m^2). Maternal age (years) and hemoglobin levels (g/dl) at delivery were not statistically significant

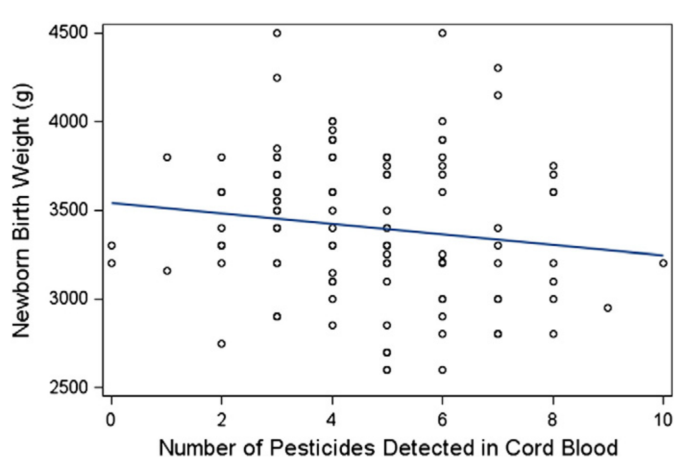

Fig. 1. Relationship between number of pesticides detected in umbilical cord blood and newborn's birth weight (g) among women in Zhejiang Province, China 2009 (n = 114). Spearman correlation coefficient (r) = −0.16, p-value = 0.09.

Table 3
Effect estimates (95% CI) for change in newborn birth weight (grams) associated with cord blood pesticides and covariates in a multivariate linear regression model (n = 112).[a]

	β (95% CI)[b]	p-Value
Constant/intercept	−3996 (−7410 to −579)	0.02
Number of detected pesticides	−37.1 (−72.5 to −1.81)	0.04
Gestational age (weeks)	174 (95.1 to 253)	<0.0001
Maternal age (years)	15.9 (−3.57 to 35.4)	0.11
Maternal body mass index (kg/m^2)	28.3 (6.60 to 50.0)	0.01
Maternal hemoglobin at delivery (g/dl)	−30.4 (−80.3 to 19.5)	0.23

[a] N = 112 due to missing data on all covariates (n = 2), missing data on maternal hemoglobin at delivery (n = 1), removal of outlier (n = 1).
[b] 95% CI = confidence interval.

predictors in the model, but were retained because they increased the model fit and were correlated with birth weight.

To determine whether the relationship with birth weight differed by pesticide type, we formed a variable for each pesticide type representing the number of pesticides of that type detected in cord blood. Table 4 provides results of the multivariable linear regression models. Birth weight averaged 116 g less (95% CI, −212 to −19.2) for detection of each additional fungicide. When OPs and carbamates were grouped together as insecticides, there was a suggestive negative association between birth weight and number of insecticides detected (p = 0.10). There were no statistically significant associations between birth weight and organophosphates, carbamates, or herbicides.

In analyses of individual pesticides modeled as dichotomous (detect/non-detect) variables (data not shown), vinclozolin (β = −174; 95% CI, −312 to −36.3; p = 0.01), a fungicide, and acetochlor (β = −165 95% CI, −325 to −5.7; p = 0.04), an herbicide, were significantly and inversely associated with birth weight. Individual pesticides were further analyzed as 3-level ordinal variables in multivariate models to explore evidence of dose-related trends, but no significant trends in relation to birth weight were observed.

4. Discussion

We found that a range of non-persistent pesticides could be detected in umbilical cord blood from a rural region in China, with considerable inter-individual variation. The pesticides detected with the greatest frequency were diethyltoluamide (DEET), a commonly used repellant, and vinclozolin, a fungicide. Most pesticides had detection rates less than 35%. Due to the exploratory nature of our study, the sensitivity of the analytical method was not as great as has been reported in three recent U.S. studies that have also measured an array of pesticides in cord blood (Barr et al., 2010; Neta et al., 2010; Whyatt et al., 2003; Yan et al. 2009). This resulted in higher limits of detection in our study, and, in some instances, lower sample detection rates in comparison to those studies. However, when comparing the high end of the exposure distributions across studies, the difference is striking. Among the pesticides that were measured in both the present study and in at least one of the U.S. studies (chlorpyrifos, diazinon, fonofos, malathion, terbufos, carbofuranphenol, propoxur, acetochlor, alachlor, atrazine, metolachlor, dicloran, metalaxyl, and DEET), the 90th percentile and maximum concentrations measured in the Chinese samples were several orders of magnitude greater than the highest levels reported in the U.S. studies. This suggests that at least a portion of pregnant women and fetuses in the study area experienced high exposures to pesticides compared to the levels found in the U.S. For example, for chlorpyrifos, the 90th percentile concentration in the present study was 0.17 ng/ml compared to the maximum values of 0.002, 0.01, and 0.07 ng/ml reported in studies of cord serum or plasma from deliveries taking place in New Jersey (Yan et al., 2009), Baltimore (Neta et al., 2010), and New York (Whyatt et al., 2003), respectively. Metolachlor, which was associated with reduced birth weight in the New Jersey study, was measured at a maximum concentration of 0.002 ng/ml in that study compared with 2.8 ng/ml in the present study (Barr et al., 2010; Yan et al., 2009). However, due to the higher limit of detection for metolachlor in this study (and <2% detection frequency), we could not adequately assess the relationship with birth weight.

Our results support the growing call for the consideration of mixtures in studies of health outcomes in relation to environmental exposures, rather than focusing only on individual agents. We found that the number of pesticides in cord blood was inversely associated with newborn birth weight. When stratified by pesticide type, the only statistically significant association was for fungicides. Among specific pesticides, detection of vinclozolin or acetochlor was inversely associated with birth weight. However, these relationships involving types or single pesticides do not explain the significant relationship observed with the exposure variable that accounted for all pesticides detected in cord blood. Thus, when assessing the relationship between pesticide exposure and fetal growth, it may be important to consider an aggregate measure of pesticide burden in addition to exposure to individual chemicals. In support of this, animal studies have recently reported that mixtures may have a greater effect than individual compounds alone. In one study of rats, no effects were seen for individual endocrine disrupting pesticides (EDPs) for particular endpoints like gestational age and mortality, while exposure to a mixture of the EDPs induced health effects on the same endpoints (Jacobsen et al., 2012). Another study showed that mixtures of compounds that act by disparate mechanisms of toxicity to disrupt complex signaling pathways result in cumulative effects, regardless of the mode of action of the individual components (Rider et al., 2010).

To our knowledge, this is the first study to investigate the association between infant birth weight and fetal exposure to multiple fungicides. The absence of a relationship between dicloran and birth weight in the present study is consistent with the New Jersey study (Barr et al., 2010). As far as we are aware birth weight in relation to metalaxyl and vinclozolin exposure has not been explored in other studies. Experimental studies of fungicides and fetal growth are also limited, and the potential mechanisms involved are unclear. However, animal studies have shown that two primary metabolites of vinclozolin are antiandrogenic (Kelce and Wilson, 1997; Kelce et al., 1994) and that maternal vinclozolin exposure is associated with lower weight offspring (Wolf et al., 2004). More research is needed on the potential adverse effects related to fungicide exposure during pregnancy. The human health effects from environmental exposure to acetochlor, the other individual pesticide associated with birth

Table 4
Effect estimates (95% CI) for change in newborn birth weight (grams) associated with the number of pesticides detected in cord blood, by pesticide type, in separate multivariate models (n = 112).[a,b,c]

Pesticide type	N	β (95% CI)	p-Value
Insecticides	10	−44.1 (−96.6 to 8.48)	0.10
Organophosphates	8	6.59 (−210 to 222)	0.95
Carbamates	2	−115 (−134 to 44.7)	0.32
Herbicides	6	−44.9 (−236 to 74.4)	0.31
Fungicides	3	−116 (−212 to −19.2)	0.02
Repellant	1	110 (−49.2 to 269)	0.17

[a] N = 112 due to missing data on all covariates (n = 2), missing data on maternal hemoglobin at delivery (n = 1), removal of outlier (n = 1).
[b] Within each pesticide category, the number of pesticides detected in cord blood was summed and used as the primary exposure variable.
[c] Models were adjusted for gestational age (weeks), maternal age (years), maternal BMI at early pregnancy (kg/m^2), and maternal Hb at delivery (g/dl).

weight in the present study, are unknown (CDC, 2009). Although chronic exposure in animal studies has not shown developmental or fetal toxicity, high doses of acetochlor have produced other negative health outcomes such as testicular atrophy or neurologic movement abnormalities (CDC, 2009).

In the present study, insecticides were further separated into two classes, OPs and carbamates. Although we found a suggestive relationship between birth weight and the number of insecticides detected in cord blood ($p = 0.10$), we did not observe differences in birth weight for the number of OPs ($p = 0.95$) or carbamates ($p = 0.32$), when analyzed separately. This may be consistent with two previous studies, one in Shanghai, China and one in an agricultural population in California, which did not find associations between birth weight and fetal pesticide exposure assessed by urinary dialkyl phosphate metabolites or other pesticide-specific OP metabolites (Eskenazi et al., 2004; Wang et al., 2011). Likewise, the lack of association between DEET and birth weight is consistent with results from the New Jersey study (Barr et al., 2010).

Past studies have found associations between certain pesticide exposures and decreased gestational duration (Eskenazi et al., 2004), increased risk of preterm delivery (Villanueva et al., 2005), risk of fetal growth restriction (Chevrier et al., 2011), and increased risk of intrauterine-growth restriction (Levario-Carrillo, 2004; Munger et al., 1997). Discrepancies in findings between our study and those which included both pre-term and full-term infants may be explained by differences in populations, exposure levels, and study exclusion criteria. Participation in our pilot study was restricted to healthy, uncomplicated, full term (>37 weeks gestation) singleton births, and no low birth weight infants were included in the study population. Thus, our exclusion criteria may have resulted in a decreased ability to detect a difference in infant birth weight related to pesticide exposure. Even though we found that pesticide exposure was associated with newborn birth weight, it is possible that the association could have been stronger if gestational age was not limited to deliveries greater than 37 weeks.

In the present study, birth weight was inversely related with maternal Hb measured at delivery, and we included it as a covariate in multivariate models. Previous studies have shown that a major factor influencing hemoglobin concentration in pregnancy is expansion of plasma volume (Steer, 2000) and that poor plasma volume expansion is associated with impaired fetal growth (Duffus et al., 1971; Gibson, 1973; Rasmussen, 2001). Our findings are consistent with those of Steer (2000) who observed that mean birth weight was higher with lower 28-week Hb. They also found that the greatest proportion of babies with low birth weight (<2500 g) occurred in association with the highest Hb concentrations (>126 g/l) (Steer, 2000).

This study had a number of strengths, such as the use of specific pesticide biomarkers in cord blood to assess individual level exposure, the analysis of a large number and variety of types of pesticides, some of which have not previously been assessed in epidemiological studies, and methods to explore impacts of pesticide mixtures. The present study also had a number of limitations. Our methods for pesticide analysis were not as sensitive as previous U.S. studies, which limited our ability to assess the relationship between exposure to individual pesticides or mixtures and birth weight more quantitatively. Also, because exposure to these non-persistent pesticides was measured at delivery, we were limited in our ability to characterize pesticide exposure throughout pregnancy and at potentially critical windows of development. One alternative that might address these two limitations is measuring pesticide metabolites in maternal urine samples collected throughout pregnancy. However, urinary metabolites are not necessarily specific to one pesticide, and measurement of parent pesticides in cord blood may be a more accurate estimation of the dose absorbed by the fetus (Lu et al., 2005). Other limitations include an inability to evaluate pesticide exposure by season, since all births occurred within a 3-month span of time, and limited statistical power for detecting subtle relationships. Additionally, we only evaluated a selection of pesticides; the number of pesticides actually present in cord blood is likely incomplete. Finally, fetal growth was assessed by infant birth weight but other metrics like birth length, abdominal and head circumference, or ultrasound scan data could also be used (Barr et al., 2010; Chevrier et al., 2011; Eskenazi et al., 2004; Wang et al., 2011; Whyatt et al., 2004).

In conclusion, our results from this hypothesis-generating investigation show the potential for high pesticide exposure in the study region, and suggest that fetal exposure to a mixture of multiple pesticides may be inversely related to birth weight. We also observed associations between fungicide exposure and decreased birth weight. Exposure to multiple pesticides may be a greater health concern to pregnant women and developing fetuses than exposure to any one pesticide, but additional research is needed to demonstrate causality and elucidate mechanistic actions of pesticide mixtures. For this Zhejiang study population, future plans include expanding our sample size, analyzing blood and urine samples for a larger number of pesticides (e.g. pyrethroids) using more sensitive methods, and follow-up of the infants to assess neurodevelopment.

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