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Authors
Kim, Joanne
Yang, Seungmi
Moodie, Erica EM
et al.

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Prenatal exposure to insecticides and child cardiometabolic risk factors in the VHEMBE birth cohort

Joanne Kim[a], Seungmi Yang[a], Erica EM Moodie[a], Muvhulawa Obida[b], Riana Bornman[b], Brenda Eskenazi[c], Jonathan Chevrier[a,*]

Background: As part of malaria control programs, many countries spray dichlorodiphenyltrichloroethane (DDT) or pyrethroid insecticides inside dwellings in a practice called indoor residual spraying that results in high levels of exposure to local populations. Gestational exposure to these endocrine- and metabolism-disrupting chemicals may influence child cardiometabolic health.

Methods: We measured the serum concentration of DDT and dichlorodiphenyldichloroethylene (DDE) and urinary concentration of pyrethroid metabolites (*cis*-DBCA, *cis*-DCCA, *trans*-DCCA, 3-PBA) in peripartum samples collected between August 2012 and December 2013 from 637 women participating in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), a birth cohort study based in Limpopo, South Africa. We applied marginal structural models to estimate the relationship between biomarker concentrations and child-size (height and weight), adiposity (body mass index [BMI], body fat percentage, waist circumference) and blood pressure at 5 years of age.

Results: Maternal concentrations of all four pyrethroid metabolites were associated with lower adiposity including reduced BMI z-scores, smaller waist circumferences, and decreased body fat percentages. Reductions in BMI z-score were observed only among children of mothers with sufficient energy intake during pregnancy ($\beta_{cis\text{-}_{DCCA,}\ trans\text{-}_{DCCA}}$=–0.4, 95% confidence interval (CI) = –0.7,–0.1; $p_{interaction}$=0.03 and 0.04, respectively) but there was no evidence of effect modification for the other measures of adiposity. Maternal *p,p'*-DDT concentrations were associated with a reduction in body fat percentage (β = –0.4%, 95% CI = –0.8,–0.0).

Conclusions: Gestational exposure to pyrethroids may reduce adiposity in children at 5 years of age.

Keywords: Indoor residual spraying; Insecticides; DDT; Pyrethroids; Cardiometabolic health; Adiposity

[a]*Department of Epidemiology, Biostatistics and Occupational Health, Faculty of Medicine, McGill University, Montreal, Canada;* [b]*University of Pretoria Institute for Sustainable Malaria Control, School of Health Systems and Public Health, University of Pretoria, Pretoria, South Africa; and* [c]*Center for Environmental Research and Children's Health, School of Public Health, University of California, Berkeley*

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Data and computing code access may be discussed by contacting Dr. Jonathan Chevrier (jonathan.chevrier@mcgill.ca).

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**Corresponding Author. Address: Department of Epidemiology, Biostatistics and Occupational Health, Faculty of Medicine, McGill University, 1020 Pine Avenue West, Montreal, QC H3A 1A2. E-mail: jonathan.chevrier@mcgill.ca (J. Chevrier).*

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Introduction

Low- and middle-income countries such as South Africa are experiencing a double burden of malnutrition characterized by a high prevalence of both under- and overnutrition. In South Africa, one in four (27%) children under 5 years of age are stunted, and in some provinces, up to 13% of children are underweight.[1] Concurrently, 13% of children under 5 are overweight,[1] more than twice the global average for this age group.[2] Early-life exposure to endocrine-disrupting chemicals may contribute to these patterns by affecting the hormonal regulation of energy, glucose, and lipid metabolism.[3,4] In malaria-endemic regions of South Africa, indoor residual spraying (IRS) of dichlorodiphenyltrichloroethane (DDT) or pyrethroid insecticides on the interior walls and eaves of homes for malaria vector control results in high levels of exposure to these endocrine-disrupting chemicals.[5–9] These chemicals can cross the placenta and may

What this study adds

As low- and middle-income countries experience the epidemiologic transition, many are faced with the double burden of malnutrition, characterized by a high prevalence of both under- and overnutrition/obesity. This may be due in part to exposure to endocrine-disrupting chemicals but the literature on this topic is scarce. This study is the first to investigate associations between prenatal exposure to DDT and pyrethroid insecticides and multiple markers of cardiometabolic health among preschool children from an area where indoor residual spraying occurs. We do so by applying marginal structural models to account for potential confounding and selection biases.

interfere with fetal development and have a long-term impact on child cardiometabolic health.[4,10]

Pyrethroid insecticides are commonly used for IRS[5] and in agriculture and retail products for domestic use. These chemicals have been shown to disrupt androgen signaling,[11,12] steroidogenesis,[13–15] and lipid metabolism in animals and *in vitro*.[15–17] Only two epidemiologic studies have examined associations between prenatal exposure to pyrethroids and adiposity in children. In the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), taking place in an area of South Africa where IRS is conducted annually, inverse associations were observed between maternal concentrations of pyrethroid metabolites and BMI z-scores at 1, 2, and 3.5 years among boys,[18,19] but no associations were found with BMI z-score among South Korean children at age 4 years.[20] It remains unclear whether the associations observed in VHEMBE persist at older ages, or whether findings from high-income populations such as South Korea are generalizable to IRS populations. Furthermore, to our knowledge, no study has investigated the potential effects of prenatal exposure to pyrethroids on other cardiometabolic risk factors such as waist circumference (a measure of the more metabolically harmful abdominal/visceral fat), body fat percentage, or blood pressure.

DDT is an estrogen agonist and its environmentally-persistent breakdown product dichlorodiphenyldichloroethylene (DDE) is an androgen antagonist.[21–23] Epidemiological studies of prenatal exposure to DDT and DDE (DDT/E) have been mixed, reporting positive[18,24–31] or null[19,32–38] associations with child adiposity. Cardiometabolic risk factors other than size and adiposity were assessed only in a Greek birth cohort, which found a positive association between maternal serum DDE and blood pressure in children at 4 years of age.[28] However, except for VHEMBE, these prior studies did not address potential selection bias from missing covariate data or loss to follow-up, and used confounder selection strategies such as stepwise and change-in-estimate approaches that may bias estimates and overestimate precision.[39–41] Furthermore, only VHEMBE occurs in the context of current exposure to DDT from IRS. The objective of this study is therefore to estimate the causal effects of prenatal exposure to DDT/E and pyrethroid insecticides on child cardiometabolic risk factors including anthropometrics, measures of adiposity (including abdominal/visceral fat), and blood pressure at 5 years of age in a population exposed annually to IRS, using inverse probability weighting methods to address confounding and selection bias.

Methods

Data source

Mothers giving birth at Tshilizidini hospital in South Africa's Limpopo Province were recruited into the VHEMBE study between August 2012 and December 2013. In this region, IRS spraying occurs at the start of the rainy season (October to April). The pyrethroids cypermethrin or deltamethrin are generally sprayed in homes with painted walls while DDT is generally sprayed in homes with unpainted walls. Eligible women were at least 18 years of age, spoke Tshivenda at home, lived within 20 km of the hospital, intended to remain in the area for at least 2 years, did not have malaria during pregnancy, had contractions at least 5 minutes apart, and delivered a live, singleton infant. Of the 920 women who met eligibility criteria, 752 provided informed consent, completed a baseline questionnaire and provided peripheral blood samples for DDT/E analysis (see Figure S1.1; http://links.lww.com/EE/A177). Follow-up consisted of a home visit 1 week postpartum and field office visits at 1, 2, 3.5, and 5 years. At the home visit, study staff recorded observations and administered a questionnaire on home materials, pesticide use and storage, and household assets. Follow-up field office visits included extensive questionnaires on various demographic and health information, biological sample collection and physical assessments of both the mother and child. Of the 640 mother-and-child pairs who presented for the 5-year visit (88% retention, excluding 25 child deaths), physical assessments were completed for 637 of the children. Of these, 628 mothers had provided sufficient urine volume for pyrethroid metabolite analysis. Ethics approval for the VHEMBE study was obtained from McGill University, the University of Pretoria, Tshilidzini Hospital, the Limpopo Department of Health and Social Development, and the University of California, Berkeley.

Maternal serum DDT/E and urinary pyrethroid metabolite concentrations

Maternal blood and urine samples were collected in Tshilidzini hospital at the time of delivery, and were processed immediately after collection and stored at −80°C until shipment on dry ice to analytical laboratories. Maternal serum concentrations of DDT/E isomers (*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, and *p,p'*-DDE) were measured by the Emory University Environmental Health Laboratory (Atlanta, USA) using gas chromatography-tandem mass spectrometry.[42] Maternal urine concentrations of the following pyrethroid metabolites were measured by the Institut National de Santé Publique du Québec (Québec City, Canada) using gas chromatography-tandem mass spectrometry:[43] *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DBCA), *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DCCA), *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*trans*-DCCA), 3-phenoxybenzoic acid (3-PBA), and 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA). Urine specific gravity was measured with a portable refractometer (Atago PAL-10S; Tokyo, Japan) and total serum lipid concentrations were estimated based on total cholesterol and triglyceride levels measured by standard enzymatic methods (Roche Chemicals; Indianapolis, USA).[44]

One 3-PBA measurement did not meet quality control standards and was discarded. Owing to low quantification frequencies, 4-F-3-PBA (8%) and *o,p'*-DDE (16%) were excluded from further analyses. For the other analytes, concentrations below the limits of detection (LOD) were imputed at random based on log-normal probability distributions whose parameters were estimated via maximum likelihood.[45] Values between the LOD and limit of quantification (LOQ) were assigned the machine-read values. Pyrethroid metabolite concentrations were specific gravity (SG)-corrected for urine dilution and expressed in μg/L.[46] DDT/E were corrected for serum lipid content and expressed in ng/g lipid.

Child cardiometabolic risk factors (size, adiposity, and blood pressure)

At the 5-year visit, trained staff measured child weight to the nearest 0.01 kg and body fat percentage to the nearest 0.1% (via. bioelectrical impedance) using a Tanita Children's Body Fat Monitor BF-689 (Arlington Heights, USA).[47] Child standing height using a Charder HM200P stadiometer (Taichung, Taiwan), waist circumference using a measuring tape, and blood pressure using an OMRON oscillometric device (Lake Forest, USA) were measured in triplicate and then averaged, following US National Health and Nutrition Examination Survey protocols.[48] Age- and sex-standardized z-scores for height, weight, and BMI were calculated using the WHO's igrowup[49] and WHO 2007[50] Stata macros, which implement the 2006–2007 WHO child growth standards.[51]

Covariates

At the baseline and follow-up visits, study staff administered questionnaires to mothers or primary caregivers on potential confounders and conducted anthropomorphic assessments. Maternal postdelivery weight was measured using a Beurer

PS06 scale (Ulm, Germany) and height was measured in triplicate using a Charder HM200P stadiometer (Taichung, Taiwan), then averaged.

The baseline questionnaire collected data on sociodemographic characteristics (e.g., date of birth, marital status, household income, and household size), diet and lifestyle (e.g., food frequency, alcohol, and smoking during pregnancy), and health. Mothers also reported their occupational and domestic use of pesticides, and the presence of agricultural workers in the household. Based on Statistics South Africa guidelines, households earning less than 386 Rands/person/month were defined as living with food poverty.[52] Food insecurity was defined as two or more affirmative responses to the US National Center for Health Statistics' Six-Item Food Security Scale.[53] Mothers' daily total energy intake was estimated based on a locally-validated quantitative food frequency questionnaire[54] by a South African nutritionist using the FoodFinder3 software (SouthAfrica Medical Research Council/WAMTechnology CC). The Institute of Medicine recommended total daily energy intake for mothers in late pregnancy was calculated based on their age (years), height (meters), and postpartum weight (kg) because prepregnancy weight was not available: 4.184 kJ/cal × (452 + 354 − [6.91 × age] + 1.27 × [9.36 × weight] + 726 × height); energy intake below this threshold was classified as insufficient.[55,56] Mothers' HIV status during pregnancy was ascertained from self-report or use of antiretroviral drugs indicated in medical records.

To capture socioeconomic status in this region where much of the economy is informal,[1,19] a family wealth index was constructed based on South Africa Demographic and Health Survey methodology, using data from the baseline questionnaire and the 1-week home visit (questionnaire and staff observations).[19] Duration of exclusive and nonexclusive breastfeeding was calculated based on responses from questionnaires administered at 1 week and 1, 2, and 3.5 years. We also constructed a child diet diversity score to explore potential confounding by child dietary intake.[19] The score was calculated as the total number of different food groups (e.g., fruit, vegetables, meat, chicken, fish, milk, or eggs) eaten in the past month by the child based on the maternal report at 3.5-years questionnaire.

Statistical analysis

The relation between a 10-fold increase in maternal lipid-corrected serum DDT/E or specific gravity-corrected urinary pyrethroid metabolite concentrations and each cardiometabolic risk factor were estimated using marginal structural models with inverse probability weights constructed from the product of two weights: inverse probability of censoring weights (IPCWs) to account for potential selection bias owing to loss to follow-up; and, inverse probability of treatment weights (IPTWs) to control for confounding.[57] Under the three identifiability assumptions of consistency, exchangeability, and positivity, and assuming no misspecification of the models used to estimate the weights, the resulting estimates have a causal interpretation.

Further details on the construction of the weights are provided in section 2 of the eAppendix; http://links.lww.com/EE/A177. Briefly, we used logistic regression to estimate the probability of the censoring status of each subject (i.e., completed the 5-year visit vs. lost to follow-up), conditional on predictors of censoring identified using directed acyclic graphs (DAGs) and constructed IPCWs based on the inverse of these probabilities and stabilized the weights with the marginal probability of the censoring status received.[57] Then, excluding censored individuals, we constructed IPTWs based on the generalized propensity score method for each exposure, using multivariable linear regression to estimate the density function conditional on potential predictors of the outcomes and confounders of exposure-outcome relationships identified using the DAG (Figure S2.1; http://links.lww.com/EE/A177).[58,59] The following covariates were included in both IPCW and IPTW models: child sex (boy/girl); household food poverty (yes/no), food insecurity (yes/no), and wealth index (continuous); maternal age (years, continuous), height (meters, continuous), postdelivery weight (kg, continuous), education (high school vs. no high school), marital status (married or living-as-married vs. not married), energy intake during pregnancy (insufficient/sufficient), alcohol use during pregnancy (yes/no), HIV status at delivery (positive/negative), duration of exclusive breastfeeding (months, continuous), and parity (continuous). In the IPCW models, we also included gestational age (preterm vs. not preterm) and DDT/E and pyrethroid metabolite concentrations. All analyte concentrations were $\log_{10}$-transformed to reduce the influence of outliers, resulting in estimates of effect per 10-fold increase in concentration.

Inverse probability weighting accounts for selection bias and confounding by creating a pseudo-population in which censoring is independent of exposure and covariates and exposure is independent of confounders.[57] This can be verified by assessing, in the weighted sample, whether exposure and covariates are equally distributed (i.e., balanced) between censored and uncensored individuals, and whether the distribution of confounders is balanced at different levels of exposure. For this purpose, we conducted the following recommended diagnostics[60,61]: (1) standardized differences, to compare proportions or means across (exposure or censorship) categories; (2) correlations, to evaluate associations between continuous covariates and the continuous exposures, and (3) variance ratios, to compare variability across (exposure or censorship) categories. Following published guidelines, variables with standardized differences below 0.2 when comparing across exposure quartiles (accounting for additional variability expected from small sample sizes),[60] below 0.1 when comparing across censoring status, and correlations below 0.1, were considered to be balanced.[60,61] Variance ratios of 1.0 describe a covariate which has equal variance across exposure categories, and a threshold of <2.0 has been suggested to indicate balance.[62] Further details on the inverse probability weights and balance assessment are provided elsewhere[63] and in section 2 of the eAppendix; http://links.lww.com/EE/A177.

To account for the small amount of missing covariate values (181 of 11,265; 1.6% missingness), we conducted multiple imputation by chained equations with imputation models including all participants enrolled at baseline (n = 751). In the imputation models, we included all outcomes, exposures, and covariates identified above and generated 10 imputed datasets[64] (see section 3 of the eAppendix; http://links.lww.com/EE/A177 for additional details). Since endocrine disruptors may differentially affect boys and girls,[65,66] and effects on cardiometabolic risk factors may differ based on socioeconomic and nutritional context,[19] we also investigated effect measure modification by child sex, food poverty, and maternal energy intake during pregnancy by including cross-product terms in models. We used a threshold of $P < 0.1$ to indicate statistical evidence of effect modification. We constructed 95% confidence intervals (CIs) from bootstrapping the entire procedure (multiple imputation, estimation of IPCW and IPTW, and outcome regressions) 500 times.[67,68] All analyses were conducted using Stata 14 (StataCorp, College Station, TX).

Results

Participant characteristics

All VHEMBE mothers (n = 637) were Black Africans. At delivery, the average age of mothers was 26.4 years, and just under half were married (46%) and had a high school education (43%) (Table 1). Most households lived below the South African food poverty line (61%), and many were food insecure (42%). The prevalence of HIV infection among mothers was 12% at delivery. Half of the children were female (49%) and 12% were

Table 1.

Characteristics of VHEMBE participants who completed the 5-year visit, Limpopo, South Africa (n = 637)

Baseline maternal characteristics		
Age, years (mean, ± SD)	26.4	±6.2
Height, cm (mean, ± SD)	158.1	±6.9
Postdelivery weight, kg (mean, ± SD)	69.1	±13.8
Postdelivery BMI, kg/m^2 (mean, ± SD)	27.7	±5.5
Married or living-as-married (n, %)	296	46%
High school diploma (n, %)	276	43%
Nulliparous (n, %)	272	43%
Insufficient energy intake during pregnancy[a] (n, %)	427	68%
Any alcohol during pregnancy (n, %)	37	6%
HIV positive (n, %)	79	12%
Baseline household sociodemographic characteristics		
Food poverty[b] (n, %)	388	61%
Food insecurity[c] (n, %)	267	42%
Child characteristics		
Female sex (n, %)	313	49%
Low birthweight, <2500 g (n, %)	47	7%
Preterm birth, <37 weeks (n, %)	79	12%
Any breastfeeding, months (mean, ± SD)	16.1	±7.0
Exclusive breastfeeding, months (mean, ± SD)	2.3	±1.9

[a]Below the Institute of Medicine recommended total daily caloric intake for mothers in late pregnancy.[55]

[b]Below the food poverty line of 386 Rand/person/month.[52]

[c]Two or more affirmative response to the US National Center for Health Statistics' Six-Item Food Security Scale.[53]

BMI, body mass index; SD, standard deviation.

preterm (<37 weeks gestational age at birth). One-quarter of the children were born small-for-gestational-age (<10th percentile) and 7% had low birthweight (<2500 g).[69] The median duration of exclusive breastfeeding without the introduction of water or solids was short (2.3 months), though breastfeeding continued for longer (median = 16.1 months) (Table 1).

DDT/E and pyrethroids were detected in virtually all participants. DDT/E concentrations varied greatly, with up to a 100,000-fold difference in exposure (Table 2). The pyrethroid metabolites *cis*-DCCA, *trans*-DCCA, and 3-PBA were highly correlated with each other (Pearson's r = 0.83 to 0.87) but were only moderately correlated with *cis*-DBCA (r = 0.33 to 0.53), and were not correlated with DDT/E (r = –0.02 to 0.04). Isomers of DDT/E were highly intercorrelated (r = 0.69 to 0.85). Occupational exposure to pesticides was infrequent, with 7% of mothers reporting use of pesticides at work during pregnancy, and 7% of households included an agricultural worker. Domestic use of pesticides was more frequent, with mothers reporting the use of pesticides in the yard (13%) and indoors (32%).

Inverse probability weights and covariate balance diagnostics

The mean of each set of inverse probability weights was 1.00 for all models and no extreme weights were observed, suggesting that the positivity assumption was not violated (range = 0.22–3.07; Table S2.1; http://links.lww.com/EE/A177). Inverse probability weighting achieved covariate balance, with all mean absolute standardized differences being below 0.2, all correlations being below 0.1 and all variance ratios being about 1.0, indicating that confounding by measured variables was controlled. Balance diagnostics for *trans*-DCCA are shown in Figure 1 for illustration purposes; diagnostics for other analytes are shown in Figures S2.3–S2.5; http://links.lww.com/EE/A177.

Effects of gestational pyrethroid exposure on child cardiometabolic risk factors at 5 years of age

Overall, maternal concentrations of all pyrethroid metabolites were associated with reduced BMI z-score, waist circumference, and body fat percentage in the children. Magnitudes were relatively consistent across metabolites, with an approximately 0.2 decrease in BMI z-score (e.g., $\beta_{cis\text{-DBCA}}$ = –0.18, 95% CI = –0.33, –0.03), 0.6 to 0.9 cm smaller waist circumference (e.g., $\beta_{cis\text{-DBCA}}$ = –0.57, 95% CI = –1.09, –0.06), and 0.7 to 0.8% reduced body fat percentage (e.g., $\beta_{cis\text{-DBCA}}$ = –0.75, 95% CI = –1.34, –0.17) per 10-fold higher concentration of each metabolite (Table 3). Pyrethroids were not associated with child height or weight z-scores or blood pressure overall (Table 3).

Inverse associations between pyrethroid metabolites and adiposity were observed only among children whose mothers had sufficient energy intake during pregnancy. In this subgroup, *cis*-DCCA (β = –0.43, 95% CI = –0.73, –0.14) and *trans*-DCCA (β = –0.40, 95% CI = –0.67, –0.12) were each associated with lower BMI z-score, with P-values for interaction (p_{inter}) of 0.03 and 0.05, respectively, and lower body fat percentage ($\beta_{cis\text{-DCCA}}$ = –1.30, 95% CI = –2.37, –0.24; $\beta_{trans\text{-DBCA}}$ = –1.32, 95% CI = –2.27, –0.37), though evidence of effect modification for this outcome was weaker (p_{inter} = 0.12 and 0.15, respectively; Table 4). Inverse associations between pyrethroid metabolites and BMI z-scores also tended to be stronger among children from nonpoor households relative to those from poor households, especially for *cis*-DBCA (β = –0.36, 95% CI = –0.61, –0.11; p_{inter} = 0.08) and to a lesser extent 3-PBA (β = –0.40, 95% CI = –0.73, –0.06; p_{inter} = 0.13) (Table 5). Associations between pyrethroids and other outcomes did not vary by maternal energy intake (Table 4, Table S4.1; http://links.lww.com/EE/A177v) or poverty (Table 5, Table S4.2; http://links.lww.com/EE/A177).

When we investigated effect modification by child sex, *trans*-DCCA concentrations were associated with higher height

Table 2.

Distribution of maternal peripartum serum DDT/E (ng/g lipid) and urinary pyrethroid metabolite (μg/L, specific gravity-corrected) concentrations among VHEMBE study participants, Limpopo, South Africa

							Percentiles			
	n	≥LOD[a], %	≥LOQ[b], %	Geometric mean	Geometric SD	Min	25	50	75	Max
o,p′-DDT	637	90.7	45.1	9.22	4.57	<LOD	3.58	7.73	23.19	2029.27
p,p′-DDT	637	98.1	90.7	71.02	6.57	<LOD	19.79	60.70	263.12	15027.56
p,p′-DDE	637	100	97.5	295.24	4.75	3.98	94.40	256.53	860.66	22613.43
cis-DBCA	628	100	99.6	0.34	3.06	0.02	0.15	0.32	0.74	13.39
cis-DCCA	628	100	99.9	0.47	2.54	0.05	0.26	0.45	0.80	209.49
trans-DCCA	628	100	99.6	0.55	3.03	0.03	0.25	0.53	1.04	268.95
3-PBA	627	100	100	1.10	2.36	0.10	0.65	1.03	1.84	88.22

[a]Limits of detection (LOD): 0.01 ng/mL (*o,p′*-DDT and *p,p′*-DDT), 0.03 ng/mL (*p,p′*-DDE), 0.0025 μg/L (*cis*-DBCA), 0.0045 μg/L (*cis*-DCCA), 0.0038 μg/L (*trans*-DCCA), and 0.0047 μg/L (3-PBA).

[b]Limits of quantification (LOQ): 0.05 ng/mL (*o,p′*-DDT and *p,p′*-DDT), 0.15 ng/mL (*p,p′*-DDE), 0.0082 μg/L (*cis*-DBCA), 0.015 μg/L (*cis*-DCCA), 0.013 μg/L (*trans*-DCCA), and 0.016 μg/L (3-PBA).

DBCA, 3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; DCCA, 3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; DDT, Dichlorodiphenyltrichloroethane; PBA, phenoxybenzoic acid; SD, standard deviation.

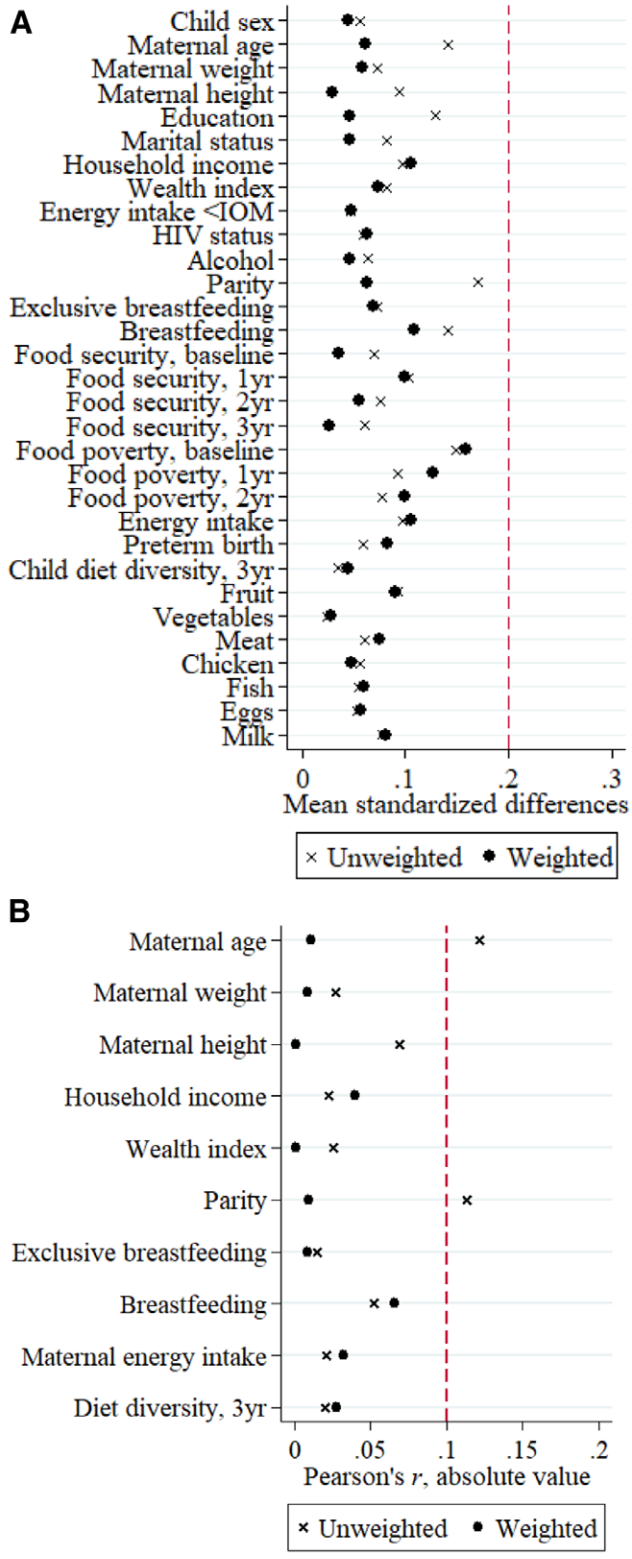

Figure 1. Balance diagnostics for the final inverse probability weight for *trans*-DCCA before (×) and after (·) weighting: (A) mean standardized differences across exposure quartiles and (B) correlations with continuous potential confounders

z-score among girls (β = 0.23, 95% CI = 0.05, 0.41) but not among boys (β = −0.06, 95% CI = −0.25, 0.14; p_{inter} = 0.04), and associations with measures of adiposity or blood pressure did not vary by sex (Table 6, Table S4.3; http://links.lww.com/EE/A177).

Table 3.

Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (μg/L) concentrations and cardiometabolic risk factors among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

		Height z-score β (95% CI)	Weight z-score β (95% CI)	BMI z-score β (95% CI)	Fat percentage, % β (95% CI)	Waist circumference, cm β (95% CI)	Systolic blood pressure, mmHg β (95% CI)	Diastolic blood pressure, mmHg β (95% CI)
DDT/E	*o,p′*-DDT	0.07 (−0.03, 0.17)	0.09 (−0.01, 0.18)	0.06 (−0.06, 0.17)	−0.24 (−0.75, 0.26)	0.09 (−0.25, 0.42)	0.35 (−0.97, 1.67)	0.49 (−0.77, 1.74)
	p,p′-DDT	−0.00 (−0.10, 0.09)	0.02 (−0.07, 0.11)	0.02 (−0.06, 0.11)	−0.39 (−0.76, −0.02)[a]	−0.02 (−0.34, 0.29)	0.04 (−0.88, 0.97)	0.22 (−0.71, 1.14)
	p,p′-DDE	0.10 (−0.01, 0.21)	0.10 (−0.01, 0.20)	0.05 (−0.06, 0.15)	−0.28 (−0.70, 0.14)	0.24 (−0.14, 0.62)	0.07 (−1.01, 1.14)	−0.00 (−1.08, 1.07)
Pyrethroid metabolites	*cis*-DBCA	0.02 (−0.11, 0.15)	−0.11 (−0.24, 0.02)	−0.18 (−0.33, −0.03)[a]	−0.75 (−1.34, −0.17)[a]	−0.57 (−1.09, −0.06)[a]	−0.19 (−1.69, 1.32)	−0.12 (−1.75, 1.50)
	cis-DCCA	0.03 (−0.14, 0.19)	−0.10 (−0.26, 0.05)	−0.19 (−0.34, −0.03)[a]	−0.65 (−1.26, −0.04)[a]	−0.88 (−1.45, −0.30)[a]	0.24 (−1.45, 1.93)	−0.65 (−2.66, 1.37)
	trans-DCCA	0.09 (−0.03, 0.21)	−0.06 (−0.19, 0.06)	−0.17 (−0.32, −0.03)[a]	−0.78 (−1.28, −0.27)[a]	−0.58 (−1.07, −0.10)[a]	0.06 (−1.27, 1.39)	−1.30 (−2.77, 0.16)
	3-PBA	0.03 (−0.15, 0.21)	−0.10 (−0.27, 0.07)	−0.18 (−0.37, 0.00)	−0.75 (−1.44, −0.05)[a]	−0.84 (−1.50, −0.17)[a]	0.15 (−1.76, 2.07)	−0.98 (−3.07, 1.10)

[a]95% CI excludes the null.

CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

Table 4.

Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (μg/L) concentrations and adiposity, by maternal energy intake sufficiency, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	BMI z-score			Fat percentage, %			Waist circumference, cm		
	Sufficient β (95% CI)	Insufficient β (95% CI)	p_{inter}	Sufficient β (95% CI)	Insufficient β (95% CI)	p_{inter}	Sufficient β (95% CI)	Insufficient β (95% CI)	p_{inter}
o,p′-DDT	−0.02 (−0.25, 0.20)	0.08 (−0.04, 0.21)	0.43	−0.80 (−1.65, 0.06)	−0.04 (−0.62, 0.55)	0.15	0.01 (−0.80, 0.82)	0.11 (−0.26, 0.48)	0.83
p,p′-DDT	−0.07 (−0.28, 0.15)	0.06 (−0.03, 0.15)	0.30	−0.81 (−1.70, 0.08)	−0.23 (−0.60, 0.14)	0.24	−0.15 (−0.92, 0.62)	0.02 (−0.31, 0.35)	0.70
p,p′-DDE	−0.07 (−0.33, 0.18)	0.10 (−0.01, 0.21)	0.24	−0.88 (−1.90, 0.14)	−0.05 (−0.47, 0.37)	0.15	−0.01 (−0.91, 0.89)	0.32 (−0.04, 0.68)	0.50
cis-DBCA	−0.17 (−0.48, 0.13)	−0.19 (−0.37, −0.01)	0.90	−1.06 (−2.09, −0.03)[a]	−0.62 (−1.32, 0.07)	0.49	−0.39 (−1.43, 0.65)	−0.71 (−1.32, −0.11)[a]	0.60
cis-DCCA	−0.43 (−0.73, −0.14)[a]	−0.05 (−0.23, 0.13)	0.03[b]	−1.30 (−2.37, −0.24)[a]	−0.31 (−1.01, 0.40)	0.12	−0.97 (−1.94, 0.00)	−0.83 (−1.54, −0.12)[a]	0.82
trans-DCCA	−0.40 (−0.67, −0.12)[a]	−0.06 (−0.23, 0.10)	0.04[b]	−1.32 (−2.27, −0.37)[a]	−0.51 (−1.10, 0.08)	0.15	−0.58 (−1.48, 0.32)	−0.58 (−1.15, 0.00)	1.00
3-PBA	−0.39 (−0.78, 0.00)	−0.10 (−0.30, 0.11)	0.19	−1.35 (−2.74, 0.03)	−0.53 (−1.35, 0.30)	0.31	−0.71 (−1.96, 0.54)	−0.94 (−1.72, −0.16)[a]	0.75

[a]95% CI excludes the null.

[b]*P*-value for interaction<0.1.

CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

Table 5.

Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (μg/L) concentrations and adiposity, by household food poverty status, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	BMI z-score			Fat percentage, %			Waist circumference, cm		
	Non-poor β (95% CI)	Poor β (95% CI)	p_{inter}	Non-poor β (95% CI)	Poor β (95% CI)	p_{inter}	Non-poor β (95% CI)	Poor β (95% CI)	p_{inter}
o,p′-DDT	0.10 (−0.04, 0.25)	0.03 (−0.13, 0.19)	0.52	−0.13 (−0.77, 0.51)	−0.30 (−1.00, 0.41)	0.75	0.14 (−0.36, 0.63)	0.05 (−0.40, 0.50)	0.80
p,p′-DDT	0.06 (−0.08, 0.19)	0.01 (−0.11, 0.14)	0.67	−0.07 (−0.63, 0.49)	−0.54 (−1.04, −0.04)[a]	0.23	0.14 (−0.26, 0.54)	−0.09 (−0.54, 0.36)	0.46
p,p′-DDE	−0.02 (−0.17, 0.14)	0.09 (−0.07, 0.24)	0.39	−0.24 (−0.87, 0.40)	−0.29 (−0.91, 0.33)	0.91	0.18 (−0.35, 0.71)	0.28 (−0.25, 0.81)	0.80
cis-DBCA	−0.36 (−0.61, −0.11)[a]	−0.07 (−0.27, 0.12)	0.08[b]	−1.10 (−2.06, −0.13)[a]	−0.55 (−1.31, 0.20)	0.40	−0.96 (−1.78, −0.13)[a]	−0.35 (−1.00, 0.31)	0.25
cis-DCCA	−0.27 (−0.53, −0.01)[a]	−0.14 (−0.34, 0.06)	0.44	−0.29 (−1.35, 0.76)	−0.85 (−1.65, −0.05)[a]	0.42	−0.90 (−1.82, 0.02)	−0.86 (−1.58, −0.14)[a]	0.94
trans-DCCA	−0.30 (−0.56, −0.03)[a]	−0.12 (−0.30, 0.07)	0.28	−0.73 (−1.74, 0.28)	−0.78 (−1.40, −0.16)[a]	0.93	−0.73 (−1.62, 0.16)	−0.51 (−1.11, 0.08)	0.69
3-PBA	−0.40 (−0.73, −0.06)[a]	−0.08 (−0.31, 0.15)	0.13	−0.79 (−2.09, 0.51)	−0.71 (−1.57, 0.15)	0.92	−0.98 (−2.10, 0.13)	−0.76 (−1.58, 0.05)	0.75

[a]95% CI excludes the null.

[b]p-value for interaction <0.1.

CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

Table 6.

Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations on adiposity, by sex, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	BMI z-score			Fat percentage, %			Waist circumference, cm		
	Boys β (95% CI)	Girls β (95% CI)	p_{inter}	Boys β (95% CI)	Girls β (95% CI)	p_{inter}	Boys β (95% CI)	Girls β (95% CI)	p_{inter}
o,p'-DDT	−0.03 (−0.16, 0.11)	0.13 (−0.05, 0.31)	0.20	−0.65 (−1.15, −0.14)[a]	0.14 (−0.67, 0.95)	0.12	−0.11 (−0.50, 0.28)	0.29 (−0.29, 0.86)	0.27
p,p'-DDT	−0.02 (−0.13, 0.10)	0.07 (−0.07, 0.21)	0.36	−0.63 (−1.08, −0.18)[a]	−0.10 (−0.71, 0.52)	0.20	−0.04 (−0.35, 0.27)	0.09 (−0.45, 0.63)	0.70
p,p'-DDE	−0.01 (−0.15, 0.13)	0.10 (−0.07, 0.28)	0.35	−0.64 (−1.12, −0.17)[a]	0.10 (−0.67, 0.87)	0.13	0.06 (−0.35, 0.48)	0.44 (−0.22, 1.11)	0.35
cis-DBCA	−0.15 (−0.35, 0.04)	−0.20 (−0.43, 0.04)	0.79	−0.43 (−1.10, 0.23)	−1.03 (−1.97, −0.09)[a]	0.31	−0.29 (−0.88, 0.29)	−0.81 (−1.66, 0.03)	0.32
cis-DCCA	−0.15 (−0.37, 0.07)	−0.22 (−0.43, −0.01)[a]	0.62	−0.14 (−0.94, 0.67)	−1.13 (−2.05, −0.21)[a]	0.11	−0.71 (−1.48, 0.06)	−1.05 (−1.87, −0.22)[a]	0.55
trans-DCCA	−0.16 (−0.36, 0.04)	−0.19 (−0.40, 0.01)	0.79	−0.40 (−1.08, 0.28)	−1.16 (−1.98, −0.34)[a]	0.17	−0.58 (−1.20, 0.04)	−0.60 (−1.34, 0.14)	0.97
3-PBA	−0.15 (−0.41, 0.11)	−0.23 (−0.48, 0.02)	0.65	−0.28 (−1.10, 0.54)	−1.22 (−2.31, −0.12)[a]	0.19	−0.46 (−1.25, 0.34)	−1.20 (−2.20, −0.20)[a]	0.24

[a]95% CI excludes the null.

CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

Effects of gestational DDT/E exposure on child cardiometabolic risk factors at 5 years of age

We observed a 0.39% (95% CI = −0.76, −0.02) reduction in body fat percentage per 10-fold higher *p,p'*-DDT concentration. Estimates of similar magnitude were observed for *o,p'*-DDT and *p,p'*-DDE, though confidence intervals included the null (Table 3). In analyses examining effect modification by child sex, greater reductions in body fat percentage were observed among boys relative to girls for all three analytes, but evidence of effect modification was limited (p_{inter} of 0.12 to 0.20; Table 6).

Discussion

Main findings and interpretation

We found that higher maternal urine concentrations of *cis*-DBCA, *cis*-DCCA, *trans*-DCCA, and 3-PBA were inversely associated with multiple measures of adiposity (BMI z-score, waist circumference, and body fat percentage) among 5-year-old South African children participating in the VHEMBE study. These results are consistent with inverse associations with BMI and/or weight-for-height z-scores reported among VHEMBE children at 1, 2, and 3.5 years.[18,19] While these previous reports suggested that associations were more pronounced among boys, in the present study we did not find evidence of effect modification by sex. In the only other study to examine gestational pyrethroid exposure and adiposity, maternal urine concentrations of 3-PBA were not associated with BMI z-score in a slightly smaller sample (n = 478) of South Korean children at 4 years of age[20]; however, because the investigators adjusted for potential mediators including gestational age and birth weight, the reported estimates may have been biased towards the null.[70–72]

Similar to our findings at age 3.5 years,[19] we observed larger reductions in BMI z-score from pyrethroid exposure among children whose mothers had sufficient energy intake during pregnancy and no effect in children of mothers with insufficient intake. It is possible that children who are in an energy-poor environment may have reached a physiological minimum that prevents them from losing additional fat mass. Some experimental data support our findings: mice chronically exposed to cypermethrin during puberty had lower body fat percentage and triglyceride levels compared with unexposed mice,[15] but no effect on body fat was observed among permethrin-exposed mice who were fed a low-fat diet.[73,74] However, evidence for effect modification by energy intake was weaker for other adiposity measures and we found no evidence of effect modification by food poverty.

The exact mechanism of a possible antiadipogenic effect of pyrethroids is unclear. Animal studies indicate that chronic exposure to pyrethroids such as permethrin and cypermethrin and deltamethrin induces changes in energy metabolism, including upregulation of pyruvate kinase,[15] an enzyme involved in glycolysis[75]; uncoupling protein 2[15] and peroxisome proliferator-activated receptor-alpha,[15,16] which promote lipid breakdown[76,77]; and hormone-sensitive lipase,[15] whose main function is to mobilize stored fats.[78] In addition, animal studies show that exposure to pyrethroids increases serum testosterone, a hormone with known antiadipogenic effects.[79,80]

In contrast to the literature which suggests an adipogenic effect of prenatal exposure to DDT/E, we found that *p,p'*-DDT was associated with a slight reduction in body fat percentage overall; however, the estimated magnitude was small and no associations were observed with the other isomers or other measures of adiposity. We therefore cannot exclude the possibility that this finding may be due to chance. We previously reported positive associations between maternal peripartum DDT concentrations and BMI z-score at ages 1 and 2 years among girls,[18] but not at 3.5 years, in VHEMBE.[19] Evidence from other birth cohorts is mixed, with some reporting greater overweight,

BMI, and/or waist circumference in boys at ages 6.5, 9, and 12 years,[29–31] and increased BMI and waist circumference among daughters at 50 years of age,[81] although other studies found no associations with adiposity at ages ranging from infancy to 20 years.[34–37]

Strengths and limitations

Our study presents several improvements over the existing literature. Importantly, other than VHEMBE[19] no prior studies used methods to address potential selection bias from loss to follow-up. In the present study, we noted imbalances for several variables when comparing participants lost to follow-up to those retained at the 5-year visit (Figure S2.2; http://links.lww.com/EE/A177); if outcomes were also related to loss to follow-up, this would create conditions for selection bias to arise. In addition, many studies used complete-case analysis in lieu of imputing missing covariate data, further increasing the potential for selection bias,[64] and used confounder selection strategies such as stepwise and change-in-estimate approaches which may bias estimates and result in inaccurate confidence intervals.[39–41]

In the current analysis, we applied inverse-probability weighting methods.[57] Though unmeasured confounding remains possible, these methods allowed us to verify that exposures and measured confounders were balanced between censored and uncensored participants and across the exposure range after weighting. We also used multiple imputation to address the small amount of missing covariate data and used bootstrapping to calculate accurate confidence intervals for our effect estimates. Nevertheless, residual confounding or chance could explain our study findings, which rely on additional untestable assumptions, such as consistency and correct model specification.

We investigated multiple measures of adiposity, each capturing slightly different aspects of body composition and together providing a more detailed portrait of child health. Although BMI is the most commonly used metric, one of its major disadvantages is that it does not distinguish between lean and fat mass.[82] Body fat percentage was measured using a bioelectrical impedance device validated in children,[83] and waist circumference measures abdominal fat which is more strongly linked to poor cardiometabolic health.[84,85] The agreement across all three measures lends greater confidence to our overall finding that pyrethroids may reduce adiposity, whereas findings with only a single measure may point to specific aspects of body composition or reflect chance findings.

In contrast to other studies investigating DDT and/or pyrethroids in an agricultural setting or in the context of historical widespread use, a major contribution of the VHEMBE study is that it takes place in the current indoor residual spraying context, addressing a key knowledge gap on the potential unintended health effects of this practice. Notably, all VHEMBE participants have detectable levels of *cis*-DBCA, a metabolite specific to deltamethrin which is the pyrethroid most commonly used for indoor residual spraying in South Africa, and we were therefore uniquely able to report on associations with child cardiometabolic risk factors. This said, pyrethroids are also commonly used in agriculture and retail products and so part of the exposure to VHEMBE participants may originate from these sources as well.

A limitation of this study is that exposure to pyrethroids was assessed based on a single measurement around the time of delivery, which may have introduced nondifferential measurement error and may thus have attenuated our effect estimates. However, the reliability of spot urine concentrations of pyrethroid metabolites in representing longer-term exposure may vary by population and context; intraclass correlation coefficients of 0.85 in Poland and 0.21 in the US have been reported.[86,87] In the context of IRS, elevated exposure to inhabitants may persist for months from repeated contact with contaminated surfaces, bedding, furniture, and stored food, especially inasmuch as the pyrethroids used for IRS remain effective for up to 10 months, and the lack of direct sunlight and external elements indoors slows their degradation.[88,89] Furthermore, indicators of regular pesticide use, such as the presence of pesticide storage containers and self-reported use of pesticides in the yard were associated with higher pyrethroid metabolite concentrations among VHEMBE mothers, suggesting that a single measurement may be representative of longer-term exposure in the VHEMBE population.[8,90]

Conclusions

This study finds that prenatal exposure to pyrethroids may be related to reduced adiposity in children at 5 years of age. Such depletion of fat stores may be most detrimental in nutrient-poor environments. Future studies should investigate whether these associations persist later in childhood and consider evaluating relations with growth trajectories, which may better predict cardiometabolic risk.[91,92]

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