

## Biomarkers in assessing residential insecticide exposures during pregnancy and effects on fetal growth

R.M. Whyatt<sup>a,\*</sup>, D. Camann<sup>c</sup>, F.P. Perera<sup>a</sup>, V.A. Rauh<sup>a</sup>, D Tang<sup>a</sup>, P.L. Kinney<sup>a</sup>, R Garfinkel<sup>a</sup>,  
H. Andrews<sup>a</sup>, L. Hoepner<sup>a</sup>, D.B. Barr<sup>b</sup>

<sup>a</sup>Columbia Center for Children's Environmental Health, Department of Environmental Health Sciences, Joseph L. Mailman School of Public Health, Columbia University, 60 Haven Avenue, B-1, New York, NY 10032, USA

<sup>b</sup>National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

<sup>c</sup>Southwest Research Institute, San Antonio, TX 78228-0510, USA

Received 1 July 2004; accepted 17 November 2004

Available online 25 April 2005

### Abstract

The Columbia Center for Children's Environmental Health is using a combination of environmental and biologic measures to evaluate the effects of prenatal insecticide exposures among urban minorities in New York City. Of the 571 women enrolled, 85% report using some form of pest control during pregnancy and 46% report using exterminators, can sprays, and/or pest bombs. Chlorpyrifos, diazinon, and propoxur were detected in 99.7–100% of 48-h personal air samples collected from the mothers during pregnancy ( $n = 394$ ) and in 39–70% of blood samples collected from the mothers ( $n = 326$ ) and/or newborns ( $n = 341$ ) at delivery. Maternal and newborn blood levels are similar and highly correlated ( $r = 0.4–0.8$ ,  $P < 0.001$ ). Levels of insecticides in blood samples and/or personal air samples decreased significantly following the 2000–2001 U.S. Environmental Protection Agency's regulatory actions to phase out residential use of chlorpyrifos and diazinon. Among infants born prior to 1/1/01, birth weight decreased by 67.3 g (95% confidence interval (CI)  $-116.6$  to  $-17.8$ ,  $P = 0.008$ ) and birth length decreased by 0.43 centimeters (95% CI,  $-0.73$  to  $-0.14$ ,  $P = 0.004$ ) for each unit increase in log-transformed cord plasma chlorpyrifos levels. Combined measures of (ln)cord plasma chlorpyrifos and diazinon (adjusted for relative potency) were also inversely associated with birth weight and length ( $P \leq 0.007$ ). Birth weight averaged 215.1 g less (95% CI  $-384.7$  to  $-45.5$ ) among those with the highest exposures compared to those without detectable levels. No association was seen between birth weight and length and cord plasma chlorpyrifos or diazinon among newborns born after 1/1/01 ( $P > 0.8$ ). Results support recent regulatory action to phase out residential uses of these insecticides.

© 2005 Elsevier Inc. All rights reserved.

**Keywords:** Insecticides; Prenatal; Residential; Minority; Urban; Women; Birth weight; Birth length

### Introduction

Residential pesticide use is widespread in the United States, with approximately 80–90% of American households using pesticides (Landrigan et al., 1999). Insecticide

use appears to be particularly high among minority populations residing in New York City (Berkowitz et al., 2003; Surgan et al., 2002; Thier et al., 1998; Whyatt et al., 2002, 2003). A 1997 study of pesticide use in New York State found that the heaviest application (in gallons and pounds) of legally registered pesticides by licensed applicators occurred not in the agricultural counties but in the boroughs of Manhattan and Brooklyn in New York City (Thier et al., 1998). Chlorpyrifos was one of the insecticides most heavily applied in New York City including by pest control operators for the New York City Housing Authority (Landrigan et al., 1999; Thier et al., 1998). Other

*Abbreviations:* LPM, liters per minute; PUF, polyurethane foam; QC, quality control; EPA, U.S. Environmental Protection Agency; CDC, Centers for Disease Control and Prevention; cm, centimeter; kg, kilogram; pg/g, picogram/gram; CI, confidence interval.

\* Corresponding author. Fax: +1 646 459 9610.

*E-mail address:* [rmw5@columbia.edu](mailto:rmw5@columbia.edu) (R.M. Whyatt).

insecticides commonly used in the home included the organophosphate diazinon and the carbamate propoxur and bendiocarb (Landrigan et al., 1999; Whitmore et al., 1994; Whyatt et al., 2002, 2003). However, use of chlorpyrifos and diazinon appears to have decreased substantially as a result of the 2000–2001 regulatory actions by the U.S. Environmental Protection Agency (EPA) to phase out their residential uses (Carlton et al., 2004; U.S. Environmental Protection Agency, 2000a, 2001a; Whyatt et al., 2003, 2004). While human data on effects of residential pesticide exposure during pregnancy are limited, experimental data in laboratory animals suggest that exposures to certain organophosphates (including chlorpyrifos and diazinon) during pregnancy or early life can impair fetal growth and neurocognitive development in the offspring (reviewed in Eskenazi et al., 1999). Reduction in birth weight was also seen experimentally in a two-generation reproductive study of propoxur in rats, but only at high exposure levels (U.S. Environmental Protection Agency, 1997). However, contrary to results presented here, two recent epidemiologic studies failed to find associations between prenatal chlorpyrifos exposure and either birth weight or birth length (Berkowitz et al., 2004; Eskenazi et al., 2004). Both studies used levels of the chemical-specific metabolite of chlorpyrifos (3,5,6-trichloro-2-pyridinol (TCPY)) in urine samples collected from the mother during pregnancy as the dosimeter of prenatal exposure. The Columbia Center for Children's Environmental Health is conducting a large-scale prospective cohort study using a combination of environmental and biologic monitoring to evaluate the effects of prenatal insecticide exposure on fetal growth and infant neurocognitive development. Enrollment began in 1998 and will be completed by 2006 with a total of 730 mother/newborn pairs enrolled; 571 pairs have been enrolled to date. Prior results on the research have been published (Perera et al., 2003; Whyatt et al., 2002, 2003, 2004). The current manuscript updates these research findings to include a larger sample size and summarizes research results to date.

## Methods

**Study subjects.** Study protocols, including eligibility requirements as well as the comparability between those who agreed to participate compared to those who refused, have been described in detail previously (Perera et al., 2003; Whyatt et al., 2002, 2003). Bilingual research workers attend the prenatal clinics at Harlem and New York Presbyterian hospitals to explain the study and determine eligibility if a woman is interested in participating. The study is restricted to women 18–35 years old who self-identified as African American or Dominican and had resided in Northern Manhattan (Central Harlem or Washington Heights/Inwood) or the South Bronx for  $\geq 1$  year prior to pregnancy. Women are excluded if they smoked

cigarettes or used other tobacco products during pregnancy, used illicit drugs, had diabetes, hypertension, or known HIV, or had their first prenatal visit after the 20th week of pregnancy. The study was approved by the Institutional Review Board of Columbia University and informed consent was obtained from all study subjects. The 571 subjects included in the current report were enrolled between January 1998 and January 2004.

**Questionnaire data.** A 45-min questionnaire, administered to each woman in her home by a trained bilingual interviewer during the 3rd trimester of pregnancy, collects information on demographics, home characteristics, lifetime residential history, history of active and passive smoking, occupational history, maternal education and income level, alcohol and drug use during pregnancy, and history of residential pesticide use. Information about pesticide use includes whether or not any pest control measures were used by an exterminator or by others (the woman herself, other household members, or the building superintendent) during pregnancy and if so what types of measures were used (Perera et al., 2003; Whyatt et al., 2002, 2003, 2004).

**Prenatal personal ambient air samples.** As described in detail previously (Perera et al., 2003; Whyatt et al., 2002, 2003, 2004), women in the cohort are asked to wear a small backpack during the 3rd trimester of pregnancy containing a personal ambient air monitor during the daytime hours for two consecutive days and to place the monitor near the bed at night. The personal air sampling pumps operated continuously at 4 liters per minute (LPM) over this period, collecting particles of  $\leq 2.5$   $\mu\text{m}$  in diameter on a precleaned quartz microfiber filter and collecting semivolatile vapors and aerosols on a polyurethane foam (PUF) cartridge back-up. Analyses for pesticide levels are carried out at Southwest Research Institute as described (Perera et al., 2003; Whyatt et al., 2002, 2003, 2004). The pesticides being measured are the organophosphates chlorpyrifos, diazinon, malathion, and methyl parathion, the carbamates propoxur, bendiocarb, carbofuran, and carbaryl, and the pyrethroids *cis*- and *trans*-permethrin. To date, air samples have been analyzed for 394 women in the cohort. The personal air monitorings for these women took place between 1998 and 2002.

**Blood samples.** A sample of umbilical cord blood is collected as close to delivery as possible by syringing the blood into a heparinized syringe to avoid clotting. A sample of maternal blood is obtained within 2 days postpartum into heparinized vacutainer tubes by the hospital staff. Blood processing and analysis have been described in detail previously (Perera et al., 2003; Whyatt et al., 2002, 2003, 2004). Analyses are undertaken at the Centers for Disease Control and Prevention (Whyatt et al., 2003). Methods for the laboratory assay, including quality control, reproducibility, and limits of detection, have been published (Barr et al., 2002). Twenty-nine pesticides are being measured

in blood as described (Whyatt et al., 2003) and include the same insecticides that are being measured in air. To date, blood samples have been analyzed for 326 mothers and 341 newborns. The deliveries for these subjects also took place between 1998 and 2002.

*Measures of fetal growth.* As described previously, information is abstracted by research workers from the mothers' and infants' medical records following delivery, including date of delivery, gestational age at birth, infant sex, birth weight, length, head circumference, infant malformations, Apgar scores, maternal height, pre-pregnancy weight, total weight gain, complications of pregnancy and delivery, and medications used during pregnancy.

*Statistical analysis.* Pesticide levels were log-transformed prior to statistical analyses to normalize positively skewed distributions. Values below the limit of detection (LOD) were assigned a value of  $0.5 \times \text{LOD}$ . Spearman's rank correlation coefficients were used to examine associations between pesticide levels in paired maternal and newborn blood samples. Spearman's rank correlation coefficients were also used to examine correlations between pesticide levels in air and blood. Analysis of variance (ANOVA) was used to test whether pesticide levels varied significantly among the following groups: (1) women not using any pest control methods; (2) women using non-spray methods only (sticky traps, bait traps, boric acid and gels); (3) women using can sprays and pest bombs (with or without non-spray methods); and (4) women using exterminators (with or without the other methods). If levels differed significantly among groups, the least significant difference test was used to determine which groups varied significantly. Analysis of variance (ANOVA) was also used to test whether pesticide levels varied significantly by year of the personal monitoring or birth. These analyses were restricted to infants born between 1999 and 2002, since only one infant was born before 1999. Multiple regression analyses were conducted to measure the contribution of antenatal insecticide exposure to birth outcomes. To eliminate possible effects related to active smoking, subjects were excluded if the mother reported any smoking during pregnancy or if plasma cotinine levels in either maternal or cord blood samples collected at delivery exceeded 15 ng/ml (Perera et al., 2003). As described previously (Whyatt et al., 2004), covariates included in the final models were race/ethnicity, gestational age, parity, maternal pre-pregnancy weight and net weight gain during pregnancy (maternal pregnancy weight gain minus the newborn's weight), maternal self-reported environmental tobacco smoke in the home, gender of the newborn, and season of delivery. Models for head circumference also included whether or not the delivery was by cesarean section. Most of the deliveries were full-term since women are not fully enrolled until environmental measures had been collected during the 3rd trimester and blood samples

(from the mother and/or newborn) had been obtained at delivery. However, restricted analyses were undertaken after removing the 12 infants born <37 weeks gestation; main effects remained unchanged from those reported here for the whole cohort. To evaluate whether stratified race/ethnicity-specific analyses should be undertaken, interaction effects of pesticide levels and race/ethnicity on birth outcomes were assessed. None of the interaction terms was significant and analyses are therefore presented for the whole cohort controlling for race/ethnicity. Since insecticide levels in maternal and umbilical cord plasma samples were highly correlated (Perera et al., 2003), in cases where the umbilical cord blood sample was not collected, the mother's values were used based on the formulas derived from regression analyses as described (Whyatt et al., 2004). To evaluate the combined effects of chlorpyrifos and diazinon on birth outcomes, a methodology developed by the U.S. EPA for conducting cumulative risk assessment for organophosphates was used (U.S. Environmental Protection Agency, 2001b). Briefly, diazinon levels were put into chlorpyrifos equivalents based on the ratio of the chlorpyrifos and diazinon relative potency factors calculated by the EPA (U.S. Environmental Protection Agency, 2002). Diazinon levels in chlorpyrifos equivalents were summed with chlorpyrifos levels using the EPA methodology. The log-transformed values were initially entered into parallel models as continuous variables. When the models indicated a significant association between a pesticide and one or more of the birth outcomes in a regression equation, the pesticide levels were also categorized into 4 exposure groups in order to evaluate the dose-response relationships. The lowest exposure group included subjects with sample results below the limit of detection and the remaining subjects were ranked into three additional equal exposure groups: group 2 contained infants with the lowest third of detectable levels; group 3 contained infants with the middle third of detectable levels; and group 4 contained infants with the highest third of detectable levels. Dummy variables were used in the regression analyses to compare birth outcomes among newborns in exposure group 1 (those with non-detectable levels) to birth outcomes among newborns in exposure groups 2, 3, and 4. Stratified analyses were also conducted to evaluate the effects of pesticide exposures on birth outcomes among newborns born before versus on or after January 1, 2001. A total of 314 infants with complete data on birth outcomes, potential confounders, and pesticide measurements in personal air and blood were included in the regression analyses. Results are considered statistically significant at  $P < 0.05$  (two-tailed).

## Results

Table 1 presents demographics for study women and shows the percentage of women who reported that pests

Table 1  
Demographics and the number of women using pest control during pregnancy,  $N = 571$

Age	24.9 ± 5.0 <sup>a</sup>	Annual household income <sup>b</sup>	
Ethnicity		<\$10,000	237 (45%)
African American	208 (37%)	\$10,000–\$30,000	213 (41%)
Dominican	357 (63%)	>\$30,000	71 (14%)
Community <sup>b</sup>		Total number with pest sightings	467 (85%)
Harlem	223 (40%)	Sightings of cockroaches	384 (68%)
Washington Heights	202 (36%)	Sightings of rodents (mice and rats)	302 (54%)
South Bronx	129 (23%)	Sightings of other pests	125 (25%)
Marital status <sup>b</sup>		Total number using pest control <sup>b</sup>	410 (85%) <sup>b</sup>
Never married	373 (66%)	By an exterminator only	36 (8%)
Married <sup>c</sup>	151 (27%)	By exterminator plus others <sup>d</sup>	136 (28%)
Separated, widowed, divorced	42 (7%)	By others <sup>d</sup> only	238 (49%)
Medicaid recipient	505 (89%)	Birth weight	3382.1 ± 485.8
Education <sup>b</sup>		Birth length	50.9 ± 2.6
<High school degree	193 (35%)	Birth head circumference	34.1 ± 1.5
High school diploma or GED	233 (42%)		
Some college (<4 years)	108 (19%)		
≤College degree (4 year)	23 (4%)		

<sup>a</sup> Maternal age, birth weight, birth weight, and birth head circumference are reported as mean ± SD; the rest of the table reports number of subjects (%) in each category.

<sup>b</sup> Missing values: maternal age ( $n = 3$ ), ethnicity ( $n = 6$ ), community ( $n = 17$ ), marital status ( $n = 5$ ), medicaid ( $n = 6$ ), education ( $n = 14$ ), income ( $n = 50$ ), pest sightings (cockroaches  $n = 8$ , rodents  $n = 7$ , other pests  $n = 73$ ), pest control ( $n = 89$ ).

<sup>c</sup> Includes women living as married with same partner >7 years. Birth outcomes provided on 314 subjects for whom medical record and pesticide data are available.

<sup>d</sup> The woman herself, other household member, or the apartment superintendent.

were sighted and that pest control measures were used during pregnancy. Among the women reporting that pest control measures were used, Fig. 1 shows the types of pest control measures and for each measure the proportion that was targeted at control of cockroaches, rodents, or other pests. In total, 85% of the women reported that pests were sighted in the home and 85% reported that some forms of pest control measures were used during pregnancy. Women who reported that pests were sighted were more likely to be users of pest control. Specifically, 91% of women with pest sightings, versus 61% without, reported that some form of pest control was used in the home during pregnancy, a difference that was highly significant (Chi-square = 46.7;

$P < 0.001$ ). Among women who reported that pest control measures were used, 40% reported using lower toxicity methods only (traps, gels, and boric acid); 6% reported using non-specified other methods with or without the lower toxicity methods; and 54% reported using one or more of the higher toxicity methods (sprays by an exterminator, can sprays, and pest bombs) with or without the other methods. Most of the pest control use was targeted at cockroach control.

Of the ten insecticides being measured in both the 48-h personal air samples collected from the mothers during pregnancy and the blood samples collected from the mothers and/or newborns at delivery, the following three were

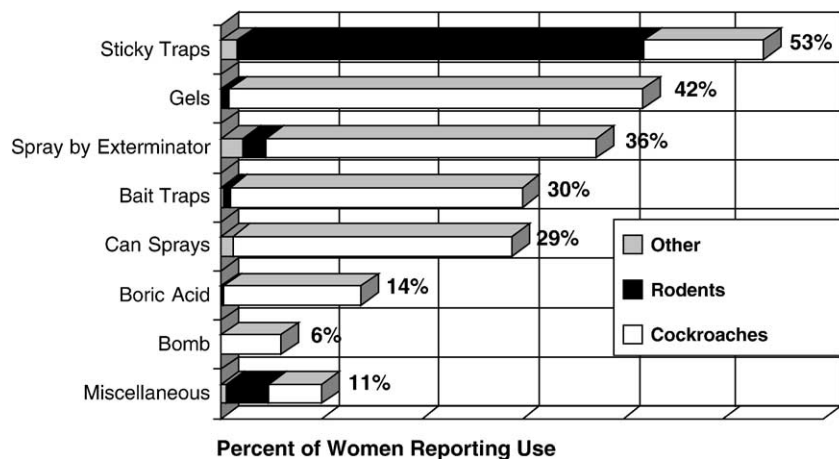


Fig. 1. Percentage of women reporting use of each of eight specific pest control methods. Analyses restricted to women who reported that some form of pest control was used during pregnancy.

Table 2

Insecticides in maternal personal air samples collected over 48 h during pregnancy and in blood samples collected from the mother and newborn at delivery

	Personal air (ng/m <sup>3</sup> ) (n = 394)		Maternal blood (pg/g) (n = 326)		Cord blood (pg/g) (n = 341)	
	Percentage > LOD <sup>a</sup>	Mean ± SD <sup>b</sup> (range)	Percentage > LOD <sup>a</sup>	Mean ± SD <sup>b</sup> (range)	Percentage > LOD <sup>a</sup>	Mean ± SD <sup>b</sup> (range)
Chlorpyrifos	99.7	14.3 ± 30.7 (0.1–344.8)	70	3.9 ± 4.8 (0.3–35)	64	3.7 ± 5.7 (0.3–63)
Diazinon	100	99.5 ± 449.8 (1.0–6000)	45	1.3 ± 1.8 (0.3–25)	44	1.2 ± 1.4 (0.3–13)
Propoxur <sup>b</sup>	100	53.5 ± 124.5 (1.2–1420)	39	2.8 ± 2.7 (1.4–32.8)	40	3.0 ± 3.0 (1.4–26)

<sup>a</sup> LOD = limit of detection.<sup>b</sup> 2-Isopropoxyphenol was measured in blood samples.

detected most frequently: the organophosphates chlorpyrifos and diazinon and the carbamate propoxur. Table 2 provides results for these three pesticides. Table 3 shows the correlations between these pesticides in personal air samples and blood samples. These insecticides were detected in 99.7–100% of 48-h personal air samples collected from the mothers during pregnancy (n = 394) and in 39–70% of blood samples collected from the mothers (n = 326) and/or newborns (n = 341) at delivery. The correlations between the insecticides in maternal personal air and blood samples were weak and generally not statistically significant. However, maternal and newborn blood levels were similar and highly correlated (Table 3). In addition, the carbamates bendiocarb and carbofuran were detected in 35–51% of maternal and cord blood samples, but were found less frequently in maternal personal air samples (detected in 2–15% of samples, data not shown). The remaining 5 insecticides were detected less frequently in both personal air and blood samples (detected in 0–23% of samples, data not shown). A significant association was seen between maternal self-reported pesticide use during pregnancy and levels of diazinon and propoxur, but not chlorpyrifos, in the maternal personal air samples collected during pregnancy (see Fig. 2). However, no association was seen between maternal self-reported pesticide use during pregnancy and levels of insecticides in blood samples collected from the mother and/or newborn at delivery. Levels of all three insecticides in maternal personal air samples decreased significantly between 1999 and 2002 (Fig. 3). A significant decrease was also seen in levels of chlorpyrifos and propoxur in cord blood samples among infants born between 1998 and 2002 (Fig. 3). Diazinon levels in cord blood samples also

differed significantly by year of birth ( $P < 0.05$ , ANOVA), but no trend was apparent. The decrease in insecticide levels in maternal blood samples was similar to that seen for cord blood samples (data not shown). By contrast, there was no significant difference in self-reported pest control use during pregnancy among women born between 1999 and 2002 ( $P > 0.1$ , Chi-square).

Table 4 shows the association between birth weight and length and levels of insecticides in umbilical cord blood samples among newborns born before and after 1/1/01. Among newborns born before 1/1/01, an inverse and highly significant association was seen between (ln)chlorpyrifos levels and both birth weight ( $B = -67.3$  g/unit,  $P = 0.008$ ) and birth length ( $B = -0.43$  cm/unit,  $P = 0.004$ ). By contrast, among newborns born after 1/1/01, the relationships were no longer inverse nor significant ( $P > 0.5$ ), although the confidence intervals were wider due to reduced sample size. Similarly, among newborns born prior to 1/1/01, a highly significant inverse association was seen between the (ln)combined levels of chlorpyrifos and diazinon (in chlorpyrifos equivalents) and both birth weight ( $B = -72.5$  g/unit,  $P = 0.007$ ) and birth length ( $B = -0.46$

Table 3

Correlation between insecticides in maternal personal air samples collected over 48 h during pregnancy and in blood samples collected from the mother and newborn at delivery

		Cord blood	Maternal blood
Chlorpyrifos	Personal air	0.19*	0.21*
	Maternal blood	0.79*	
Diazinon	Personal air	-0.06	0.004
	Maternal blood	0.69*	
Propoxur	Personal air	0.11	0.06
	Maternal blood	0.41*	

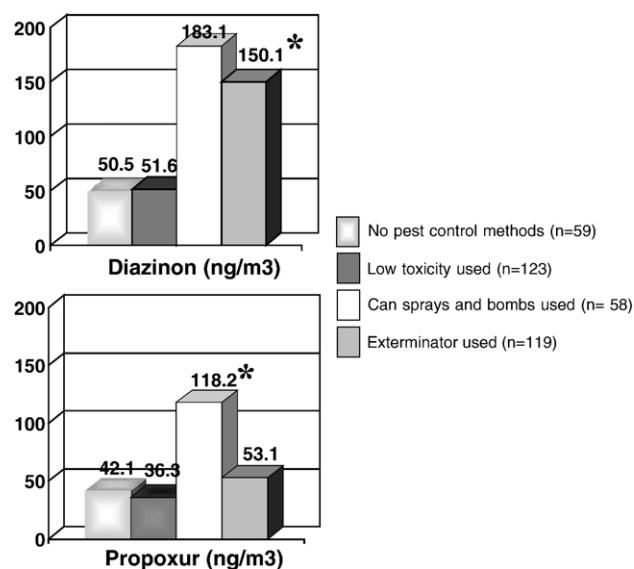
\*  $P \leq 0.001$  Spearman's rank.

Fig. 2. Mean air concentrations (ng/m<sup>3</sup>) in maternal personal air samples collected over 48 h during the 3rd trimester stratified by maternal self-reported pesticide use during pregnancy. \* $P < 0.05$  ANOVA.

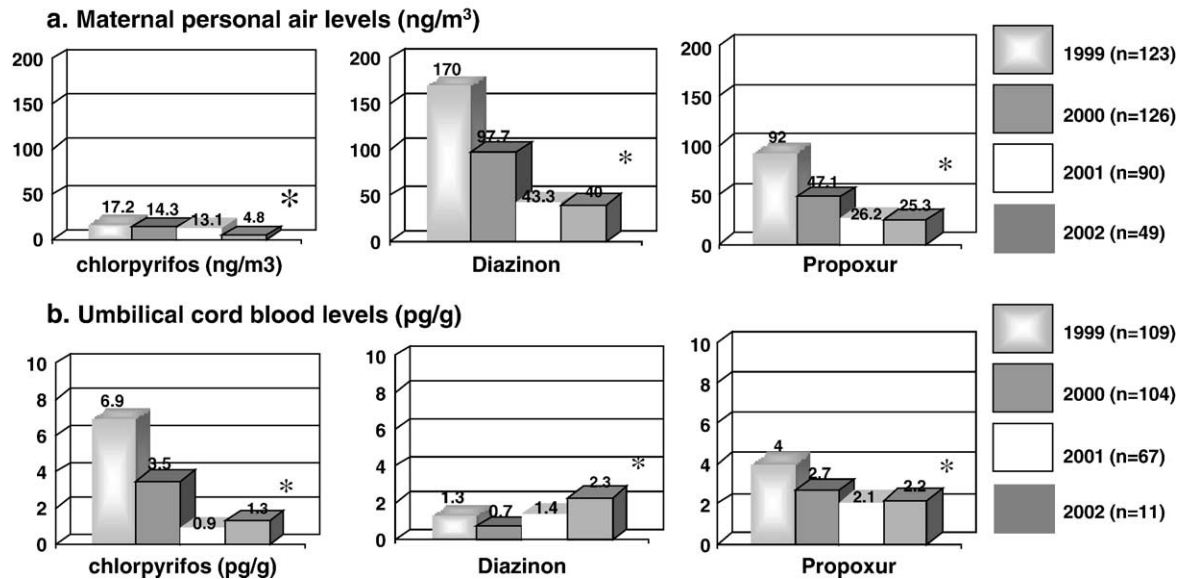


Fig. 3. Mean insecticide levels in maternal personal air sample collected over 48 h during the 3rd trimester of pregnancy by year of delivery (a) and in umbilical cord blood samples at delivery (b) by year of delivery.

cm/unit,  $P = 0.004$ ). Among newborns born after 2001, the magnitude of the effect was much less and no longer significant ( $P > 0.8$ , Table 4). It is of note that among newborns born prior to 1/1/01, 34% had combined group 4 exposure levels of chlorpyrifos and diazinon while among newborns born after 1/1/01 only one (1.5%) had combined group 4 exposure levels, a difference that was highly significant ( $\chi^2 = 50$ ,  $P < 0.001$ ). Birth weight averaged 215.1 g less (95% CI  $-384.7$  to  $-45.5$ ,  $P = 0.01$ ) among those with the highest combined cord plasma chlorpyrifos and diazinon exposure levels (group 4) compared to newborns without detectable levels of either pesticide in cord plasma (group 1, data not shown). After additional controlling for cord plasma 2-isopropoxyphenol as well as the other potential confounders, the associations between birth weight and length and cord plasma (ln)chlorpyrifos,

as well as the sum of cord plasma (ln)chlorpyrifos and diazinon (in chlorpyrifos equivalents adjusted for relative potency), remained significant ( $P \leq 0.02$ ) and the effect size remained similar to that seen without 2-isopropoxyphenol in the model (data not shown).

As seen from Table 4, among newborns born prior to 2001, the association between (ln)2-isopropoxyphenol and birth length was statistically significant ( $B = -0.73$  cm/unit,  $P = 0.01$ ). However, after additionally controlling for chlorpyrifos and diazinon as well as the other potential confounders, the association between 2-isopropoxyphenol and birth length among newborns born prior to 1/1/01 remained inverse but the effect size was reduced ( $B = -0.57$  cm/unit compared to  $B = -0.73$  cm/unit) and the association dropped to borderline significance ( $P = 0.05$ , data not shown). Among newborns born after 1/1/01, the

Table 4

Regression analyses<sup>a</sup> of birth weight and length and organophosphate levels in umbilical cord plasma samples for infants born before and after 1/1/01

	Birth weight (gm)		Birth length (cm)	
	B (95% CI)	P value	B (95% CI)	P value
Born before 1/1/01 ( $n = 237$ )				
Chlorpyrifos	-67.3 (-116.6--17.8)	0.008	-0.43 (-0.73--0.14)	0.004
Sum chlorpyrifos and diazinon <sup>b</sup>	-72.5 (-125.0--20.0)	0.007	-0.46 (-0.77--0.14)	0.004
2-Isopropoxyphenol	-75.8 (-171.1--19.5)	0.12	-0.73 (-1.3--0.17)	0.01
Born after 1/1/01 ( $n = 77$ )				
Chlorpyrifos	30.7 (-108.6--169.9)	0.66	0.07 (-0.65--0.79)	0.85
Sum chlorpyrifos and diazinon <sup>b</sup>	0.6 (-144.7--145.9)	0.99	-0.07 (-0.82--0.67)	0.84
2-Isopropoxyphenol	-107.3 (-298.7--84.2)	0.27	-0.30 (-1.3--0.71)	0.56

<sup>a</sup> Each (ln)insecticide levels was entered as the independent variable into a parallel multiple linear regression model. Model covariates were gestational age of the newborn (in weeks), maternal pre-pregnancy weight and weight gain during pregnancy (in pounds), newborn gender (0 = male; 1 = female), parity (0 = nulliparous; 1 = at least one prior live birth), ethnicity (0 = Dominican; 1 = African American), ETS in the home (0 = no; 1 = yes), and season of delivery (dummy variable 1: 0 = summer; 1 = winter; dummy variable 2: 0 = summer; 1 = spring; dummy variable 3: 0 = summer; 1 = fall); models for head circumference included whether or not the delivery was by cesarean section ( $n = 0, 1 = \text{yes}$ ).

<sup>b</sup> Sum of chlorpyrifos and diazinon in chlorpyrifos equivalents adjusted for relative potency.

association remained inverse but the magnitude of the effect was smaller and no longer significant ( $B = -0.30$  cm/unit,  $P = 0.56$ ). No association was seen among levels of these insecticides in maternal personal air samples and the birth outcomes (data not shown). Nor was any association seen between infant head circumference and levels of insecticides in either maternal personal air and cord blood samples (data not shown).

## Discussion

These results show widespread insecticide exposures during pregnancy among African American and Dominican women from minority communities of New York City. Findings are consistent with our published reports in the same cohort (Whyatt et al., 2002, 2003, 2004). They are also consistent with prior research. A 1997 study indicated that the heaviest application of legally registered pesticides in New York State occurred not in the agricultural communities, but in the boroughs of Manhattan and Brooklyn (Thier et al., 1998). Chlorpyrifos was the pesticide most heavily applied throughout New York State (Thier et al., 1998). The number of gallons of chlorpyrifos applied in Manhattan exceeded the total number of gallons of all pesticides applied in any other single county in the State. Similarly, chlorpyrifos has been one of the most heavily used insecticides by pest control operators for the New York City Housing Authority over the last 10 years (Landrigan et al., 1999). A more recent survey of pest control measures used by residents of public housing in New York State, conducted during 2000–2001 by the New York State Attorney General's Office, concluded that pest problems and pesticide use were related to housing density (Surgan et al., 2002). Specifically, 93% of the residents of public housing in New York City reported applying pesticides in their homes and more than half said they did so once per week (Surgan et al., 2002). By contrast, only 41% of public housing residents in Syracuse, New York, a less densely populated area, applied pesticides and more than half of them applied the pesticides once per year or less (Surgan et al., 2002). An ongoing prospective cohort study of mothers and newborns delivered at Mount Sinai Hospital has also documented considerable indoor pesticide exposure during pregnancy among minority women in New York City (Berkowitz et al., 2003). Our data indicate that most of the insecticide use was targeted at cockroach control (Whyatt et al., 2002).

Chlorpyrifos and diazinon were among the insecticides detected most frequently in both personal air and blood samples in the current cohort. Further, combined exposures to these insecticides were common. Specifically, both were detected simultaneously in 100% of the maternal personal air samples and in over a third of cord blood samples. A significant correlation was seen between the two insecticides in personal air ( $r = 0.3$ ,  $P < 0.001$ ) and cord blood ( $r = 0.57$ ,

$P < 0.001$ , Spearman's rank) (Whyatt et al., 2004). However, blood and air levels were generally not correlated. This may be due to the fact that pesticide levels in blood reflect exposures from all routes, including dermal absorption and ingestion, as well as inhalation. It is likely that the women in the current cohort received some exposures to these insecticides through diet. Both chlorpyrifos and diazinon are registered for use on multiple food crops (Smegal, 1999; U.S. Environmental Protection Agency, 2000b). A recent aggregate exposures study of four pesticides, including chlorpyrifos and diazinon, among 102 children from Minnesota concluded that ingestion was the dominant route of exposure (Clayton et al., 2003). Another study found chlorpyrifos residues in 38% of the food samples collected over 4 days from 75 individuals (MacIntosh et al., 2001), although dietary intakes were estimated to account for only approximately 13% of aggregate exposures (Pang et al., 2002). Dermal absorption and non-intentional ingestion may also be significant sources of exposure to residues of the insecticides on surfaces in the home following residential use (Camann et al., 1995; Gordon et al., 1999; Gurunathan et al., 1998; Whitmore et al., 1994). In addition, the pesticides are rapidly excreted (with biologic half-life on the order of a few days) and blood levels provide short-term dosimeters (Barr et al., 1999, 2002; Nolan et al., 1984). The personal air monitoring of the mother was generally completed more than a month before collection of the blood samples at delivery.

The current study also saw a highly significant inverse association between umbilical cord chlorpyrifos levels and both birth weight and birth length among infants in the current cohort born prior to U.S. EPA regulatory actions to phase out residential uses of the insecticide (Whyatt et al., 2003). Results also suggest the possibility that prenatal diazinon exposures may have contributed to fetal growth deficits. These findings are consistent with experimental evidence in laboratory animals, which has shown a link between both chlorpyrifos and diazinon exposures during pregnancy and reduced fetal growth (Eskenazi et al., 1999; Smegal, 1999; U.S. Environmental Protection Agency, 2000b). However, in contrast to our findings, two recent epidemiologic studies failed to find associations between prenatal chlorpyrifos exposure and either birth weight or length (Berkowitz et al., 2004; Eskenazi et al., 2004). Both studies used levels of the chemical-specific metabolite of chlorpyrifos (3,5,6-trichloro-2-pyridinol (TCPY)) in urine samples collected from the mother during pregnancy as the dosimeter of prenatal exposure. In a thoughtful commentary, Needham discusses factors that may have contributed to these disparate findings, including the possibility that cord plasma chlorpyrifos may provide a better dosimeter of fetal exposures than maternal urinary TCPY levels during pregnancy as the latter reflects exposure of the mother to the parent compound, as well as to chlorpyrifos-methyl, and to their degradation products in the environment and is a measure of the amount of these exposures excreted during

pregnancy (Needham, 2005). By contrast, cord plasma chlorpyrifos reflects the amount of the parent compound that is actually transferred from the mother to the fetus during pregnancy. In addition, it is possible that blood levels provide a better dosimeter for steady state exposures than urinary levels, although this has not been assessed (Needham, 2005). Finally, Needham cautions that the findings could be due to chance and that additional research is clearly warranted given these discrepancies (Needham, 2005).

Results from this research support the recent U. S. EPA regulatory action to phase out residential uses of these insecticides and indicate that it has been effective at reducing exposures. In June 2000, EPA entered into an agreement with the registrant to begin phasing out residential uses of chlorpyrifos and to terminate all retail sales for indoor use by December 2001 (U.S. Environmental Protection Agency, 2000a). In January 2001, EPA entered into an agreement with the registrant to begin phasing out residential uses of diazinon and to terminate all retail sales for indoor use by December 2002 (U.S. Environmental Protection Agency, 2001a). Prior to this regulatory action, EPA estimated that approximately 75% of U.S. diazinon use and 50% of U.S. chlorpyrifos use were for residential pest control (U.S. Environmental Protection Agency, 2000a, 2001a). We conducted a survey during June–July 2002 of pesticide products sold in stores in minority communities of New York City and found that only 4/135 stores sold products containing chlorpyrifos, although 40% still had products containing diazinon (Carlton et al., 2004). In a follow-up 1 year later, chlorpyrifos was found in only one store and diazinon was found in 18%, although it was still available in 80% of the supermarkets (Carlton et al., 2004). Our data also show that levels of these insecticides in personal air and blood samples collected from our cohort mothers and newborns have been decreasing between 1998 and 2002 (Whyatt et al., 2003).

## Acknowledgments

The OB/GYN staffs at Harlem and NY Presbyterian Hospitals, Darrell Holmes, Mejico Borjas, Jing Lai, Lirong Qu, Xinhe Jin. Work supported by NIEHS P50 ES09600, RO1 ES08977, and RO1 ES11158, RO1 ES06722, U.S. EPA R827027, R8260901, and R82860901, Irving General Clinical Research Center (grant #RR00645), Bauman Family Foundation, Gladys and Roland Harriman Foundation, Hansen Foundation, W. Alton Jones Foundation, New York Community Trust, Educational Foundation of America, The New York Times Company Foundation, Rockefeller Financial Services, Horace W. Smith Foundation, Beldon Fund, The John Merck Fund, September 11th Fund of the United Way and New York Community Trust, The New York Times 9/11 Neediest Fund, and V. Kann Rasmussen Foundation.

## References

- Barr, D.B., Barr, J.R., Driskell, W.J., Hill Jr., R.H., Ashley, D.L., Needham, L.L., 1999. Strategies for biological monitoring of exposure to contemporary-use pesticides. *Toxicol. Ind. Health* 15, 1–2.
- Barr, D.B., Barr, J.R., Maggio, V.L., Whitehead, R.D., Sadowski, M.A., Whyatt, R.M., Needham, L.L., 2002. A multi-analytic method for the quantification of contemporary pesticides in human serum and plasma using high resolution mass spectrometry. *J. Chromatogr., B, Biomed. Sci.* 778, 99–111.
- Berkowitz, G.S., Obel, J., Deych, E., Lapinski, R., Godbold, J., Liu, Z., Landrigan, P.J., Wolff, M.S., 2003. Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. *Environ. Health Perspect.* 111, 79–84.
- Berkowitz, G.S., Wetmur, J.B., Birman-Deych, E., Obel, J., Lapinski, R.H., Godbold, J.H., Holzman, I.R., Wolff, M.S., 2004. In utero pesticide exposure, maternal paraoxonase activity, and head circumference. *Environ. Health Perspect.* 112, 388–391.
- Camann, D.E., Harding, H.J., Clothier, J.M., Kuchibhatla, R.V., Bond, A.E., 1995. Dermal and In-Home Exposure of the Farm Family to Agricultural Pesticides: Measurement of Toxic and Related Air Pollutants. Air and Waste Management, Pittsburgh, PA, pp. 548–554.
- Carlton, E.J., Moats, H.L., Feinburg, M., Shepard, P., Garfinkel, R., Whyatt, R., Evans, D., 2004. Pesticide sales in low-income, minority neighborhoods. *J. Commun. Health* 29, 231–244.
- Clayton, A.C., Pellizzari, E.D., Whitmore, R.W., Quackenboss, J.J., Adgate, J., Sefton, K., 2003. Distributions, associations, and partial aggregate exposure of pesticides and polynuclear aromatic hydrocarbons in the Minnesota Children's Pesticide Exposure Study (MNCPEs). *J. Expo. Anal. Environ. Epidemiol.* 13, 100–111.
- Eskenazi, B., Bradman, A., Castorina, R., 1999. Exposures of children to organophosphate pesticides and their potential adverse health effects. *Environ. Health Perspect.* 107, 409–419.
- Eskenazi, B., Harley, K., Bradman, A., Weltzien, E., Jewell, N.P., Barr, D.B., Furlong, C.E., Holland, N.T., 2004. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environ. Health Perspect.* 1116–1124.
- Gordon, S.M. C.P., Nishioka, M.G., Brinkman, M.C., O'Rourke, M.K., Lebowitz, M.D., Moschandreas, D.J., 1999. Residential environmental measurements in the national human exposure assessment survey (NHEXAS) pilot study in Arizona: preliminary results for pesticides and VOCs. *J. Expo. Anal. Environ. Epidemiol.* 9, 456–470.
- Gurunathan, S., Robson, M., Freeman, N., Buckley, B., Roy, A., Meyer, A., Bukowski, J., Liyo, P.J., 1998. Accumulation of chlorpyrifos on residential surfaces and toys accessible to children. *Environ. Health Perspect.* 106, 9–16.
- Landrigan, P.J., Claudio, L., Markowitz, S.B., Berkowitz, G.S., Brenner, B.L., Romero, H., Wetmur, J.G., Matte, T.D., Gore, A.C., Godbold, J.H., Wolff, M.S., 1999. Pesticides and inner-city children: exposures, risks, and prevention. *Environ. Health Perspect.* 107, 431–437.
- MacIntosh, D.L., Kabiru, C.W., Echols, S.L., Ryan, P.B., 2001. Dietary exposure to chlorpyrifos and levels of 3,5,6-trichloro-2-pyridinol in urine. *J. Expo. Anal. Environ. Epidemiol.* 11, 279–285.
- Needham, L.L., 2005. Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes. *Environ. Health Perspect.* 113, 494–498.
- Nolan, R.J., Rick, D.L., Freshour, N.L., Saunders, J.H., 1984. Chlorpyrifos: pharmacokinetics in human volunteers. *Toxicol. Appl. Pharmacol.* 73, 8–15.
- Pang, Y., MacIntosh, D.L., Camann, D.E., Ryan, P.B., 2002. Analysis of aggregate exposure to chlorpyrifos in the NHEXAS-Maryland investigation. *Environ. Health Perspect.* 110, 235–240.
- Perera, F., Rauh, V., Tsai, W.Y., Kinney, P., Camann, D., Barr, D., Bernert, T., Garfinkel, R., Tu, Y.H., Diaz, D., Dietrich, J., Whyatt, R.M., 2003. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multi-ethnic population. *Environ. Health Perspect.* 111, 201–205.



- Smegal, D.C. 99. Washington, DC: United States Environmental Protection Agency.
- Surgan, M.H., Congdon, T., Primi, C., Lamster, S., Louis-Jacques, J. 2002. Pest Control in Public Housing, Schools and Parks: Urban Children at Risk. LAW 180-4 PESP 202-7643 Albany New York: State Department of Law, Environmental Protection Bureau.
- Thier, A., Enck, J., Klossner, C., 1998. Plague by Pesticides: An Analysis of New York State and New York City's 1997 Pesticide Use and Sales Data. New York Public Research Group, Albany, NY, pp. 1–43.
- U.S. Environmental Protection Agency, 1997. Reregistration Eligibility Decision (RED) Propoxur. U. S. Environmental Protection Agency, EPA# 738-R-97-009.
- U.S. Environmental Protection Agency, 2000a. Chlorpyrifos revised risk assessment and agreement with registrants. U.S. Environmental Protection Agency, Washington, DC.
- U.S. Environmental Protection Agency. 2000. Diazinon. Revised HED Preliminary Human Health Risk Assessment for the Reregistration Eligibility Decision (RED) D262343. PC Code: 057801. List, A., Case No. 0238. U. S. Environmental Protection Agency, Washington, D.C.
- U.S. Environmental Protection Agency, 2001a. Diazinon Revised Risk Assessment and Agreement with Registrants. US EPA, Washington, DC.
- U.S. Environmental Protection Agency, 2001b. Organophosphate Pesticides: Preliminary OP Cumulative Risk Assessment. U.S. Environmental Protection Agency, Washington DC. <http://www.epa.gov/pesticides/cumulative/pra-op>.
- U.S. Environmental Protection Agency, 2002. Organophosphate Pesticides: Preliminary OP Cumulative Risk Assessment. U.S. Environmental Protection Agency, Washington DC. <http://www.epa.gov/pesticides/cumulative/pra-op>.
- Whitmore, R., Immerman, F.W., Camann, D.E., Bond, A.E., Lewis, R.G., Schaum, J.L., 1994. Non-occupational exposures to pesticides for residents of two U.S. cities. Arch. Environ. Contam. Toxicol. 26, 47–59.
- Whyatt, R.M., Camann, D.E., Kinney, P.L., Reyes, A., Ramirez, J., Dietrich, J., Diaz, D., Perera, F.P., 2002. Pesticide exposure during pregnancy among minority women residing in Northern Manhattan and the South Bronx. Environ. Health Perspect. 110, 507–514.
- Whyatt, R.M., Barr, D.B., Camann, D.E., Kinney, P.L., Barr, J.R., Andrews, H.F., Hoepner, L.A., Garfinkel, R., Hazi, Y., Reyes, A., Ramirez, J., Cosme, Y., Perera, F.P., 2003. Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ. Health Perspect. 111, 749–756.
- Whyatt, R.W., Rauh, V., Barr, D.B., Camann, D.E., Andrews, H.F., Garfinkel, R., Hoepner, L.A., Diaz, D., Dietrich, J., Tang, D., Kinney, P.L., Perera, F.P., 2004. Prenatal insecticide exposures and birth weight and length among an urban minority cohort. Environ. Health Perspect. 112, 1125–1132.