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Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey

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ABSTRACT

We evaluated *in utero* exposures to pesticides by measuring maternal and cord serum biomarkers in a New Jersey cohort of pregnant women and the birth outcomes of their neonates. The study was based on 150 women that underwent an elective cesarean delivery at term in a hospital in central New Jersey. We evaluated the following pesticide compounds in both maternal and umbilical cord sera: chlorpyrifos, diazinon, carbofuran, chlorothalonil, dacthal, metolachlor, trifluralin and diethyl-m-toluamide (DEET). Of these compounds, chlorpyrifos, carbofuran, chlorothalonil, trifluralin, metolachlor and DEET were the pesticides most frequently detected in the serum samples. We found high (≥75th percentile) metolachlor concentrations in cord blood that were related to birth weight (3605 g in upper quartile vs 3399 g; $p = 0.05$). We also observed an increase in abdominal circumference with increasing cord dichloran concentrations ($p = 0.031$). These observations suggest that *in utero* exposures to certain pesticides may alter birth outcomes.

1. Introduction

Approximately 912 million pounds of conventional pesticides are used annually in the United States (Kiely et al., 2004). Because of their widespread use, virtually everyone will come in contact with pesticide residues regularly whether from dietary, non-dietary ingestion, residential, or occupational pathways.

Pesticide exposures have been widely studied in various populations over the past several decades (Adgate et al., 2001; Swan et al., 2003; Berkowitz et al., 2003; Whyatt et al., 2003; Hore et al., 2005; Bradman et al., 2005; Barr et al., 2005; Arcury et al., 2005; Curwin et al., 2005); however, more studies are beginning to focus on pregnant women and their fetuses because of the vulnerability of this subpopulation to harmful effects from exposure to environmental contaminants (Bradman et al., 2003; Perera et al., 2003; Whyatt et al., 2004; Eskenazi et al., 2004; Berkowitz et al., 2004; Bradman et al., 2005; Young et al., 2005). Human fetuses are particularly sensitive to pesticide exposure because the brain is developing very rapidly and the fetus' ability to detoxify contaminants is not fully developed (Eskenazi et al., 2008). Toxicological studies have shown organophosphorus OP insecticides, along with other insecticides such as p,p′-DDT, hexachlorobenzene and chlordane, can cross the placental and blood–brain barriers (Whyatt et al., 2003; Barr et al., 2007). In addition, these studies using biomarkers of exposure have found that *in utero* exposure to OP insecticides may impact birth length and weight (Perera et al., 2003; Whyatt et al., 2004), length of gestation (Eskenazi et al., 2004), and in some susceptible subpopulations, other measures of growth such as head circumference (Berkowitz et al., 2004). Perera et al. (2003) reported an inverse relationship between cord blood chlorpyrifos concentrations and birth length and birth weight in an African American and Dominican population in Northern Manhattan. Further studies of this cohort found a stronger effect when cumulative cord blood chlorpyrifos and diazinon were evaluated in relation to birth length and weight; however, diazinon concentrations alone were not significantly associated with the birth outcomes (Whyatt et al., 2004). A marginally significant

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inverse association was also observed between cord blood concentrations of a metabolite of the carbamate insecticide propoxur and birth length (Whyatt et al., 2004). Follow up evaluations in the children born during this study showed a relationship between cord chlorpyrifos and developmental delays and early onset of attention deficit disorder (Rauh et al., 2006). Although other studies have focused on pesticide exposures and birth outcomes and development (Eskenazi et al., 2004; Berkowitz et al., 2004; Young et al., 2005), their exposure measurements did not involve measurement of the parent pesticides in either maternal or cord blood (Needham 2005). To date, the observations of the Northern Manhattan study remain unreplicated.

We previously reported the prevalence of pesticides or their metabolites in a variety of maternal–fetal matrices collected from pregnant women in New Jersey (Yan et al., 2009). Certain pesticides including the acetylcholinesterase inhibitors chlorpyrifos and carbofuran were frequently detected in maternal and umbilical cord sera. In this paper, we explore the relation between maternal and cord pesticide concentrations and birth outcomes including birth weight, birth length, and abdominal and head circumferences.

2. Methods

This was a prospective cohort analysis of pesticide exposures in maternal and fetal compartments (Yan et al., 2009). All subjects provided informed consent prior to participation. The protocol was approved by the Institutional Review Boards at Rutgers University, UMDNJ-Robert Wood Johnson Medical School, NJ, and Saint Peter's University Hospital, NJ with a collaborative agreement with the Centers for Disease Control and Prevention (CDC).

We recruited 150 mothers and their newborns at Saint Peter's University Hospital in New Brunswick. Subjects were recruited from July 2003 to May 2004 and represent a convenience sample of non-consecutive cases based on availability of research personnel for recruitment. Subjects eligible for recruitment included women with singleton pregnancies and non-anomalous fetus scheduled for an elective cesarean birth at term (≥37 weeks) and if the hemoglobin level was ≥8 mg/dl. Women were excluded if there was evidence for labor or rupture of membranes at the time of operative delivery and if they were taking medications that could potentially interfere with metabolism of environmental chemicals. Subjects were identified prior to admission from a scheduling list and the potential subjects' physicians were asked for permission to offer participation. Each potentially eligible subject was approached to verify eligibility and to obtain informed consent.

Following enrollment and prior to placement of intravenous and bladder catheters in the pre-operative holding area, maternal blood samples (10 ml) were obtained. Alternatively, maternal blood was obtained from extra specimens available from pre-operative maternal blood testing stored in undisturbed Vacutainer tubes. Catheters were then placed and intravenous fluids (lactated Ringer's solution) were started.

After maternal samples were collected, the patient was taken to the operating room where anesthesia was administered. The operative procedure was carried out based on the technique preference of the attending physician. After delivery of the newborn, the umbilical cord was clamped, and 30–60 ml of cord blood was aspirated directly from the umbilical vein after cleaning the cord. All umbilical vein samples were obtained within 15 min of delivery. An investigator was present at each delivery to assure correct sampling procedures were followed. Finally, maternal pregnancy characteristics and neonatal outcome data were also recorded from the medical record prior to hospital discharge. All consenting and data collection were performed by one of 2 individuals, an investigator (SL) or a research coordinator.

2.1. Sample collection and processing

Prior to initiation of the study, representative samples of all equipment used for either collection or storage of samples were examined by the Centers for Disease Control and Prevention to verify the absence of chemicals that could lead to specimen contamination.

Maternal blood (30 ml) was collected from a direct blood draw with a 21 gauge needle or at the time of the intravenous catheter placement (prior to fluid instillation) using a Vacutainer barrel and 3 Vacutainer serum separator tubes. Samples were rested upright for at least 30 min at room temperature to allow clot formation. The specimens were centrifuged at 3000 rpm for 15 min until the serum layer appeared clear. Up to 3 ml aliquots of the serum were transferred using glass pipettes into cryovials.

Umbilical vein blood specimens were collected using 21 gauge needles with 30 ml syringes or with a multiple tube adaptor for 10 ml Vacutainer tubes containing no preservatives. Syringe collected samples were transferred into similar Vacutainer tubes. Samples were processed using the same procedures as for maternal blood samples.

After processing, all samples were stored at −70 °C until transferred to the Centers for Disease Control and Prevention for analysis. Specimens were batched and sent overnight express packed in dry ice to avoid the potential for freeze-thaw effects. They were maintained at −70 °C until analyzed.

A questionnaire was distributed to the pregnant women to collect information on pest control use and frequency of use in the pre-operative holding area. In addition, maternal age, gravidity, race, maternal prepregnancy BMI, infant sex and gestational age were also collected.

Serum samples were analyzed using gas chromatography–high resolution mass spectrometry to measure synthetic pyrethroid, carbamate and OP insecticides and other pesticides including herbicides, repellents, and fungicides (Table 1). The laboratory method involved a solid-phase extraction for analyte isolation with analysis using high resolution mass spectrometry with isotope dilution calibration. The method was previously developed and validated at the CDC (Barr et al., 2002). Positive and negative control samples were analyzed concurrently with participant samples to ensure quality laboratory measurements.

2.2. Statistical methods

The statistical analysis consisted of assessments of distributional properties of each metabolite, including means, medians, distribution percentiles, ranges, and correlation analyses among analytes from the various matrices. In all analyses, concentration values below the LOD were assigned a concentration equal to LOD/$\sqrt{2}$ for the calculations (Hornung and Reed 1990). We performed 3 sets of analyses. In the first, we examined the (Pearson) correlation in each pesticide compound between maternal and cord sera. In the second set of analyses, we compared differences in the mean pesticide exposure between the maternal and cord sera, and applied the *t*-test. Finally, we examined the association between pesticide exposures and birth outcomes (birth weight, head circumference, abdominal circumference and birth length). For this latter analysis, we created indicators for pesticide compounds as exposures ≥75th or <75th percentile, as well as exposures ≥90th or <90th percentile. These percentiles were derived from the total cohort, but specific to exposures in the maternal and umbilical cord sera. Since results for pesticide exposures ≥90th

Table 1
Pesticides measured in maternal and umbilical cord sera in a New Jersey cohort.

Pesticide	Class
Chlorpyrifos	Organophosphorus insecticide
Diazinon	Organophosphorus insecticide
Carbofuran	Carbamate insecticide
Chlorothalonil	Fungicide
Dacthal	Terephthalic acid herbicide
Metolachlor	Chloroacetanilide herbicide
Trifluralin	Dinitroaniline herbicide
Diethyl-m-toluamide	Repellent