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Pesticide exposure, birth size, and gestational age in the ISA birth cohort, Costa Rica

Berna van Wendel de Joode[a,*] Jorge Peñaloza-Castañeda[a], Ana M. Mora[a,b], Andrea Corrales-Vargas[a], Brenda Eskenazi[b], Jane A. Hoppin[c,d], Christian H. Lindh[e]

Purpose: To examine associations of prenatal biomarkers of pesticide exposure with birth size measures and length of gestation among newborns from the Infants' Environmental Health (ISA) birth cohort, Costa Rica.

Methods: We included 386 singleton liveborn newborns with data on birth size measures, length of gestation, and maternal urinary biomarkers of chlorpyrifos, synthetic pyrethroids, mancozeb, pyrimethanil, and 2, 4-D during pregnancy. We associated biomarkers of exposure with birth outcomes using multivariate linear regression and generalized additive models.

Results: Concentrations were highest for ethylene thiourea (ETU, metabolite of mancozeb), median = 3.40; p10–90 = 1.90–6.79 µg/L, followed by 3,5,6-trichloro-2-pyridinol (TCP, metabolite of chlorpyrifos) p50 = 1.76 p10–90 = 0.97–4.36 µg/L, and lowest for 2,4-D (p50 = 0.33 p10–90 = 0.18–1.07 µg/L). Among term newborns (≥37 weeks), higher prenatal TCP was associated with lower birth weight and smaller head circumference (e.g., β per 10-fold-increase) during the second half of pregnancy = –129.6 (95% confidence interval [CI] = –255.8, –3.5) grams, and –0.61 (95% CI = –1.05, –0.17) centimeters, respectively. Also, among term newborns, prenatal 2,4-D was associated with lower birth weight (β per 10-fold-increase = –125.1; 95% CI = –228.8, –21.5), smaller head circumference (β = –0.41; 95% CI = –0.78, –0.03), and, during the second half of pregnancy, with shorter body length (β = –0.58; 95% CI = –1.09, –0.07). Furthermore, ETU was nonlinearly associated with head circumference during the second half of pregnancy. Biomarkers of pyrethroids and pyrimethanil were not associated with birth size, and none of the biomarkers explained the length of gestation.

Conclusions: Prenatal exposure to chlorpyrifos and 2,4-D, and, possibly, mancozeb/ETU, may impair fetal growth.

[a]*Infants' Environmental Health (ISA) Program, Central American Institute for Studies on Toxic Substances (IRET), Universidad Nacional, Heredia, Costa Rica;* [b]*Center for Environmental Research and Community Health (CERCH), University of California at Berkeley;* [c]*Center for Human Health and the Environment, North Carolina State University, North Carolina;* [d]*Department of Biological Sciences, North Carolina State University, North Carolina; and* [e]*Division of Occupational and Environmental Medicine, Lund University, Sweden*

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**Corresponding Author. Address: Infants' Environmental Health (ISA) Program, Central American Institute for Studies on Toxic Substances (IRET), Universidad Nacional, Campus Omar Dengo, Edificio de Bioprocesos Industriales, Apartado 86-3000, Heredia, Costa Rica. E-mail: berendina.vanwendel.dejoode@una.cr (B. van Wendel de Joode).*

Introduction

Pesticides may pass the blood-placenta barrier, possibly affecting fetal development and growth.[1,2] Harmful effects in the developing fetus may result from lower concentrations of chemical exposures as compared to adults, due to rapid cell turnover and tissue growth during fetal development.[3] Prematurity, low birth weight, and intrauterine growth restriction are risk factors for neurodevelopmental delay.[4] Similarly, smaller head circumference has been related to reduced brain weight and size[5,6] and impaired cognitive development.[7]

Fetal growth can be restricted because of suboptimal uterine-placental perfusion and fetal nutrition.[8] Maternal conditions that may affect fetal growth include pregnancy-related hypertensive diseases, gestational diabetes, and tobacco use.[8,9] In addition, nonpersistent chemicals may disturb fetal growth as indicated by knowledge of biological mechanisms and evidence from animal models.[10] For example, pesticides may disrupt maternal thyroid hormone concentrations that are essential for fetal growth and endocrine regulation.[10,11] Also, pesticides may

What this study adds

This study adds to our knowledge about the effects of pesticides on fetal growth measured at birth and gestational age. Most cohorts studied the effects of organophosphate insecticide exposure, but results have not been consistent across cohorts, and few addressed the effects of non-organophosphate insecticide pesticides.

We evaluated if biomarkers of prenatal pesticide exposure explained a newborn's birth size measures and gestational age in the Infants' Environmental Health study, Costa Rica. Our findings indicate chlorpyrifos and 2,4-D, and possibly mancozeb, may impair fetal growth, particularly during the second half of pregnancy. Our results are of concern as these pesticides are used worldwide.

generate oxidative stress and maternal inflammation, which have been associated with reduced fetal growth.[3,10] However, relatively few birth cohorts have examined the effects of pesticide exposure on fetal growth and gestational age.[10] Most studies have studied the effects of organophosphate insecticide (OP) exposure,[12–20] but results have not been consistent across cohorts,[10,21,22] and few addressed effects of non-OP pesticides[15,23–27]

The infant's environmental health (ISA, for its acronym in Spanish) birth cohort[28] is situated in a rural area in Costa Rica with extensive banana production for export to Europe and the United States of America, in which pesticides are intensively applied year-round.[29] This use has included the application of chlorpyrifos-treated bags and aerial spraying of the fungicides mancozeb and pyrimethanil (Table S1; http://links.lww.com/EE/A260).[11,30,31] Furthermore, the herbicide 2,4-D is used on pastures and, sometimes, soccer fields, and synthetic pyrethroid insecticides are used for vector control in the home environment.[32] These pesticides are commonly used worldwide,[33–36] although in 2020, the use of both chlorpyrifos and mancozeb was not renewed in the European Union[37,38] due to concerns regarding neurodevelopmental toxicity,[39] reprotoxic, and endocrine disrupting effects,[40] respectively. Also, the United States Environmental Protection Agency has published requests for the voluntary cancellation of chlorpyrifos products and to end food use.[41] In Costa Rica, its use is restricted to agricultural use.[42]

In the current study, we evaluated if exposure to chlorpyrifos, synthetic pyrethroids, mancozeb, pyrimethanil, and 2,4-D was associated with measures of birth size and gestational age among newborns from the ISA birth cohort while adjusting for possible confounders.

Methods

Study population

As part of a community-based cohort study, we enrolled pregnant women (n = 451) (98% response rate) between March 2010 and June 2011.[28] Women were eligible if: aged ≥15 years, gestational age <33 weeks, living at <5 kilometers from a banana plantation in Matina County. Out of the 451 pregnancies, 21 (5%) resulted in miscarriage, stillbirth, or neonatal death; 39 (9%) women were lost-to-follow-up before childbirth, and a set of twins (n = 2) were excluded from the analysis.[43] Of the remaining 389 mother-child pairs, 386 singleton liveborn infants (86% of the enrolled population) had data on maternal urinary pesticide concentrations during pregnancy, birth weight, and length of gestation. The Scientific Ethics Committee of the Universidad Nacional, Costa Rica (CECUNA) approved all study activities. All women supplied written informed consent before enrollment. For women aged <18 years, we obtained additional written informed consent from their parents or legal guardians.

Data collection

Depending on their gestational age at enrollment, we interviewed the 386 women with singleton liveborn infants 1–3 times during pregnancy (first, second, and third trimester: n = 103, 314, and 307, respectively; median gestational ages at the first, second, and third study visit = 19, 30, and 33 weeks, respectively), and postpartum (median = 7 weeks) as described previously.[43] In short, we collected information about sociodemographics, occupational and lifestyle characteristics, and medical history. We also abstracted data from medical records completed by clinic/hospital personnel and provided to the pregnant women (e.g., maternal prepregnancy weight, timing of prenatal care initiation, blood pressure (BP), and other medical conditions). We then calculated prepregnancy body mass index (BMI) as (weight in kilograms)/(height in meters),^{2} using maternal prepregnancy weight (when available) or weight at the first prenatal care visit (if <14 weeks gestation) and height measured by the ISA study interviewers. We identified women with gestational hypertension using BP levels abstracted from medical records, that is, women with ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic in two or more prenatal control visits at <20 weeks of pregnancy, but with normal BP before pregnancy, diagnosis abstracted from medical records, and maternal report of hypertensive drug use during pregnancy. We identified women with gestational diabetes using maternal reports and diagnoses from physicians' reports abstracted from medical records.

Birth size and length of gestation

We evaluated fetal growth by recollecting data on birth size, and abstracted data on birth weight (grams), body length (cm), and head circumference (cm) from medical records provided to the study participants. We calculated the infant ponderal index, a measure of proportionality of growth as (birth weight in grams × 100)/(body length in centimeters).[3]

We generally estimated the length of gestational age using the date of last menstrual period (LMP) but used the estimate from: (1) an ultrasound during the first trimester if its difference with LMP was >7 days; (2) medical record estimate at birth if the difference with LMP was ≥14 days, and; (3) first measure of fundal height registered in the medical prenatal control record to determine gestational age if no other data were available. The gestational age at the time of biological sample collection was calculated using data on the length of gestation and date of sample collection.

Urinary pesticide metabolites measurements

We assessed prenatal pesticide exposure by measuring biomarkers in maternal urine obtained 1–3 times during pregnancy for a total of 828 urine samples from 386 women (n = 233 from 207 women during the first half [<20 weeks] of gestation and n = 595 from 375 women during the second half [≥20 weeks] of gestation). We collected the samples at the same time as the interviews in 100 mL beakers (Vacuette, sterile), and aliquoted them into 15 mL tubes (Performer Centrifuge tubes, Labcon, sterile), and then stored them at −20 °C until shipment to the Division of Occupational and Environmental Medicine at Lund University, Sweden, for analysis.

We analyzed urine samples for the following pesticide metabolites: ethylene thiourea (ETU, metabolite of mancozeb); hydroxypyrimethanil (OHP, metabolite of pyrimethanil); 5-hydroxythiabendazole (OHT, metabolite of thiabendazole), 3,5,6-trichloro-2-pyridinol (TCP, metabolite of chlorpyrifos), 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCCA, sum of cis and trans, metabolite of permethrin, cypermethrin, cyfluthrin, as well as other pyrethroids); 3-phenoxybenzoic acid (3PBA, metabolite of permethrin, cypermethrin, deltamethrin, allethrin, resmethrin, fenvalerate, and other pyrethroids); and the herbicide 2,4-D (Table S1; http://links.lww.com/EE/A260)

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Urinary metabolites were analyzed in duplicate using a liquid chromatography mass spectrometry (LC-MS/MS; QTRAP 5500; AB Sciex) as described by Norén et al.[44] For TCPy and 3PBA analysis, the laboratory participates in the German External QUality Assessment Scheme (G-EQUAS) coordinated by University of Erlangen-Nuremberg, Germany (Figure S1; http://links.lww.com/EE/A260). Mean concentrations of the duplicate samples were used in further calculations. Between-run and between-batch precisions were 4–18% and 8–19%, respectively.[30] ETU and TCP were detected in all samples, while 2,4-D and 3PBA were detected in 99.8%, DCCA in 99.3%, OHP in 86.6%, and OHT in 64.7% of the samples (n = 828), respectively. We used concentrations indicated by LC-MS/MS for values ≥ limit of detection (LOD)/2 and imputed values <LOD with LOD/2.

Urinary specific gravity (kg/L) was determined using a hand refractometer, and pesticide metabolite concentrations were normalized for dilution using the formula $M_{SG} = M \times [(1.017 - 1)/(SG - 1)]$, where M_{SG} is the specific-gravity-corrected metabolite concentration (µg/L), M is the observed metabolite concentration (µg/L), SG is the specific gravity of the urine sample, and 1.017 kg/L is the average specific gravity for all urine samples included in these analyses (n = 828).

Statistical analysis

We limited our analysis to biomarkers of exposure that were detected in at least 85% of the samples and therefore excluded OHT. We calculated descriptive statistics and distributional plots for all variables. We then estimated bivariate associations between covariates and outcomes using t-tests, chi-square tests, and linear regression models. We also estimated correlations between specific gravity-corrected urinary pesticide metabolite concentrations using Spearman's correlation coefficients (R_s). We calculated intraclass correlation coefficientsusing mixed effect models to assess the within- and between-woman variability of metabolite concentrations in maternal urine samples collected during pregnancy.[45] We then averaged individual specific gravity-corrected urinary pesticide metabolite concentrations across the repeated samples collected for each woman throughout pregnancy. To evaluate the effect of time of exposure during pregnancy, we also calculated averaged concentrations for the first (<20 weeks) and for the second (≥20 weeks) half of pregnancy. We transformed specific gravity-corrected urinary pesticide biomarkers to the $\log_{10}$ scale to normalize the residuals and to reduce the influence of outliers. With respect to the analyses on pesticide exposure and birth weight, birth length, ponderal index, and head circumference, we excluded preterm newborns (<37 weeks), as birth weight in preterm newborns may reflect growth restriction and/or prematurity.

We then examined associations of maternal urinary pesticide metabolite concentrations during pregnancy with birth outcomes using separate multivariate linear regression models for each metabolite, using a similar approach as published previously.[43] We included maternal age, newborn's sex, parity, smoking during pregnancy, pre-pregnancy BMI, and maternal country of birth *a priori* in the models for being known predictors of fetal growth and gestational age,[43] or possible confounders (Figure S2; http://links.lww.com/EE/A260, directed acyclic graph). In addition, we evaluated the effect of the following covariables on effect estimates: history of miscarriage, previous low birth weight delivery, interpregnancy interval, timing of prenatal care initiation, caffeine consumption during pregnancy, maternal marital status, maternal education, maternal occupation, family income, alcohol consumption, illegal drug use, iron intake, vaginal bleeding, high blood pressure, and gestational diabetes. We added them to the multivariable models if they were associated with at least one of the outcomes ($P < 0.10$) and kept them in the model if they changed the effect estimates in any of the models by at least 10%. Nevertheless, none of these other covariables met these criteria.

Missing covariate values were imputed at random based on observed probability distributions (<5% missing).[46] For women missing information about prepregnancy weight (n = 21), we predicted this variable from a regression model including maternal weight 1-year postpartum and parity (0/≥1) ($R^2 = 0.84$) for 18 of the women. For the remaining three women, without information about their weight at the 1-year visit, we predicted prepregnancy weight by a regression model including maternal education and maternal age at enrollment ($R^2 = 0.12$).[11]

In addition to the linear regression models, we fitted covariate-adjusted generalized additive models with penalized spline smooth terms for continuous exposures (constrained to a maximum of 4 knots). We considered models with estimated degrees of freedom (edf) ≥2 and $P < 0.05$ nonlinear.

We conducted several sensitivity analyses to assess the robustness of our results. First, we reran models after excluding outliers of residuals defined as the 1% of the highest values of Cook's distance. If the effect estimates for a specific biomarker of exposure changed by 10% or more, we presented models without outliers in our results section. Second, to understand the effect of the temporality of exposure, we included, in the same model but separately for each biomarker, the concentrations measured during the first and second half of pregnancy. Third, we evaluated the effect of summed pyrethroid metabolites. Finally, we considered the effect of multiple pesticides by including biomarkers of exposure associated with outcomes ($P < 0.10$) in the same models.

Results

Table 1 describes the characteristics of the 386 newborns and their mothers. About half (49.3%) of the newborns were girls, 6.5% were born preterm, and 3.1% had low birthweight. Mothers were young (18% <18 years old and 47% between 18 and 24 years), and about half (51%) only had primary school or less. Few women reported cigarette smoking (4.7%), using alcohol (3.1%), or illegal drugs (1%) during pregnancy. Only some women suffered from gestational diabetes (3.9%) and gestational high blood pressure (3.6%). Only 8.5% of the women (n = 33) worked in agriculture during pregnancy, mostly on banana plantations (n = 30, 8%). About a quarter of the women (26%) lived less than 50 meters from a banana plantation.

Overall, specific-gravity-corrected urinary biomarker concentrations were highest for ETU (p50 = 3.40; p10–p90 = 1.90–6.79 µg/L), followed by TCP (p50 = 1.76 p10–p90 = 0.97–4.36 µg/L) and DCCA (p50 = 1.30 p10–p90 = 0.50–4.30 µg/L), and were lowest for 2,4-D (p50 = 0.33 p10–90 = 0.18–1.07 µg/L) (Table 2). Exposure levels were similar for the first and second half of pregnancy (Table 2), also when we restricted our analysis to women with urine samples in both the first and second halves of pregnancy (Table S2; http://links.lww.com/EE/A260). Except for DCCA and 3PBA (*rho* = 0.82), both metabolites of synthetic pyrethroids, biomarkers of exposure were only weakly correlated (range of *rho*'s = 0.15–0.25; Table S3; http://links.lww.com/EE/A260).

Results presented in Table 3 (see Table S4; http://links.lww.com/EE/A260 for crude beta estimates) show term newborns (≥37 weeks) with higher prenatal urinary TCP and/or 2,4-D concentrations had decreased birth weight, body length, and head circumference, especially during the second half of pregnancy. For example, for birth weight (grams): $\beta_{\text{per-ten-fold-increase}}$ for urinary TCP and 2,4-D, respectively = −129.6 (95% confidence interval [CI] = −255.8 to −3.5) and −127.4 (95% CI = −223.3, −31.5) during the second half of pregnancy. In addition, for head circumference (centimeters) $\beta_{\text{per-ten-fold-increase}}$

Table 1.

Characteristics of newborns and their mother from the ISA study for all (n = 386) and full-term (n = 361) newborns with information about gestational age and birth weight and at least one urine sample during pregnancy

		n(%)	
Characteristics	**Value**	**All**	**Term**
Sex	Boy	194 (50.3%)	177 (49%)
	Girl	192 (49.7%)	184 (51%)
Preterm birth		25 (6.5%)	-
Low birthweight		12 (3.1%)	4 (1.1%)
Maternal age (years)	<18	68 (17.6%)	66 (18.3%)
	18–24	181 (46.9%)	166 (46%)
	25–29	67 (17.4%)	64 (17.7%)
	30–34	37 (9.6%)	35 (9.7%)
	35	33 (8.5%)	30 (8.3%)
Maternal education	≤6th grade	198 (51.3%)	185 (51.2%)
	7–11th grade	177 (45.9%)	165 (45.7%)
	Completed high school	11 (2.8%)	11 (3%)
Mother is married or living as married		292 (75.6%)	272 (75.3%)
Born in Costa Rica		312 (80.8%)	295 (81.7%)
Income per capita[a]	Above poverty line	154 (40.6%)	141 (39.8%)
	Below poverty line and above extreme poverty	154 (40.6%)	144 (40.7%)
	Below extreme poverty line	71 (18.7%)	69 (19.5%)
Parity (≥1)[a]		243 (63.6%)	226 (63.1%)
History of miscarriage[a]		65 (17.4%)	59 (16.9%)
Prepregnancy BMI (kg/m^2)	Underweight	12 (3.1%)	12 (3.3%)
	Normal	189 (49%)	177 (49%)
	Overweight	101 (26.2%)	94 (26%)
	Obese	84 (21.8%)	78 (21.6%)
Start of prenatal care initiation[a]	First trimester	291 (76.4%)	276 (77.5%)
	Second trimester	76 (19.9%)	67 (18.8%)
	Third trimester	14 (3.7%)	13 (3.7%)
Smoking during pregnancy		18 (4.7%)	18 (5%)
Second-hand smoking during pregnancy		100 (25.9%)	97 (26.9%)
Cotinine detected in urine (>1 µg/L)		61 (15.8%)	59 (16.3%)
Alcohol consumption during pregnancy		12 (3.1%)	11 (3%)
Drug use during pregnancy[a]		4 (1%)	4 (1.1%)
Caffeinated tea consumption during pregnancy[a]		49 (12.8%)	44 (12.3%)
Caffeinated coffee consumption during pregnancy	No	112 (29%)	107 (29.6%)
	1 or less	163 (42.2%)	148 (41%)
	2 or more	111 (28.8%)	106 (29.4%)
Iron intake during pregnancy		354 (91.7%)	332 (92%)
Vitamin consumption during pregnancy		375 (97.2%)	350 (97%)
Gestational diabetes		15 (3.9%)	15 (4.2%)
Diabetes before pregnancy		5 (1.3%)	5 (1.4%)
Gestational high blood pressure[a]		14 (3.6%)	12 (3.3%)
Preeclampsia		2 (0.5%)	1 (0.3%)
Gestational anemia		156 (40.4%)	149 (41.3%)
Vaginal bleeding during first trimester		14 (3.9%)	12 (3.6%)
Maternal occupation	Agricultural work	33 (8.5%)	32 (8.9%)
	Other work (nonagricultural)	65 (16.8%)	61 (16.9%)
	No paid job	288 (74.6%)	268 (74.2%)
Paternal occupation[b]	Agricultural work	230 (62%)	215 (61.8%)
	Other work (nonagricultural)	119 (32.1%)	111 (31.9%)
	No paid job	22 (5.9%)	22 (6.3%)
Residential distance to banana plantations <50 meters		99 (25.6%)	91 (25.2%)

[a]Missing values were random imputed for: income per capita n=7, parity n = 4, history of miscarriage n=12, start of prenatal care initiation n=5, drug use during pregnancy n=2, caffeinated tea consumption during pregnancy n= 2, gestational high blood pressure n = 1, and vaginal bleeding during first trimester n = 29.
[b]Missing information for 15 cases.

for TCP and 2.4-D, respectively = −0.61 (95% CI = −1.05, −0.17) and −0.47 (−0.81, −0.12) during the second half of pregnancy. Also, higher prenatal 2,4-D concentrations were associated with shorter body length, especially for exposure during the second half of pregnancy: $\beta_{per\text{-}ten\text{-}fold\text{-}increase}$ = −0.58 (95% CI = −1.09, −0.07). Urinary ETU concentrations during the second half of pregnancy were nonlinearly associated with head circumference (Figure 1). Finally, DCCA, 3PBA, and OHP were not associated with any birth size measure (Table 3) and biomarkers of pesticide exposure did not explain newborns' gestational age or ponderal index.

When we restricted our analysis to newborns with information on maternal urinary pesticide metabolite concentrations in both the first and second half of pregnancy (n = 196), we observed similar results, although the association during the second half of pregnancy became stronger for TCP and weaker for 2.4-D (Table S5; http://links.lww.com/EE/A260). Finally, when we included both TCP and 2,4-D in the same models, results were comparable to the models that only included a single biomarker, although associations were somewhat attenuated for TCP (Figure 2, Table S6; http://links.lww.com/EE/A260).

Table 2.

Distribution of maternal urine biomarker concentrations during pregnancy (n = 828 samples from 386 women) and grouped by first (n = 233, 207 women) and second (n = 595, 375 women) half of pregnancy, Infant's Environmental Health Study ISA 2010–2011

Metabolite[a]			Prenatal concentrations[b]			Mean prenatal concentrations						
Period of pregnancy	Women	n	GM	GSD	ICC	Min	P10	P25	P50	P75	P90	Max
TCP												
Overall	386	828	1.95	2.00	0.38	0.41	0.97	1.31	1.76	2.54	4.36	62.96
First half	207	233	1.71	2.17	0.53	0.28	0.72	1.06	1.60	2.44	4.22	50.01
Second half	375	595	1.92	2.06	0.46	0.41	0.89	1.22	1.77	2.59	4.25	91.10
DCCA												
Overall	386	828	1.32	2.32	0.26	0.15	0.50	0.76	1.30	2.23	3.65	23.56
First half	207	233	1.12	2.87	0.46	0.06	0.33	0.59	1.12	1.82	4.30	45.77
Second half	375	595	1.24	2.35	0.23	0.13	0.43	0.67	1.20	2.20	3.53	18.06
3PBA												
Overall	386	828	0.83	2.23	0.28	0.10	0.30	0.48	0.79	1.30	2.43	16.96
First half	207	233	0.75	2.54	0.41	0.07	0.25	0.42	0.72	1.26	2.21	32.61
Second half	375	595	0.77	2.31	0.33	0.06	0.27	0.42	0.71	1.31	2.28	16.81
2.4-D												
Overall	386	828	0.39	2.33	0.26	0.09	0.18	0.23	0.33	0.53	1.07	79.76
First half	207	233	0.29	2.31	0.49	0.04	0.12	0.17	0.26	0.47	1.01	3.50
Second half	375	595	0.39	2.52	0.38	0.05	0.16	0.23	0.32	0.55	1.12	159.21
ETU												
Overall	386	828	3.56	1.74	0.16	0.81	1.90	2.41	3.40	4.92	6.79	127.38
First half	207	233	3.48	1.92	0.46	0.58	1.74	2.17	3.53	5.00	7.70	31.06
Second half	375	595	3.37	1.82	0.13	0.65	1.67	2.28	3.27	4.76	6.67	127.38
OHP												
Overall	386	828	0.57	4.16	0.27	0.03	0.12	0.21	0.50	1.30	2.80	368.55
First half	207	233	0.37	4.77	0.71	0.02	0.05	0.15	0.31	0.84	1.94	946.36
Second half	375	595	0.51	4.37	0.33	0.02	0.08	0.19	0.49	1.25	2.84	368.55

[a]ETU and TCP: 100% >limit of detection (LOD); 3PBA: 99.8% >LOD; 2,4-D: 99.8% >LOD; DCCA 99.3% >LOD; and OHP 86.6% >LOD.

[b]Estimates from mixed regression models.

3PBA indicates 3-phenoxybenzoic acid; DCCA, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid; ETU, ethylenethiourea; ICC, intraclass correlation coefficients; OHP, hydroxy pyrimethanil; TCP, 3,5,6-trichloro-2-pyridinol.

Table 3.

Beta estimates with 95%CI for gestational age and birth size measures for each 10-fold increase in specific-gravity-corrected pesticide metabolite concentration, adjusted for newborn's sex, maternal age, parity, smoking during pregnancy, pre-pregnancy BMI, and country of birth

	All newborns			Newborns with gestational age of 37 weeks or more										
	Gestational age at birth (weeks)			Birth weight (g)			Body length (cm)			Ponderal index (g/cm^3)			Head circumference (cm^2)		
Metabolite (µg/L)	**n**	**ß**	**95% CI**	**n**	**ß**	**95% CI**	**n**	**ß**	**95% CI**	**n**	**ß**	**95% CI**	**n**	**ß**	**95% CI**
TCP															
Mean	382	0.24	−0.34, 0.83	357	−81.2	−203.8, 41.4	352	−0.23	−0.89, 0.44	352	−0.02	−0.10, 0.06	348	−0.45	−0.90. 0.01
1st half	204	0.56	−0.19, 1.30	192	39.2	−107.8, 186.3	190	0.29	−0.48, 1.06	190	0.01	−0.09, 0.12	188	0.17	−0.34, 0.68
2nd half	371	0.06	−0.51, 0.63	352	−129.6	−255.8, −3.5	347	−0.50	−1.21, 0.22	347	−0.03	−0.13, 0.07	339	−0.61	−1.05, −0.17
DCCA															
Mean	382	−0.05	−0.53, 0.44	357	−10.0	−112.4, 92.4	352	−0.06	−0.62, 0.51	352	0.01	−0.06, 0.09	352	−0.21	−0.59, 0.19
1st half	204	−0.03	−0.57, 0.51	192	−22.7	−131.0, 85.6	190	0.21	−0.35, 0.78	192	−0.03	−0.11, 0.04	188	0.10	−0.28, 0.47
2nd half	371	0.14	−0.34, 0.62	348	3.01	−99.5, 105.5	343	−0.14	−0.70, 0.42	343	0.02	−0.05, 0.09	343	−0.20	−0.59, 0.20
3PBA															
Mean	386	−0.19	−0.74, 0.37	357	−14.9	−120.0, 90.1	352	−0.26	−0.84, 0.31	352	0.04	−0.03, 0.12	352	−0.19	−0.60, 0.21
1rst half	204	0.10	−0.51, 0.70	192	13.4	−109.3, 136.1	190	0.37	−0.27, 1.00	190	−0.03	−0.12, 0.05	188	0.11	−0.31, 0.53
2nd half	371	−0.14	−0.64, 0.35	348	−9.1	−110.9, 92.6	343	−0.33	−0.88, 0.23	343	0.05	−0.02, 0.12	343	−0.13	−0.52, 0.26
ΣDCCA, 3PBA															
Mean	382	−0.21	−0.73, 0.31	357	−15.42	−123.6, 92.8	352	−0.17	−0.76, 0.43	352	0.03	−0.04, 0.1	348	−0.22	−0.63, 0.18
1st half	204	−0.02	−0.62, 0.58	192	−18.49	−138.5, 101.5	190	0.26	−0.36, 0.89	190	−0.04	−0.12, 0.04	188	0.08	−0.33, 0.49
2nd half	371	0.00	−0.51, 0.51	348	−1.7	−108.5, 105.1	343	−0.22	−0.81, 0.36	343	0.04	−0.03, 0.11	339	−0.19	−0.58, 0.21
2.4-D															
Mean	382	−0.16	−0.67, 0.35	361	−125.1	−228.8, −21.5	352	−0.46	−1.00, 0.07	352	−0.02	−0.09, 0.05	352	−0.41	−0.78, −0.03
1st half	204	0.06	−0.64, 0.75	192	−30.5	−165.6, 104.6	190	0.07	−0.67, 0.81	190	−0.06	−0.15, 0.04	188	0.03	−0.46, 0.52
2nd half	371	−0.20	−0.64, 0.24	352	−127.4	−223.3, −31.5	343	−0.58	−1.09, −0.07	343	−0.01	−0.08, 0.05	343	−0.47	−−0.81, −0.12
ETU															
Mean	382	−0.08	−0.82, 0.66	361	104.3	−58.0, 266.5	352	0.06	−0.78, 0.90	352	0.07	−0.04, 0.17	348	0.13	−0.49, 0.74
1st half	204	0.20	−0.70, 1.11	192	0.7	−180.5, 181.8	190	0.09	−0.85, 1.04	190	−0.02	−0.14, 0.11	190	−0.15	−0.79, 0.50
2nd half	371	0.05	−0.63, 0.74	348	74.5	−68.1, 217.0	343	−0.04	−0.82, 0.74	343	0.04	−0.06, 0.14	339	0.16[a]	−0.41, 0.73
OHP															
Mean	382	0.13	−0.16, 0.42	357	7.2	−52.4, 66.9	352	0.01	−0.32, 0.34	352	0.00	−0.04, 0.04	352	−0.07	−0.30, 0.16
1st half	204	−0.01	−0.40, 0.39	192	24.7	−52.1, 101.5	190	0.04	−0.37, 0.45	190	0.00	−0.05, 0.06	190	0.08	−0.20, 0.36
2nd half	371	0.13	−0.14, 0.41	352	−15.8	−76.7, 45.2	343	−0.05	−0.37, 0.26	343	−0.01	−0.04, 0.03	343	−0.10	−0.32, 0.12

$P < 0.05$ in bold.

[a]Nonlinear association was observed in the second half of pregnancy for ETU and head circumference edf = 2.8; $P = 0.02$, see Figure 1.

3PBA indicates 3-phenoxybenzoic acid; DCCA, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid; ETU, ethylenethiourea; OHP, hydroxy pyrimethanil; TCP, 3,5,6-trichloro-2-pyridinol.

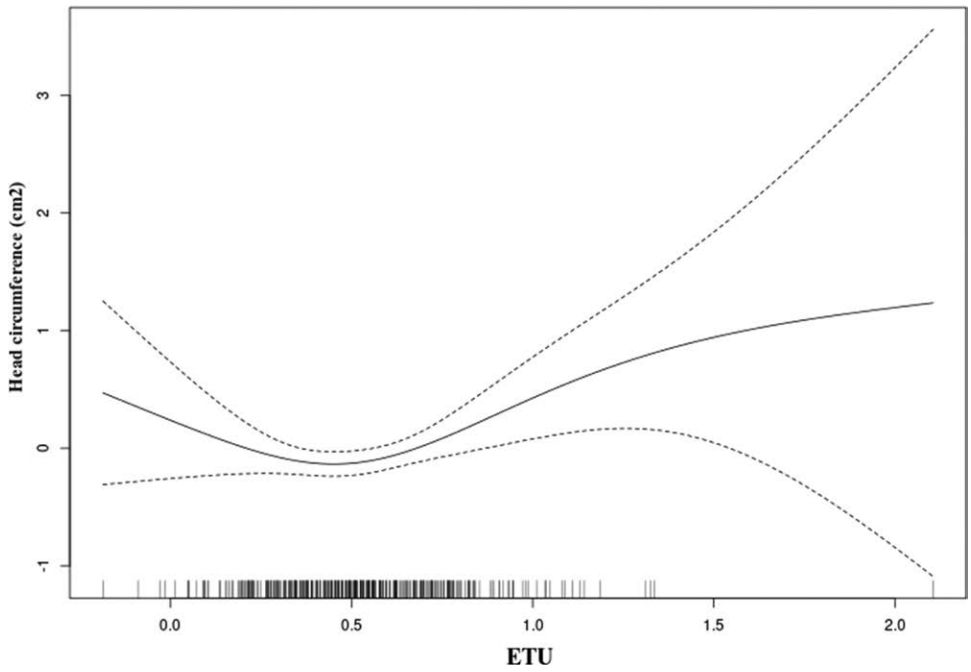

Figure 1. ETU non-linear association with head circumference, the second half of pregnancy, edf = 2.8; *P* = 0.02. edf indicates estimated degrees of freedom; ETU, ethylene thiourea.

Discussion

Our results showed maternal urinary TCP and 2,4-D, especially during the second half of pregnancy, were associated with lower birth weight, smaller head circumference, and, for 2,4-D, also smaller birth length among on-term newborns. In addition, urinary ETU during the second half of pregnancy showed a nonlinear association with head circumference. The stronger associations observed for these pesticide exposures during the second half of pregnancy as compared to the first half of pregnancy may be because fetal growth occurs mainly during the second half of pregnancy.[10] The estimated effects for each 10-fold increase (~ range of concentration) in TCP and 2,4-D were of the same order of magnitude as the effect reported from exposure to cigarette smoke (~150 grams decrease in birth weight).[10] We observed null associations for markers of pyrethroids and pyrimethanil.

Our finding that urinary TCP was associated with decreased birth size measures coincides with results from several other birth cohorts that showed specific biomarkers of chlorpyrifos exposure were associated with reduced birth weight[17,47] and decreased head circumference.[15,19] However, in contrast with some of the cohorts,[17,24,47] increased TCP in our study was not statistically significantly associated with shorter body length, although for exposure during the second half of pregnancy, the direction of the association aligned with previous studies. A cohort situated in New Jersey reported null findings for chlorpyrifos exposure on fetal growth measured at birth, possibly due to its small sample size (n = 150) or relatively low exposure levels.[26] Results from cohort studies using nonspecific biomarkers to evaluate OP exposure showed inconclusive findings with respect to birth size measures,[10,16,21] and some of these studies found inverse associations with gestational age.[48,49] In general, inconsistent findings between studies may be explained by differences in routes, levels, timing, and duration of exposure. Also, socioenvironmental factors and genetic susceptibility may influence the effect of the exposures[21,50] Finally, nonspecific OP biomarkers reflect exposures from different OP-insecticides as well as exposure to the less toxic OP-metabolites[10]

With respect to 2,4-D, our finding that 2,4-D was associated with reduced birth weight, length, and head circumference may be explained by dose-dependent cell membrane damage, uncoupling of oxidative phosphorylation, and disruption of acetyl-coenzyme A metabolism.[51] Our results are partially consistent with findings from two birth cohorts in New York City with similar levels of prenatal urinary 2,4-D as our study;[15] the authors reported 2,4-D was associated with decreased head circumference in boys and girls, but the sex-specific associations were incoherent between the cohorts, and 2,4-D was associated with increased birth length in boys in one of the cohorts. In contrast, both a Danish[27] and French[24] general population cohort reported null associations for 2,4-D exposure with birth size measures.[27] However, the urinary 2,4-D concentrations measured in the Danish cohort were about half the concentrations measured in our study (medians = 0.16 µg/L and 0.33 µg/L, respectively), and the French cohort used 2.4-D in hair samples as a biomarker of exposure and urine and hair exposure concentrations cannot be compared.

To our knowledge, results on prenatal exposure to mancozeb/ETU and birth size measures have not been reported previously. The nonlinear association of ETU with head circumference observed in this study should be interpreted with caution because data were sparse at the higher end of the exposure. Yet, the nonlinear association might be due to changes in thyroid functioning, as increased ETU exposure has been associated with decreased free thyroxine, a thyroid hormone important for fetal growth, in pregnant women from the ISA cohort.[11] Regarding our null findings for pyrethroid insecticides, birth cohorts in South Africa and Denmark also showed null associations for synthetic pyrethroid metabolites and birth size measures.[25,27] However, a Chinese birth cohort reported that newborns from women with increased summed urinary pyrethroid metabolite concentrations during pregnancy were associated with lower birth weight,[23] exposure levels in the Chinese cohort were similar to our study.

Our study has several limitations and strengths. First, a general concern with cohort studies is the possibility of selection bias due to loss-to-follow-up; yet, as 86% of the originally enrolled population had data on birth outcomes and prenatal pesticide exposure, loss-to-follow-up was small in our study. Moreover, the participation rate was high (98%), therefore it

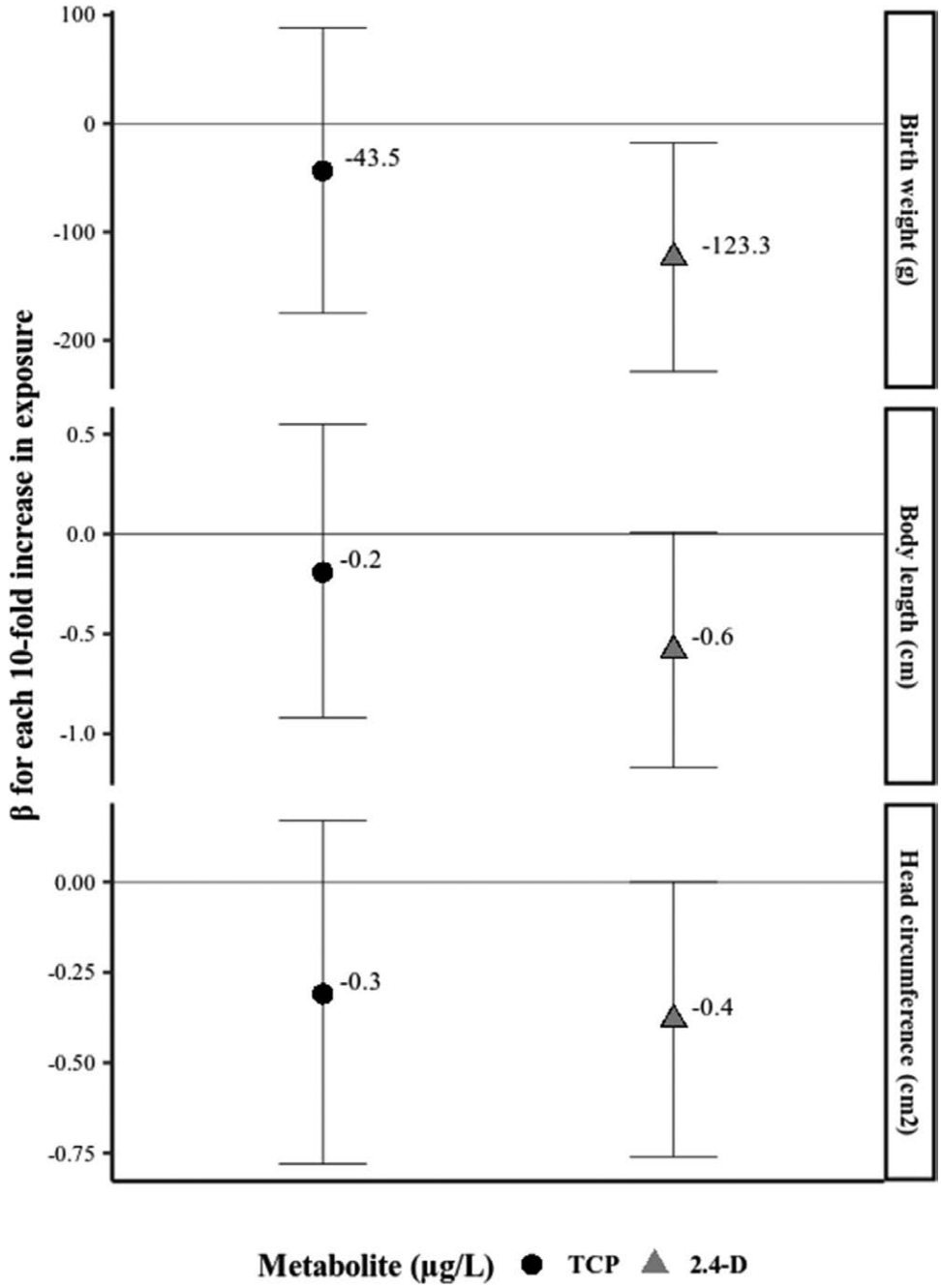

Figure 2. Adjusted beta estimates with 95%CI for newborn's birth weight, head circumference, and body length per 10-fold increase in TCPy and 2,4-D in the same model. TCP indicates 3,5,6-trichloro-2-pyridinol.

is unlikely that selection bias occurred. Second, with respect to exposure assessment, although biomarkers of exposure are considered the gold standard as their concentrations reflect all routes of exposure,[52] a limitation of evaluating pesticide exposure with urinary pesticide metabolites is their short half-life of approximately 1 day or less. The measured concentrations mainly reflect exposures during the 24 hours before the moment of sampling, which is illustrated by the considerable intraindividual variability. However, as we obtained repeated urine measures for a substantial part of the women, this allowed us to evaluate associations for exposure during both early and late pregnancy. With respect to external validity, we expect that our findings are applicable to other agricultural populations who are environmentally exposed to the pesticides studied in this cohort, particularly to populations living in a vulnerable socioeconomical context. Compared to other studies in Latin America, the prevalence of gestational diabetes and hypertensive disorders of pregnancy among women from our study was low, which may be explained because of their young age.[53]

In conclusion, our data showed both chlorpyrifos and 2,4-D exposure were associated with decreased birth weight and head circumference, and 2,4-D also decreased body length at birth, particularly during the second half of pregnancy. Exposure to mancozeb/ETU may also influence fetal growth as urinary maternal ETU concentrations during the second half of pregnancy were nonlinearly associated with the newborn's head circumference. These findings are of concern as reduced fetal growth has been associated with infant mortality and morbidity, including decreased cognitive development in childhood.

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