Journal of Exposure Science and Environmental Epidemiology (2015) 25, 264–270
© 2015 Nature America, Inc. All rights reserved 1559-0631/15
www.nature.com/jes

ORIGINAL ARTICLE

Prenatal exposure to pyrethroid insecticides and birth outcomes in Rural Northern China

Guodong Ding[1], Chang Cui[2], Limei Chen[2], Yu Gao[2], Yijun Zhou[2], Rong Shi[2] and Ying Tian[1,2]

Although pyrethroid insecticides are widely used, little is known about potential adverse effects on fetal growth. Participating 454 mother–infant pairs were recruited from a prospective birth cohort in rural northern China between September 2010 and 2012. We measured five non-specific pyrethroid metabolites in maternal urine at delivery and examined their association with birth outcomes including birth weight, length, head circumference, and gestational duration. The creatinine-adjusted medians of pyrethroid metabolites in urine were 0.51 μg/g for cis-DCCA, 0.65 μg/g for trans-DCCA, and 0.68 μg/g for 3-PBA. The pregnant women had substantially higher levels of urinary pyrethroid metabolites compared with those reported in developed countries. A increase in total (the sum of cis-DCCA, trans-DCCA, and 3-PBA) but not individual urinary metabolite levels was associated with a decrease in birth weight (adjusted $\beta = -96.76$ g per log10 unit increase, 95% confidence interval = −173.15 to −20.37). No associations were found between individual or total metabolite levels and birth length, head circumference, or gestational duration. We report an adverse association of prenatal exposure to pyrethroids as measured by urinary metabolites with birth weight. More studies are warranted in China given the relatively high levels of urinary metabolites in our study population.

Journal of Exposure Science and Environmental Epidemiology (2015) **25,** 264–270; doi:10.1038/jes.2014.86; published online 17 December 2014

Keywords: pyrethroid; pesticide; pregnant woman; birth weight; China

INTRODUCTION

Pyrethroids, a group of synthetic insecticides, were produced in the 1970s after the removal of organochlorine insecticides from the consumer market.[1] The synthetic pyrethroids not only display the biologic activity of their natural pyrethrins, but also display enhanced environmental stability.[2,3] With the phaseout of organophosphate insecticides use in residential environments and some agricultural applications, pyrethroids have been subsequently developed and used extensively. There is a significant trend that amount of pyrethroids introduced into the environment will continuously grow to replace more toxic organophosphates. Currently, pyrethroid insecticides are the most common class of insecticides in global widespread use and account for approximately one-fourth of the worldwide insecticide market.[4]

A number of epidemiological studies have investigated the possible relationships between low-level prenatal or early postnatal pesticide exposures and children's health consequences such as fetal growth, neurodevelopment, and childhood cancer.[5] However, the majority of these studies have focused on organochlorine or organophosphate insecticides, and growing evidence supports that exposure to pesticides could adversely affect child health. Unlike many other classes of insecticides, pyrethroid insecticides display dissimilar structure and biological persistence and their modes of action are strikingly different. Pyrethroids are rapidly metabolized by esterases in humans with the elimination half time ($t_{1/2}$) being about 6 h,[6] and thus they are generally considered among the less significant insecticides regarding human toxicity.[7] However, toxicological studies have shown that they are considered to be endocrine disrupting chemicals and have been classified as potential toxicants at relative high exposure levels.[8] Pyrethroids could induced significant suppression of thyroid hormone (TH) levels, and concomitant stimulation of thyrotrophin (TSH) concentrations.[9,10] Pyrethroids have also been linked to adverse effects on reproduction and sexual development as well as the immune system.[11,12] Furthermore, fetus and children are not "little adults", they are more susceptible to the potentially adverse effects of pesticides, not only because their organ systems are developing rapidly but also because they have lower levels of the enzymes that detoxify pyrethroids than adults.[13,14] Therefore, their unique physiological characteristics leave them particularly susceptible and vulnerable to the health effects of pesticide exposures.

Today, approximately 3000 tons of pyrethroids are applied each year throughout China and are widely used in agriculture, horticulture, public health (e.g. hospitals), and homes.[11] Recent epidemiological studies have shown that exposure to pyrethroid insecticides in China and around the world is widespread among some susceptible populations, including pregnant women and children.[7,13,15–18] However, a very limited number of studies have examined the association of pyrethroid exposures during gestation or the early postnatal period and children's health risks, and they also have reported less consistent results. For example, Hanke et al.[19] found that maternal exposure to pyrethroids during pregnancy was associated with a significant decrease in birth weight. In contrast, Dabrowski et al.[20] observed no significant reduction in birth weight after reported farm use of pyrethroids during pregnancy.[20] In light of the potential adverse effects of insecticide exposures on child health, the US Environmental

[1]MOE and Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China and [2]Department of Environmental Health, School of Public Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China. Correspondence: Dr. Ying Tian, MOE and Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, 200092 Shanghai, China. Tel./Fax: +86 21 64663944.
E-mail: tianmiejp@sjtu.edu.cn
Received 11 April 2014; revised 2 October 2014; accepted 5 October 2014; published online 17 December 2014

Protection Agency has recently released the revised developmental and cumulative risk assessment of insecticides, and has requested the registrants of insecticides to provide developmental toxicity studies to the agency.[4,21] Therefore, there is an urgent need for research to fill the gaps of information on adverse effects of pyrethroid exposures to the fetus and children.

As the metabolites of currently used pyrethroid insecticides are usually excreted in urine, the biological monitoring of exposure to pyrethroids has typically involved the quantification of urinary metabolites in many epidemiological studies.[13,22] Human dose-excretion studies and occupational exposure studies have confirmed that the five non-specific metabolites including cis-DCCA, trans-DCCA, 3-PBA, 4F3PBA, and cis-DBCA are important indicators of pyrethroid exposures in humans.[13,23,24] Until now, little information about pyrethroid exposures and child health has been available in China. In this study, we investigated the five non-specific urinary metabolites of pyrethroids in pregnant women, recruited from a rural community in northern China, and we evaluated the relationship between prenatal exposure to pyrethroids and fetal growth. We tested the hypothesis that after adjusting for potential confounders, prenatal exposure to pyrethroids would be associated with reduced birth weight, length, head circumference, and gestational duration.

METHODS

Participants and Recruitment

This study was a prospective birth cohort study begun in 2010 to examine the relationship of pesticides and other environmental exposures with the health of pregnant women and their children living in a rural community in the southern coastal area of Laizhou Bay of the Bohai Sea in northern China (Shandong province). These women have worked in agricultural fields, and they are more likely to have physical contact with pyrethroids used on crops and/or dietary exposure to pyrethroids. The adverse consequences of the children were assessed at delivery and during the follow-up, which lasted over a period of at least 2 years.

Pregnant women preparing for labor and delivery in Binhai Hospital (the only county hospital in the southern coastal area of Laizhou Bay) were recruited for the study. Eligibility criteria included a singleton pregnancy; age over 18 years old; residence in the area for at least 3 years; and no report of assisted reproduction, pregestational or gestational diabetes, chronic or pregnancy-associated hypertension, HIV infection or AIDS, and illicit drug use.[25] Between September 2010 and September 2012, a total of 636 women met the eligibility criteria, and among whom 568 women agree to take part in this study (response rate 89.3%). Of these, 63 women without urine samples, 32 women with missing values for major confounders, 17 infants with missing neonatal anthropometric data, and 2 women with urinary creatinine concentrations < 0.1 g/l were excluded. Therefore, 454 mother–infant pairs were included in the final analysis. Each woman participating in the study signed an informed consent form, and the research protocol for this study was approved by the Ethics Committees of Shanghai Jiao Tong University School of Medicine. Detailed methods for the study have been described elsewhere.[25]

Maternal Interviews and Assessments

Standardized face-to-face interviews were conducted with the women after delivery by specially trained nurses in the hospital. The questionnaire included the following: demographic and socioeconomic information (maternal age, height, pre-pregnancy weight, education level, household income, and address) and maternal characteristics (cigarette smoking, alcohol use, dietary habits, and employment).[25] All the women have attended at least one prenatal visit at Binhai Hospital during the current pregnancy. Unfortunately, we have no more details on their prenatal care from the questionnaire (e.g. Have you received prenatal care at Binhai Hospital during the current pregnancy? Yes or no). Other relevant information, such as previous pregnancies, current pregnancy complications, weight gain, and self-reported last menstrual period (LMP), was obtained by interview and confirmed by medical records.

Measures of Birth Outcomes

Infant sex, birth date, parity, body weight, length (crown–heel), and head circumference were obtained from medical records. Data on prior medical history, current health status, and clinical estimates of gestational age (ultrasound) were also collected. Gestational age was estimated based on the date of the LMP; if the LMP was unreliable or if there was a significant discordance between the clinical estimate and LMP (>2 weeks), the first clinical estimation of gestational age was used.[25] Low birth weight was defined as < 2500 g, and excessive birth weight (macrosomia) was defined as ≥ 4000 g. Preterm delivery was defined as birth at less than 37 completed gestational weeks. The low and excessive birth weight infants and preterm infants were also included in our final analysis.

Urine Collection and Urinalysis

Spot urine specimens were collected during hospital admission for delivery (within 3 days before delivery) and were aliquoted and stored at –80°C until shipment on dry ice to the Shanghai Municipal Center for Disease Control and Prevention (CDC, Shanghai). The five non-specific metabolites (cis-DCCA, trans-DCCA, 3-PBA, 4F3PBA, and cis-DBCA) of pyrethroids in urine were measured using a sensitive and selective capillary gas chromatography–mass spectrometric detection (GC–MS) (Shmadzu, Kyoto, Japan) based on a slightly modified method described by Kühn et al.[26] The limits of detection (LOD) for all targeted metabolites was defined as a signal-to-noise ratio of three, the LOD for the five metabolites was 0.1 μg/l. Individual metabolite levels below the LOD were assigned a value equal to the LOD divided by the square root of two,[27] and this value was included in each sum. Details of urine analysis and quality control procedures are described elsewhere.[13,16]

Metabolite levels were adjusted using creatinine concentrations to correct for variable urine dilutions in the spot urine samples. Urinary creatinine was measured by the modified Jaffe colorimetric method using automated Beckman Coulter analyzers (Beckman Coulter, Fullerton, CA, USA). Urine samples with creatinine concentrations < 0.1 g/l were considered too dilute for accurate analysis, and two women were excluded because of low creatinine concentrations.

Data Analysis

SPSS 16.0 software (SPSS, Chicago, IL, USA) was used for all analyses. Initial descriptive statistics were calculated for the individual pyrethroid metabolites (cis-DCCA, trans-DCCA, and 3-PBA) and birth outcomes (birth weight, length, head circumference, and length of gestation). 4F3PBA and cis-DBCA were not included in the statistical analysis due to their low detection frequency in the study sample ($< 5\%$). To ensure that the creatinine concentrations did not affect the results, we calculated the urinary levels of pyrethroid metabolites with an adjustment for creatinine. In light of the skewed nature of our data, the urinary metabolite levels were log-transformed ($\log_{10}$) to minimize the disproportional impact of extreme values. To assess the associations between prenatal pyrethroid metabolite levels and birth outcomes such as birth weight, length, head circumference, and length of gestation, we constructed separate linear regression models for each of the four outcomes. We selected potential confounders for the multivariate models based on associations reported in the literature, and we included in the models those that were significantly associated ($P < 0.10$) with two or more outcomes in the present study (e.g. infant gender, parity, pre-pregnancy BMI, pregnancy weight gain, maternal age, household monthly income, passive smoking, and length of gestation). All regression analyses were run using log-transformed creatinine-adjusted metabolites.

RESULTS

Table 1 describes the sociodemographic characteristics of the study population. The average maternal age was 28.0 years (SD = 4.6), two-thirds of the women (67.0%) were primiparous, and half of them (52.2%) had graduated from high school or above. The majority of the women (91.0%) lived in households with a monthly income of less than RMB (¥) 5000 yuan. Nearly three-fifths (56.8%) of the women had a normal weight before pregnancy, and half of them (52.0%) gained 10–15 kg of weight during pregnancy. Almost one-third (31.3%) of the women lived with a smoker during pregnancy, although few smoked themselves or consumed alcohol regularly. Nearly two-thirds (63.9%) of the

© 2015 Nature America, Inc.

Table 1. Demographic characteristic of the study population ($n = 454$).

Characteristic	No. (%), mean ± SD
Maternal characteristic	
Age (years)	
< 25	122 (26.9%)
25–29	181 (39.9%)
30–34	107 (23.6%)
≥ 35	44 (9.7%)
Parity	
0 (primiparous)	304 (67.0%)
≥ 1 (multiparous)	150 (33.0%)
Education (years)	
≤ 9 (middle school)	217 (47.8%)
10–12 (high school)	130 (28.6%)
≥ 13 (greater than high school or college)	107 (23.6%)
Pre-pregnancy BMI (kg/m^2)	
< 18.5 (underweight)	59 (13.0%)
18.5 to < 23.0 (normal weight)	258 (56.8%)
≥ 23.0 (overweight)	137 (30.2%)
Pregnancy weight gain (kg)	
< 10	76 (16.7%)
10–15	236 (52.0%)
> 15	142 (31.3%)
Household monthly salary (RMB)	
< 3000	284 (62.6%)
3000 – 5000	129 (28.4%)
> 5000	41 (9.0%)
Smoking during pregnancy	
Yes	4 (0.9%)
Lived with smoker	142 (31.3%)
No	308 (67.8%)
Alcohol use during pregnancy	
Yes	3 (0.7%)
No	451 (99.3%)
Household pesticide use during pregnancy	
Never	290 (63.9%)
Occasionally (1–5 times)	81 (17.8%)
Often (≥6 times)	83 (18.3%)
Washing fruits and vegetables before eating	
Always (8–10/10 times)	160 (35.2%)
Often (5–7/10 times)	152 (33.5%)
Occasionally (2–4/10 times)	85 (18.7%)
Rarely or never (0–1/10 times)	57 (12.6%)
Infant characteristic	
Gender	
Male	233 (51.3%)
Female	221 (48.7%)
Length of gestation (weeks)	
< 37	25 (5.5%)
≥ 37	429 (94.5%)
Birth weight (g)	
< 2500	11 (2.4%)
2500–4000	401 (88.3%)
≥ 4000	42 (9.3%)
Birth weight (g)	3393.8 ± 437.9
Birth length (cm)	50.7 ± 2.7
Head circumference (cm)	33.3 ± 1.4
length of gestation (weeks)	39.4 ± 1.5

families did not use household pesticide during pregnancy, and one-fifth (18.3%) reported to use household pesticide frequently. The majority (68.7%) of the women washed their fresh vegetables and fruits under running water before eating or cooking, and only 12.6% of them seldom or never washed their fresh fruit and vegetables.

Overall, 51.3% of the newborn infants were male. The average (SD) birth weight was 3394 (438) g; average body (crown–heel) length was 50.7 (2.7) cm; average head circumference was 33.3 (1.4) cm; and average length of gestation was 39.4 (1.5) weeks. A total of 2.4% ($n = 11$) of infants were born of low birth weight, 5.3% ($n = 24$) were small-for-gestational-age (SGA) births, and 5.5% ($n = 25$) were preterm.

Urinary pyrethroid metabolite levels of the study sample, both unadjusted and adjusted for creatinine, are summarized in Table 2. The most frequently detected metabolite was 3-PBA, a non-specific urinary metabolite for many pyrethroid insecticides such as permethrin, cypermethrin, and deltamethrin, followed by trans- and cis-DCCA, two urinary metabolites for permethrin, cypermethrin, and cyfluthrin.[1,7] Very few urine samples had detectable levels of 4F3PBA and cis-DBCA, specific metabolites for cyfluthrin and deltamethrin, respectively.[1,7]

The percentages of samples in which the pyrethroid metabolites were more than the LODs were 82% for 3-PBA, 77% for trans-DCCA, and 71% for cis-DCCA, respectively. The median levels without creatinine adjustment for cis-DCCA, trans-DCCA, and 3-PBA were 0.50, 0.57, and 0.62 μg/l, respectively. The median concentration (interquartile range) of urinary creatinine of the study population was 0.93 g/l (0.72 – 1.30). After creatinine adjustment, the median levels for cis-DCCA, trans-DCCA, and 3-PBA were 0.51, 0.65, and 0.68 μg/g, respectively.

4F3PBA and cis-DBCA were detected in 3.7% and 2.4% of the urine samples analyzed in the study population, respectively. Both 4F3PBA and cis-DBCA were detected in too few of the samples tested to allow for a reliable estimation of the distribution percentiles and geometric mean (GM) levels.

Table 3 presents the adjusted regression coefficients and 95% confidence intervals (CIs) for individual (e.g. cis-DCCA, trans-DCCA, or 3-PBA) and total metabolite levels (sum of the three individual metabolites) in maternal urine with birth outcomes including birth weight, length, head circumference, and length of gestation. We observed that after adjusting for confounders, a 10-fold increase (i.e. one log-unit increase) in total metabolite levels was associated with a small but statistically significant decrease in birth weight of 96.76 g (95% CI = –173.15 to –20.37, $P = 0.013$). However, we did not find associations between individual metabolite levels and birth weight. Similarly, individual or total metabolite levels were not found to be associated with birth length, head circumference, or length of gestation.

We also analyzed the possible relationship of unadjusted individual and total metabolites (not adjusted for creatinine, μg/l) with birth outcomes, but pyrethroid metabolites were not found to be associated with any of the four outcomes (data not shown). We further examined the association between urinary metabolite levels and SGA births using logistic regression models after adjustment for potential confounders. Similarly, Cis-DCCA, trans-DCCA, 3-PBA, and total metabolite levels were not found to be associated with the risk of SGA births (data not shown).

DISCUSSION

Pyrethroid Insecticide Exposure in Northern China

Despite the wide use of pyrethroid insecticides, few studies have assessed the pyrethroid exposure in the general population, particularly for pregnant women. This study provides urinary pyrethroid metabolite levels observed at delivery in pregnant women, recruited from a rural community in northern China. Our findings can be contrasted with those of studies elsewhere. As shown in Table 4, the urinary metabolite levels (cis-DCCA, trans-DCCA, and 3-PBA) in the pregnant women from northern China were substantially higher than those for the US women who participated in the NHANES 1999–2000, 2001–2002, and 2007–2008 studies,[7,28] and those for the US pregnant women who participated in CHAMACOS cohort, 1999–2000.[29] For example, the median levels of urinary metabolites in our sample ranged from 0.51 (cis-DCCA) to 0.68 (3-PBA) μg/g, whereas the median levels of metabolites ranged from less than the LOD to 0.43 μg/g (for 3-

© 2015 Nature America, Inc.

Table 2. Detection frequency, creatinine unadjusted and adjusted geometric mean, range and percentile of urinary pyrethroid pesticide metabolites ($n = 454$)[a].

Metabolites[b]	Detection no. (%)	GM	Range	Percentile of distribution			
				25th	50th	75th	95th
Not adjusted for creatinine (μg/l)							
cis-DCCA	322 (71%)	0.37	< LOD–7.11	< LOD	0.50	0.79	2.60
trans-DCCA	349 (77%)	0.47	< LOD–8.36	0.27	0.57	0.96	2.79
3-PBA	372 (82%)	0.54	< LOD–10.61	0.33	0.62	1.17	3.27
Creatinine adjusted (μg/g) [c]							
cis-DCCA	—	0.39	< LOD–10.30	< LOD	0.51	0.91	2.65
trans-DCCA	—	0.49	< LOD–16.59	0.23	0.65	1.10	3.21
3-PBA	—	0.57	< LOD–13.63	0.32	0.68	1.24	3.31

[a]LOD, limit of detection; GM, geometric mean. The LOD for the three metabolites was 0.1 μg/l. [b]4F3PBA and cis-DBCA were not shown due to their very low frequencies of detection in the study sample (< 5%). [c] < LOD means less than the limit of detection for the urine levels not corrected for creatinine.

Table 3. Adjusted coefficients (95% CIs) in points on the birth weight, length, head circumference, and length of gestation for a $\log_{10}$ unit increase in metabolite levels of pyrethroid insecticides in maternal urine ($n = 454$).

Metabolites	Birth outcomes							
	Birth weight (g)[a]		Birth length (cm)[a]		Head circum ference (cm)[a]		Length of gestation (weeks)[b]	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
Cis-DCCA	−37.67 (−108.11, 32.78)	0.294	0.18 (−0.27, 0.63)	0.431	0.01 (−0.22, 0.24)	0.928	0.05 (−0.22, 0.31)	0.728
Trans-DCCA	22.60 (−49.46, 94.65)	0.538	0.13 (−0.32, 0.57)	0.574	0.06 (−0.17, 0.29)	0.613	0.17 (−0.09, 0.43)	0.194
3-PBA	−36.61 (−107.03, 33.80)	0.307	0.18 (−0.28, 0.63)	0.447	−0.07 (−0.30, 0.17)	0.590	0.10 (−0.17, 0.36)	0.482
$\sum_3$pyrethroids[c]	−96.76 (−173.15, −20.37)	0.013	0.25 (−0.32, 0.82)	0.393	−0.01 (−0.31, 0.29)	0.957	0.23 (−0.10, 0.56)	0.177

[a]Model included infant sex, parity, pre-pregnancy BMI, pregnancy weight gain, maternal age, household monthly income, passive smoking, and length of gestation. [b]Model included infant sex, parity, maternal age, household monthly income, passive smoking. CI, confidence interval. [c]$\sum_3$ pyrethroids mean the sum of three metabolites (cis-DCCA, trans-DCCA, and 3-PBA).

PBA) in the US women. Similarly, the urinary metabolite levels in this study were much higher than the urinary metabolite levels reported in general population in Germany and Poland.[17,30] It should be noted that a pregnant woman is likely to have a lower urinary creatinine concentration than would a non-pregnant woman; therefore, a metabolite level in the pregnant woman may be somewhat overcorrected when adjusting for creatinine. We also found that the pregnant women from northern China had higher levels of Cis-DCCA and equivalent levels of trans-DCCA and 3-PBA comparing with the corresponding population from the Caribbean countries.[31] In contrast, we found that the urinary metabolite levels in pregnant women from a rural area in Jiangsu province, southern China were much higher than the urinary metabolite levels in the corresponding population from our study (northern China), and the levels in rural southern China were roughly 1 – 3 times higher than those in rural northern China.[16]

These phenomena are probably attributable to several features of pesticide use for agriculture in China. First, people in China may have great exposure levels because of heavy use and high residue in agricultural crops. China is now becoming the largest consumer of pesticides in the world, and approximately 3000 tons (6.6 million pounds) of pyrethroids are applied annually.[13] However, the estimated annual use of pyrethroids in the US ranges from several thousand to a million pounds.[1] Indeed, increasing food production has long been the priority of the Chinese government to meet food requirements for its large population. Under the adage "if little is good, more is better", many farmers may overuse or improper use pesticides to get greater yields, which in turn, could contribute to the excess residues of agricultural crops. A recent study in China found that among the 1024 fresh vegetable and fruit samples examined, 412 samples (40.2%) contained detectable residues of at least one of the six target pyrethroids (fenpropathrin, permethrin, cypermethrin, cyfluthrin, fenvalerate, and deltamethrin), and 20 samples (1.9%) exceeded the national maximum residue limits (0.05 – 2 mg/kg for the six pyrethroids list above).[32,33] Second, the amount of pesticides used in China is not evenly distributed and rice is the major agricultural crop accounting for pesticides.[34] China is the largest rice producer with 30.7% of the global production, and southern China is the main rice cultivation region accounting for 93% of national rice production.[35] In contrast, northern China is too cold and dry for rice cultivation; wheat, rather than rice, is the staple food of the populations of this region. It was estimated in 2007 that the amount of insecticides used in Jiangsu province was nearly three times of that in Shandong province (140 *vs* 50 thousand tons).[36] This factor may help to explain why urinary metabolite levels in rural southern China are much higher than those in rural northern China.

In addition, our results also showed that the most frequently detected metabolite was 3-PBA, followed by trans- and cis-DCCA, and very few urine samples had detectable levels of 4F3PBA and cis-DBCA, which were in agreement with the previous domestic reports.[16] Interestingly, the common parent pesticides of 3-PBA, trans-DCCA, and cis-DCCA were permethrin and cypermethrin, suggesting that the parent pesticides exposed to the study population may be predominantly permethrin or cypermethrin.

© 2015 Nature America, Inc.

Table 4. Comparison of cis-DCCA, trans-DCCA, and 3-PBA urinary metabolite levels observed in several epidemiological studies.

Metabolites	Study/author	Country	Sample size/study population/age	LOD (μg/l)	Detection frequency	GM	Percentile of distribution			
							25th	50th	75th	95th
Cis-DCCA										
	Shandong, 2010–2012	China	$n=454$, pregnant women, 18–45 years	0.1	71%	0.39[a]	< LOD	0.51	0.91	2.65
						0.37[b]	< LOD	0.50	0.79	2.60
	NHANES, 1999–2000[7]	USA	$n=1004$, women, 6–59 years	0.1	47%	NC[a]	< LOD	< LOD	0.20	1.55
						NC[b]	< LOD	< LOD	0.28	1.47
	NHANES, 2001–2002[7]	USA	$n=1617$, women, ≥6 years	0.1	33%	NC[a]	< LOD	< LOD	0.27	0.96
						NC[b]	< LOD	< LOD	0.19	0.90
	NHANES, 2007–2008[28]	USA	$n=1292$, women, 6–59 years	0.5	NA	NC[a]	< LOD	< LOD	< LOD	< LOD
						NC[b]	< LOD	< LOD	< LOD	< LOD
	CHAMACOS, 1999–2000[29]	USA	$n=481$, pregnant women, ≥18 years	0.2	9%	NA[a]	NA	NA	NA	NA
						NC[b]	< LOD	< LOD	< LOD	0.5
	Heudorf and Angerer (2001)[17]	Germany	$n=483$, ≥20 years	0.1 – 0.2	28%	NC[a]	< LOD	< LOD	0.07	0.53
						NC[b]	< LOD	< LOD	0.10	0.65
	Wielgomas et al. (2013)[30]	Poland	$n=132$, 5–77 years	0.05 – 0.1	8%	NC[a]	< LOD	< LOD	< LOD	0.17
						NC[b]	< LOD	< LOD	< LOD	0.15
	Jiangsu, 2009–2010[16]	China	$n=1149$, pregnant women, 17–45 years	0.1	95%	0.73[a]	0.30	0.69	1.59	6.15
						0.47[b]	0.18	0.44	1.04	4.64
	Dewailly et al. (2014) [31]	Ten Caribbean countries	$n=295$, pregnant women	0.007	99%	NA[a]	NA	NA	NA	NA
						0.17[b]	NA	NA	NA	0.98
trans-DCCA										
	Shandong, 2010–2012	China	$n=454$, pregnant women, 18–45 years	0.1	77%	0.49[a]	0.23	0.65	1.10	3.21
						0.47[b]	0.27	0.57	0.96	2.79
	NHANES, 1999–2000[7]	USA	$n=1015$, women, 6–59 years	0.4	33%	NC[a]	< LOD	< LOD	0.88	3.65
						NC[b]	< LOD	< LOD	0.55	4.19
	NHANES, 2001–2002[7]	USA	$n=1612$, women, ≥6 years	0.4	27%	NC[a]	< LOD	< LOD	0.85	2.98
						NC[b]	< LOD	< LOD	0.44	2.62
	NHANES, 2007–2008[28]	USA	$n=1286$, women, ≥6 years	0.6	NA	NC[a]	< LOD	< LOD	< LOD	6.23
						NC[b]	< LOD	< LOD	< LOD	5.08
	CHAMACOS, 1999–2000[29]	USA	$n=481$, pregnant women, ≥18 years	0.4	17%	NA[a]	NA	NA	NA	NA
						NC[b]	< LOD	< LOD	< LOD	1.5
	Heudorf and Angerer (2001)[17]	Germany	$n=483$, ≥20 years	0.1 – 0.2	62%	NA[a]	< LOD	0.17	0.39	1.28
						NA[b]	< LOD	0.23	0.45	1.67
	Wielgomas et al. (2013)[30]	Poland	$n=132$, 5–77 years	0.05 – 0.1	7%	NC[a]	< LOD	< LOD	< LOD	0.17
						NC[b]	< LOD	< LOD	< LOD	0.12
	Jiangsu, 2009–2010[16]	China	$n=1149$, pregnant women, 17–45 years	0.1	98%	1.91[a]	0.80	1.86	4.50	15.07
						1.22[b]	0.48	1.17	2.97	11.73
	Dewailly et al. (2014) [31]	Ten Caribbean countries	$n=296$, pregnant women	0.01	99%	NA[a]	NA	NA	NA	NA
						0.40[b]	NA	NA	NA	2.44
3-PBA										
	Shandong, 2010–2012	China	$n=454$, pregnant women, 18–45 years	0.1	82%	0.57[a]	0.32	0.68	1.24	3.31
						0.54[b]	0.33	0.62	1.17	3.27
	NHANES, 1999–2000[7]	USA	n =1024, women, 6–59 years	0.1	68%	0.32[a]	< LOD	0.28	0.60	5.03
						0.31[b]	< LOD	0.25	0.74	6.03
	NHANES, 2001–2002[7]	USA	$n=1617$, women, ≥6 years	0.1	73%	0.39[a]	< LOD	0.33	0.73	4.43
						0.31[b]	< LOD	0.25	0.73	3.76
	NHANES, 2007–2008[28]	USA	$n=1238$, women, ≥6 years	0.1	NA	0.50[a]	NA	0.43	1.28	7.20
						0.39[b]	NA	0.36	1.07	6.79
	CHAMACOS, 1999–2000[29]	USA	$n=481$, pregnant women, ≥18 years	0.1	27%	NA[a]	NA	NA	NA	NA
						NC[b]	< LOD	< LOD	0.1	1.1
	Heudorf and Angerer (2001)[17]	Germany	$n=483$, ≥20 years	NA	NA	NA[a]	NA	NA	NA	NA
						NA[b]	NA	NA	NA	NA
	Wielgomas et al. (2013)[30]	Poland	$n=132$, 5–77 years	0.05 – 0.1	80%	0.22[a]	0.12	0.19	0.42	0.95
						0.26[b]	0.14	0.26	0.47	1.15
	Jiangsu, 2009–2010[16]	China	$n=1149$, pregnant women, 17–45 years	0.1	99%	1.53[a]	0.85	1.55	2.95	8.11
						0.97[b]	0.52	1.01	1.94	5.39
	Dewailly et al. (2014) [31]	Ten Caribbean countries	$n=297$, pregnant women	0.01	100%	NA[a]	NA	NA	NA	NA
						0.54[b]	NA	NA	NA	3.51

LOD, limit of detection; NC, not calculated because proportion of results below the LOD was too high to provide reliable result; NA, not applicable; Unit is μg/g creatinine (creatinine-adjusted levels) for individual metabolites; < LOD means less than the limit of detection for the urine levels not corrected for creatinine. [a]Creatinine-adjusted concentrations (μg/g creatinine). [b]Volume-based concentrations (μg/l, not adjusted for creatinine).

© 2015 Nature America, Inc.

The low frequency of detection of 4F3PBA and cis-DBCA indicated that exposure to cyfluthrin and deltamethrin was infrequent.

Effects of Prenatal Pyrethroid Exposures on Birth Outcomes

This study not only investigated the pyrethroid metabolite levels in maternal urine but also examined the possible relationships of pyrethroid exposures with birth outcomes. Our results indicated that a 10-fold increase in total but not individual urinary metabolite levels was associated with a decrease in birth weight (adjusted $\beta = -96.76$ g, 95% CI = −173.15 to −20.37). No associations were found between individual or total metabolite levels and birth length, head circumference, or gestational duration.

Few epidemiological studies have examined the effects of synthetic pyrethroids on developmental outcomes, and they also have reported inconsistent results.[19,20,37–39] However, only one study that has measured biomarker (phenoxybenzoic acid) of pyrethroid exposures in maternal urine during the third trimester of pregnancy found no association with birth weight, length, head circumference, or gestational age.[40] Unlike our study, most of previous studies examining the relationship of pyrethroid exposures with fetal growth have assessed exposure based on the reported home use or parental occupation. Because exposure is measured indirectly and is self-reported, it may be substantially different from the actual measured value. Furthermore, the lack of information about specific chemicals has made it difficult to pinpoint risks associated with specific pesticides or classes of chemicals.[13] Direct comparison with available studies is complicated by differences in the exposure scenarios, method of exposure assessment, and statistical approaches. Our study observed that the total metabolite levels of pyrethroids in maternal urine were negatively associated with birth weight.

The precise mechanism for the potential adverse fetal growth effects associated with pyrethroids is not well understood, particularly in the absence of a clinically defined adverse outcome. Fetuses may be more susceptible to pyrethroids toxicity because their organ systems are developing rapidly, and they have lower than adult levels of detoxifying enzymes. Differences in toxicity are most likely the result of the pyrethroid-specific metabolism by carboxylesterase and cytochrome P450 enzymes, which are expressed at much lower levels in the developing mammal.[41] Indeed, the use of esterase and P450 inhibitors was observed to alter the toxicity of pyrethroids in animals.[42] Pyrethroids have been considered as potential endocrine disruptors, which could adversely affect fetal growth and development.[8–12] In addition, sodium and calcium channels as well as $GABA_A$ receptors have also been reported to be molecular targets of these compounds.[43–45] Recent data supported the concept that these alternate targets may contribute to differences in toxicity observed with these compounds.[46]

To our knowledge, this study is the first in China to examine possible adverse effects of prenatal exposure to pyrethroids on fetal growth. However, our study also has several limitations. First, as in most studies on the effects of pesticide exposure, we did not adjust for other environmental pollutants (e.g. organophosphate insecticides, lead, and mercury) or possible developmental benefits (e.g. prenatal nutrients and prenatal care) which may be potential confounders, since we had little detailed data on these information. Although several important factors have been adjusted as potential confounders for the final analysis, it is possible that unassessed confounding effects may still exist. Second, we measured urinary metabolites of pyrethroids at a single time point only (delivery). We are limited in our ability to understand what the average cumulative dose from different sources was and to what extent these measurements reflect exposures over critical windows before conception and during entire pregnancy.[47] However, in cases of chronic exposure, a biomarker measured at a single time point may provide a representative dosimeter even if the toxicant has a short half-life.[48] Cotinine, for example, has a half-life of 15–40 h in plasma but is well validated as a biomarker of cigarette smoke exposure.[49,50] Third, the non-specific metabolite measurements do not allow differentiation between exposures that result from more or from less toxic pyrethroids. However, they are important indicators and excellent integrated measures of exposure to a class of insecticides in humans.[51] Currently, there are no analytical methods for measurement of specific exposure to many important pyrethroids in urine or in blood. Fourth, the measurements of urinary non-specific metabolites characterize and integrate multiple sources of pyrethroid exposure, but the measurements reflect both exposure to parent compounds and non-toxic preformed metabolites in the environment. In addition, questionnaires were administered to women after delivery, use of these questionnaire data may be a limitation; however, the majority of outcome measures used in the study were based on medical records.

In summary, we observed an adverse association of prenatal exposure to pyrethroids as measured by urinary metabolites with birth weight, and these findings should be further replicated in other populations. Given the relatively high levels of urinary metabolites in pregnant women in China, a large longitudinal study with repeated measurement of exposure levels in urine samples is needed to further examine the possible relationship between prenatal exposures and children's long-term health. In the meantime, it is important to take the precaution of minimizing pesticide exposures to pregnant women and children wherever possible.

ABBREVIATIONS

CI, confidence interval; cis-DBCA, cis-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid cis-DCCAcis-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid 4F3PBA4-fluoro-3-phenoxybenzoic acid; GM, geometric mean; LOD, limit of detection; 3-PBA3-phenoxybenzoic acid; trans-DCCA, trans-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We acknowledge the Department of Environmental Health staff, students, hospital partners, participants, and families without whom this study would have not been possible. We specifically thank Guoquan Wang and Chao Feng (CDC, Shanghai) for their work on specimen management and pyrethroid insecticide measurement. This research was partially supported by the Natural Science Foundation of China (Grant No. 81172625 and 81402645) and the Key Discipline Construction Project of Shanghai Municipal Public Health (Grant No. 12GWZX0401).

REFERENCES

1 Lu C, Barr DB, Pearson M, Bartell S, Bravo R. A longitudinal approach to assessing urban and suburban children's exposure to pyrethroid pesticides. *Environ Health Perspect* 2006; **114**: 1419–1423.
2 Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D *et al*. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology* 2002; **171**: 3–59.
3 Agency for Toxic Substances and Disease Registry (Atlanta, GA). Toxicological Profile for Pyrethrins and Pyrethroids, September, 2003. < http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id = 787&tid = 153 >. (accessed 3 March 2011).
4 Shafer TJ, Meyer DA, Crofton KM. Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs. *Environ Health Perspect* 2005; **113**: 123–136.
5 Weselak M, Arbuckle TE, Foster W. Pesticide exposures and developmental outcomes: the epidemiological evidence. *J Toxicol Environ Health B Crit Rev* 2007; **10**: 41–80.

© 2015 Nature America, Inc.

6 Leng G, Leng A, Kühn KH, Lewalter J, Pauluhn J. Human dose-excretion studies with the pyrethroid insecticide cyfluthrin: urinary metabolite profile following inhalation. *Xenobiotica* 1997; **27**: 1273–1283.
7 Barr DB, Olsson AO, Wong LY, Udunka S, Baker SE, Whitehead RD *et al*. Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999-2002. *Environ Health Perspect* 2010; **118**: 742–748.
8 Du G, Shen O, Sun H, Fei J, Lu C, Song L *et al*. Assessing hormone receptor activities of pyrethroid insecticides and their metabolites in reporter gene assays. *Toxicol Sci* 2010; **116**: 58–66.
9 Akhtar N, Kayani SA, Ahmad MM, Shahab M. Insecticide-induced changes in secretory activity of the thyroid gland in rats. *J Appl Toxicol* 1996; **16**: 397–400.
10 Maiti PK, Kar A. Is triiodothyronine capable of ameliorating pyrethroid-induced thyroid dysfunction and lipid peroxidation? *J Appl Toxicol* 1998; **18**: 125–128.
11 Bian Q, Xu LC, Wang SL, Xia YK, Tan LF, Chen JF *et al*. Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers. *Occup Environ Med* 2004; **61**: 999–1005.
12 Pine MD, Hiney JK, Lee B, Dees WL. The pyrethroid pesticide esfenvalerate suppresses the afternoon rise of luteinizing hormone and delays puberty in female rats. *Environ Health Perspect* 2008; **116**: 1243–1247.
13 Ding G, Shi R, Gao Y, Zhang Y, Kamijima M, Sakai K *et al*. Pyrethroid pesticide exposure and risk of childhood acute lymphocytic leukemia in Shanghai. *Environ Sci Technol* 2012; **46**: 13480–13487.
14 Landrigan PJ, Kimmel CA, Correa A, Eskenazi B. Children's health and the environment: public health issues and challenges for risk assessment. *Environ Health Perspect* 2004; **112**: 257–265.
15 Wu C, Feng C, Qi X, Wang G, Zheng M, Chang X *et al*. Urinary metabolite levels of pyrethroid insecticides in infants living in an agricultural area of the Province of Jiangsu in China. *Chemosphere* 2013; **90**: 2705–2713.
16 Qi X, Zheng M, Wu C, Wang G, Feng C, Zhou Z *et al*. Urinary pyrethroid metabolites among pregnant women in an agricultural area of the Province of Jiangsu, China. *Int J Hyg Environ Health* 2012; **215**: 487–495.
17 Heudorf U, Angerer J. Metabolites of pyrethroid insecticides in urine specimens: current exposure in an urban population in Germany. *Environ Health Perspect* 2001; **109**: 213–217.
18 Oulhote Y, Bouchard MF. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in canadian children. *Environ Health Perspect* 2013; **121**: 1378–1384.
19 Hanke W, Romitti P, Fuortes L, Sobala W, Mikulski M. The use of pesticides in a Polish rural population and its effect on birth weight. *Int Arch Occup Environ Health* 2003; **76**: 614–620.
20 Dabrowski S, Hanke W, Polańska K, Makowiec-Dabrowska T, Sobala W. Pesticide exposure and birthweight: an epidemiological study in Central Poland. *Int J Occup Med Environ Health* 2003; **16**: 31–39.
21 US Environmental Protection Agency. Organophosphate Pesticides: Revised Cumulative Risk Assessment, June 2002. < http://www.epa.gov/oppsrrd1/cumulative/rra-op/ >. (accessed 9 May 2012).
22 Barr DB, Needham LL. Analytical methods for biological monitoring of exposure to pesticides: a review. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002; **778**: 5–29.
23 Eadsforth CV, Bragt PC, van Sittert NJ. Human dose-excretion studies with pyrethroid insecticides cypermethrin and alphacypermethrin: relevance for biological monitoring. *Xenobiotica* 1988; **18**: 603–614.
24 Leng G, Kühn KH, Idel H. Biological monitoring of pyrethroid metabolites in urine of pest control operators. *Toxicol Lett* 1996; **88**: 215–220.
25 Ding G, Cui C, Chen L, Gao Y, Zhou Y, Shi R *et al*. Prenatal low-level mercury exposure and neonatal anthropometry in rural northern China. *Chemosphere* 2013; **92**: 1085–1089.
26 Kühn K, Leng G, Bucholski K, Dunemann L, Idel H. Determination of pyrethroid metabolites in human urine by capillary gas chromatography – mass spectrometry. *Chromatographia* 1996; **43**: 285–292.
27 Hornung RW, Reed DL. Estimation of average concentration in the presence of nondetectable values. *Appl Occup Environ Hyg* 1990; **5**: 46–51.
28 US Centers for Disease Control and Prevention. National Report on Human Exposure to Environmental Chemicals, August 2014. < http://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Aug2014.pdf >. (accessed 3 June 2014).
29 Castorina R, Bradman A, Fenster L, Barr DB, Bravo R, Vedar MG *et al*. Comparison of current-use pesticide and other toxicant urinary metabolite levels among pregnant women in the CHAMACOS cohort and NHANES. *Environ Health Perspect* 2010; **118**: 856–863.
30 Wielgomas B, Nahorski W, Czarnowski W. Urinary concentrations of pyrethroid metabolites in the convenience sample of an urban population of Northern Poland. *Int J Hyg Environ Health* 2013; **216**: 295–300.
31 Dewailly E, Forde M, Robertson L, Kaddar N, Laouan Sidi EA, Côté S *et al*. Evaluation of pyrethroid exposures in pregnant women from 10 Caribbean countries. *Environ Int* 2014; **63**: 201–206.
32 Qin L, Yang M, Wang J, Jiang D, Cao Q. Surveillance on pesticides residues in vegetables and fruits [in Chinese]. *Shi Yong Yu Fang Yi Xue* 2011; **8**: 2315–2316.
33 GB2763 – 2005 *National Standard of Maximum Residue Limits for Pesticides in Food [in Chinese]*. Nation Programme on Food Safety: Beijing, China, 2005.
34 Zhang W, Jiang F, Ou J. Global pesticide consumption and pollution: with China as a focus. *Proc Int Ecol Environ Sci*2011; **1**: 125–144.
35 Li P, Feng X, Yuan X, Chan HM, Qiu G, Sun GX *et al*. Rice consumption contributes to low level methylmercury exposure in southern China. *Environ Int* 2012; **49**: 18–23.
36 Ministry of Environmental Protection of China. Organophosphate Pesticide Wastewater Discharge Standards, November, 2008 [in Chinese] < http://www.zhb.gov.cn/ >. (accessed 24 November 2008).
37 Rupa DS, Reddy PP, Reddi OS. Reproductive performance in population exposed to pesticides in cotton fields in India. *Environ Res* 1991; **55**: 123–128.
38 Bell EM, Hertz-Picciotto I, Beaumont JJ. A case-control study of pesticides and fetal death due to congenital anomalies. *Epidemiology* 2001; **12**: 148–156.
39 Koureas M, Tsakalof A, Tsatsakis A, Hadjichristodoulou C. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. *Toxicol Lett* 2012; **210**: 155–168.
40 Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, Godbold JH *et al*. In utero pesticide exposure, maternal paraoxonase activity, and head circumference. *Environ Health Perspect* 2004; **112**: 388–391.
41 Ross MK, Borazjani A, Edwards CC, Potter PM. Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. *Biochem Pharmacol* 2006; **71**: 657–669.
42 Cantalamessa F. Acute toxicity of two pyrethroids, permethrin, and cypermethrin in neonatal and adult rats. *Arch Toxicol* 1993; **67**: 510–513.
43 Burns CJ, McIntosh LJ, Mink PJ, Jurek AM, Li AA. Pesticide exposure and neurodevelopmental outcomes: review of the epidemiologic and animal studies. *J Toxicol Environ Health B Crit Rev* 2013; **16**: 127–283.
44 Hildebrand ME, McRory JE, Snutch TP, Stea A. Mammalian voltage-gated calcium channels are potently blocked by the pyrethroid insecticide allethrin. *J Pharmacol Exp Ther* 2004; **308**: 805–813.
45 Crofton KM, Reiter LW. Pyrethroid insecticides and the gamma-aminobutyric acidA receptor complex: motor activity and the acoustic startle response in the rat. *J Pharmacol Exp Ther* 1987; **243**: 946–954.
46 Breckenridge CB, Holden L, Sturgess N, Weiner M, Sheets L, Sargent D *et al*. Evidence for a separate mechanism of toxicity for the Type I and the Type II pyrethroid insecticides. *Neurotoxicology* 2009; **30**: S17–S31.
47 Ding G, Wang P, Tian Y, Zhang J, Gao Y, Wang X *et al*. Organophosphate pesticide exposure and neurodevelopment in young Shanghai children. *Environ Sci Technol* 2012; **46**: 2911–2917.
48 Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R *et al*. Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environ Health Perspect* 2004; **112**: 1125–1132.
49 Kemmeren JM, van Poppel G, Verhoef P, Jarvis MJ. Plasma cotinine: stability in smokers and validation of self-reported smoke exposure in nonsmokers. *Environ Res* 1994; **66**: 235–243.
50 Woodward M, Tunstall-Pedoe H, Smith WC, Tavendale R. Smoking characteristics and inhalation biochemistry in the Scottish population. *J Clin Epidemiol* 1991; **44**: 1405–1410.
51 Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB *et al*. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environ Health Perspect* 2004; **112**: 1116–1124.

© 2015 Nature America, Inc.