Prenatal Exposure to Insecticides and Weight Trajectories Among South African Children in the VHEMBE Birth Cohort

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The VHEMBE study was funded by the Canadian Institutes of Health Research and the US National Institute of Environmental Health Sciences (grant R01ES020360). J.C. holds a Canada Research Chair in Global Environmental Health and Epidemiology. J.K. is supported by a Doctoral Award from the Fonds de recherche du Québec—Santé, with prior funding from McGill University's Faculty of Medicine.

ABSTRACT

Background: Dichlorodiphenyltrichloroethane (DDT) or pyrethroid insecticides are sprayed inside dwellings for malaria vector control, resulting in high exposure to millions of people, including pregnant women. These chemicals disrupt endocrine function and may affect child growth. To our knowledge, few studies have investigated the potential impact of prenatal exposure to DDT or pyrethroids on growth trajectories.

Methods: We investigated associations between gestational insecticide exposure and child growth trajectories in the Venda Health Examination of Mothers, Babies and their Environment, a birth cohort of 751 children born between 2012 and 2013 in South Africa. Based on child weight measured at follow-up and abstracted from medical records, we modeled weight trajectories from birth to 5 years using SuperImposition, Translation and Rotation, which estimated two child-specific parameters: size (average weight) and tempo (age at peak weight velocity). We estimated associations between peripartum maternal concentrations of serum DDT, dichlorodiphenyldichloroethylene, or urinary pyrethroid metabolites and SuperImposition, Translation and Rotation parameters using marginal structural models**.**

Results: We observed that a 10-fold increase in maternal concentrations of the pyrethroid metabolite *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid was associated with a 21g (95% confidence interval = −40, −1.6) smaller size among boys but found no association among girls ($P_{interaction} = 0.07$). Estimates suggested that pyrethroids may be associated with earlier tempo but were imprecise. We observed no association with serum DDT or dichlorodiphenyldichloroethylene.

Conclusions: Inverse associations between pyrethroids and weight trajectory parameters among boys are consistent with hypothesized disruption of androgen pathways and with our previous research in this population, and support the endocrine-disrupting potential of pyrethroids in humans.

Keywords: Child growth trajectory; Indoor residual spraying; Insecticides; Prenatal exposure; South Africa

Child physical growth is a global metric for development and wellbeing, and an important indicator of current and future health status. Although slow growth can predict poor health outcomes,[1] accelerated growth may increase the risk of obesity and cardiometabolic disease.[2] Aside from genetics and nutrition, exposure to endocrine-disrupting chemicals may influence child growth by interfering with sex hormones involved in critical periods of rapid growth during hypothalamic–pituitary–gonadal (HPG) axis activation.[3] Each year, millions of individuals, including pregnant women, are exposed to high levels of such chemicals from indoor residual spraying, a malaria control method that consists of the application of insecticides on the interior walls of dwellings.[4–6]

Pyrethroids, commonly used in agriculture and commercial pest control products globally and the most frequently used class of insecticides for indoor residual spraying,[6] disrupt androgen function.[7,8] However, few human studies have investigated their potential impact on child weight. In the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), a birth cohort conducted in Limpopo, South Africa where indoor residual spraying occurs annually, maternal urinary pyrethroid metabolite levels were not associated with weight at birth,[9] but were inversely associated with weight z-scores among boys at 1, 2, and 3.5 years.[10–12] In a Chinese birth cohort, the sum of three maternal pyrethroid metabolite concentrations was inversely associated with weight at birth.[13] However, other studies found no associations with weight at birth,[14] 1 year,[15] and 4 years of age.[16]

The insecticide dichlorodiphenyltrichloroethane (DDT) is a well-established estrogen agonist.[17] Though it is allowed for public health uses including indoor residual spraying,[6] it has otherwise been banned in Western countries since the 1970s and internationally since 2001, leading to low detection frequencies in most populations. Detection frequencies were sufficient to investigate associations between gestational exposure to DDT and postnatal child weight in only two cohorts (VHEMBE and a US study initiated before DDT was banned), but a larger number of studies have investigated DDT's more persistent, antiandrogenic breakdown product dichlorodiphenyldichloroethylene (DDE). In VHEMBE, maternal serum DDT but not DDE was associated with increased weight among girls at birth,[9] 1 and 2 years,[10] but no associations were observed at 3.5 years,[11] and in the US study, neither was associated with child weight at birth or 5 years.[18] Similarly, maternal serum DDE levels were not associated with weight measured from birth to 12 months in a Mexican birth cohort,[19] but other studies reported positive associations with offspring weight at older ages.[20–22]

Although weight measured at specific ages remains an important marker of child development, dynamic child growth metrics based on measures taken at two or more time points have been found to predict later metabolic and cardiovascular health better than single time point measures.[23,24] For example, rapid infant weight gain has been linked to: a 3.7-fold (95% confidence interval [CI] = 2.6, 5.2) increased risk for overweight or obesity[25]; hypertension[2,26,27]; lower HDL cholesterol[27]; insulin resistance and/or diabetes[26,27]; and metabolic syndome[26] in adolescence or adulthood. However, the few prior studies that investigated associations between exposure to pyrethroids or DDT/E and dynamic growth metrics primarily relied on changes between two-time points (e.g., birth to 1, 6, or 24 months), which does not capture the complex nature of growth dynamics.[28–33] One study applied a grouping-based classification based on latent growth patterns[29]; however, these methods pose challenges for inference including the subjective selection of the number and interpretation of observed patterns, loss of information from categorization of the outcome, and lack of comparability of observed patterns between studies. SuperImposition, Translation And Rotation (SITAR) models, which have been used widely in the perinatal epidemiologic literature,[34–36] overcome these limitations and estimate three biologically interpretable parameters: overall weight or other growth metric over time (size), and the timing (tempo) and velocity (intensity) of the growth spurt.[37]

Here, we seek to estimate the effect of gestational exposure to DDT/E and pyrethroid insecticides on child weight trajectories from birth through 5 years of age, in a population residing in an area where these insecticides are applied annually as part of indoor residual spraying programs. This study presents a novel application of SITAR to investigate environmental chemical influences on child weight trajectories.

METHODS

Data Source

The VHEMBE study recruited mothers giving birth between August 2012 and December 2013 at Tshilizidini hospital in the Vhembe district of Limpopo province, South Africa. Study staff approached 1,649 mothers, 920 of whom met eligibility criteria. 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 at the time of enrollment, and delivered a live, singleton infant. Of the eligible women, 752 provided informed consent; baseline questionnaire and peripheral blood samples for DDT and DDE analysis were available from 751 mothers, 738 of whom provided sufficient urine samples for pyrethroid analysis. Follow-up continued with a home visit 1 week postpartum and field office visits were completed at 1, 2, 3.5, and 5 years, with retention rates of 96%–99% after each visit, excluding child deaths.

All participating mothers provided informed consent. Ethics approval for the VHEMBE study was obtained from McGill University (Montreal, Quebec, Canada), the University of Pretoria (Pretoria, Gauteng, South Africa), Tshilidzini Hospital (Thohoyandou, Limpopo, South Africa), the Limpopo Department of Health and Social Development (Polokwane, Limpopo, South Africa), and the University of California, Berkeley (Berkeley, California, USA).

Exposure Measurement: Maternal Serum DDT/E and Urinary Pyrethroid Metabolites

Maternal urine and venous blood samples collected at delivery were processed immediately after collection and stored at −80 °C until shipment to analytical laboratories on dry ice. Maternal serum concentrations of DDT/E isomers (*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, and *p,p'*-DDE) were measured using gas chromatography-tandem mass spectrometry by the Emory University Environmental Health Laboratory (Atlanta, Georgia).[38] We estimated total serum lipid concentrations based on total cholesterol and triglycerides measured by standard enzymatic methods (Roche Chemicals, Indianapolis, Indiana).[39] Maternal urine concentrations of the following five pyrethroid metabolites were measured using gas chromatography-tandem mass spectrometry by the Institut National de Santé Publique du Québec (Quebec City, Quebec, Canada)[40]: *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 (SG) was measured with a portable refractometer (Atago PAL-10S; Atago, Tokyo, Japan). Limits of detection (LOD) were: 0.01 ng/mL (*o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDE), 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), 0.0047 µg/L (3-PBA), and 0.005 µg/L (4-F-3-PBA). Limits of quantification (LOQ) were: 0.05 ng/mL (*o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDE), 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), 0.016 µg/L (3-PBA), and 0.011 µg/L (4-F-3-PBA).

Urinary concentrations of *o,p'*-DDE and 4-F-3-PBA were above the LOQ in only 16% and 8% of samples, respectively, and thus were excluded from further analyses. For the other analytes, concentrations below the LOD were imputed at random based on log-normal probability distributions whose parameters were estimated via maximum likelihood methods.[41] Machine-read values were used for values between the LOD and the LOQ. DDT and DDE were corrected for serum lipid content and expressed in ng/g lipid. Pyrethroid metabolite concentrations (C_{meas}) were corrected (C_{corr}) for urine dilution via SG, based on the formula from Levine and Fahy[42]: $C_{corr} = C_{meas} \times (1.024 - 1)/(SG - 1)$.

Outcome Measurement: Child Weight

Child weight was measured by trained study staff to the nearest 10 g at the 1- and 2-year visits using a pediatric digital scale (Tanita BD-590; Tokyo, Japan), and at the 3.5- and 5-year visits using a standard digital scale (Tanita HD-351; Tokyo, Japan); a single measure was taken at each time point based on high reliability during testing. In addition, birthweight and body weight measurements recorded at the hospital, during well-child appointments, and other clinic visits were abstracted from medical records by a registered nurse, including the child's age in weeks or months when measurements were taken.

Covariates from Maternal Questionnaires and Anthropometrics

Trained bilingual (Tshivenda and English) local study staff administered questionnaires at baseline and follow-up field office visits to collect sociodemographic, nutrition, and health information (Table 1). At baseline, mothers reported their age, marital status, total household income, and total household size. Food poverty was defined as earning less than 386 Rands/person/month based on Statistics South Africa guidelines.[44] Food insecurity was defined as two or more affirmative responses to the US National Center for Health Statistics'

Six-Item Food Security Scale.[45] Daily total energy intake in kilojoules (kJ) was estimated using the FoodFinder 3 software (SouthAfrica Medical Research Council/WAMTechnology CC) based on a quantitative food frequency questionnaire designed by a South African nutritionist and validated in the local population.[46] Insufficient energy intake during pregnancy was defined according to the Institute of Medicine-recommended total daily caloric intake for high-activity mothers in the third trimester, which was calculated based on their age, height, and postdelivery weight.[43,47] Maternal HIV status during pregnancy was ascertained from self-report or medical records indicating use of antiretroviral drugs, which were abstracted by registered nurses on the study team. In addition, mothers' 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.

TABLE 1. - Selected Characteristics of VHEMBE Study Participants, Limpopo, South Africa (n = 751)

	n		
Maternal baseline characteristics			
Age, years (mean ± SD)	751	26.4	±6.3
Height, cm (mean ± SD)	739	158.2	±6.8
Weight, kg (mean ± SD)	740	68.8	±13.7
Body mass index, kg/m² (mean ± SD)	735	27.5	±5.4
Married or living-as-married (freq, %)	751	359	48%
High school diploma (freq, %)	751	339	45%
Nulliparous (freq, %)	751	326	43%
Insufficient energy intake during pregnancy[a] (freq, %)	735	444	68%
Ever smoker (freq, %)	751	6	1%
Any alcohol during pregnancy (freq, %)	751	69	5%
HIV positive (freq, %)	751	103	14%
Household sociodemographic characteristics			
Food poverty[b] (freq, %)	748	460	61%
Food insecurity[c] (freq, %)	750	329	44%
Child characteristics			
Child sex, female (freq, %)	751	364	48%
Low birthweight, <2500 g (freq, %)	750	63	8%
Preterm birth, <37 weeks (freq, %)	751	103	14%
Small for gestational age, <10th percentile (freq, %)	750	182	24%
Any breastfeeding, months (mean ± SD)	695	15.9	±7.0
Exclusive breastfeeding, months (mean ± SD)	702	2.3	±1.9

[a]Below the Institute of Medicine-recommended total daily caloric intake for mothers in late pregnancy.[43]

[b]Below the food poverty line of 386 Rand/person/month.[44]

[c]Two or more affirmative responses to the US National Center for Health Statistics' Six-Item Food Security Scale.[45]

freq indicates frequency.

Information on duration of breastfeeding was obtained from the 1-week and 1-, 2-, and 3.5-year questionnaires. A family wealth index was constructed based on data obtained from the

1-week home visit (questionnaire and staff observations), following South Africa's Demographic and Health Surveys methodology to capture socioeconomic status in this region where much of the economy is informal.[11,48]

To explore potential confounding by child dietary intake, we constructed a child diet diversity score reflecting the number of different food groups eaten, as reported by mothers at the 3.5-year visit.[47]

Statistical Analysis

Child Weight Trajectories Estimated Using SITAR

After removing 22 outliers (>3 SD from expected values conditional on preceding measurements),[49] we modeled child weight measurements from the study visits and medical records (n = 13,489; median: 12 measurements per child, interquartile range: 4–24) using the sitar package (version 1.1.1) in R (version 3.6.1).[50,51] SITAR fits a natural cubic spline to the average population growth curve (in this case, weight in kilograms vs. age in months) from which individual deviations are captured by three random effects parameters,[37] where:

$$y_{it} = a_i + h\left(\frac{t - b_i}{exp(-c_i)}\right)$$

> (1) Size (*a*) indicates the child's mean weight compared with the average (in kilograms), representing vertical translation of the weight curve;
>
> (2) Tempo (*b*) indicates the child's age at peak weight velocity compared with the average (in months), representing horizontal translation of the weight curve; and
>
> (3) Intensity (*c*) indicates the child's growth rate compared with the average (expressed as a fraction).

After fitting candidate models including all children, as well as models stratified by sex, the random effects parameters estimated by the best-fitting SITAR model were used as outcomes in marginal structural models as described below (see eAppendix 1; https://links.lww.com/EDE/B914, for details).

Inverse Probability of Treatment Weight Construction and Balance Assessment

To control for confounding, we constructed stabilized inverse probability of treatment weights (IPTW) based on the generalized propensity score method.[52] To construct the generalized propensity score, we used multivariable linear regression to estimate the conditional density of each participant's exposure. The lipid- or SG corrected exposure concentrations were log_{10}-transformed to reduce the influence of outliers. We included the following potential confounders and predictors of the outcomes as the independent variables, which were identified based on a directed acyclic graph (eFigure 3.1; https://links.lww.com/EDE/B914): 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 vs. sufficient), alcohol use during pregnancy (yes vs. no), HIV status (positive vs. negative), duration of exclusive breastfeeding (months, continuous), and parity (continuous).

Applying IPTWs generates a pseudo-population in which exposure is independent of the measured confounders.[53] To confirm this, we assessed whether the distribution of covariates was similar, or "balanced," across the exposure range in the IPTW-weighted sample by calculating correlations between each exposure and continuous covariate, as well as the absolute standardized difference of covariates in each exposure quartile versus all other exposure quartiles.[52] As recommended by Austin (2018), we considered correlations below 0.1 and absolute standardized differences below 0.2 as indicating balance.[54] We also calculated the average of variance ratios for all covariates across exposure quartiles, using a threshold of 2.0 to indicate balance.[55] Following best practices for specifying propensity score models, we considered log-transformations and machine learning methods in an iterative process to improve balance, resulting in the $\log_2$-transformation of all continuous covariates except for the wealth index. Further details on constructing the IPTW and balance assessment are provided in eAppendix 3; https://links.lww.com/EDE/B914.

Estimating Effects of Prenatal Insecticide Exposure on Child Weight Trajectory Parameters

The effects of a 10-fold increase in maternal lipid-corrected serum DDT/E or SG corrected urinary pyrethroid metabolite concentrations on each estimated child-specific random effects SITAR parameter were estimated based on marginal structural models with IPTW. Under the four assumptions of consistency, exchangeability, positivity, and no misspecification of the propensity score model, marginal structural models generate effect estimates that have a causal interpretation.[53]

Since these insecticides disrupt sex hormones and sex-specific effects on child weight have been reported,[9–11] we conducted secondary analyses investigating effect modification by child sex. In addition, since socioeconomic status has often been found to modify the health effects of environmental exposures,[9,56–58] and previous analyses in VHEMBE pointed to undernutrition as a possible explanation,[11,47,59] we also investigated potential effect modification by food poverty, food insecurity, and maternal energy intake during pregnancy. This was done by including an interaction term between the effect modifier and the exposure, using the threshold of $P < 0.1$ to indicate statistical evidence of effect measure modification. Effect modifiers were also taken into account in the IPTW for each analysis (see eAppendix 3; https://links.lww.com/EDE/B914, for more details).

We imputed missing covariate values using multiple imputation by chained equations[60] (see eAppendix 2; https://links.lww.com/EDE/B914) and constructed 95% CI by bootstrapping the multiple imputation, estimation of IPTW and outcome regressions 500 times.[61,62] We treated SITAR parameters as fixed parameters in this analysis. We conducted all analyses other than SITAR using Stata version 14.2 (StataCorp, College Station, Texas).

RESULTS

Participant Characteristics

The average age of mothers participating in the study was 26.4 years (Table 1). All were Black Africans, and just over half were unmarried (52%), had less than a high school diploma (55%), and lived below the South African food poverty line (61%). Many lived in food-insecure households (44%). The prevalence of HIV among mothers was 14%. Half of the children were female (49%), 24% were small for gestational age (<10th percentile) at birth and 14% were born preterm (<37 weeks gestational age at birth). The median duration of exclusive breastfeeding without introduction of water or solids was short (1.5 months), though breastfeeding continued for longer (median = 15.9 months; Table 1).

Virtually all participants had detectable levels of DDT/E and pyrethroid metabolites (Table 2). Correlations were high between congeners of DDT/E (Pearson's $r = 0.69–0.85$) and between the pyrethroid metabolites *cis*-DCCA, *trans*-DCCA, and 3-PBA ($r = 0.83–0.88$). However, *cis*-DBCA was only moderately correlated with the other pyrethroid metabolites ($r = 0.32–0.51$), and pyrethroid metabolites were not correlated with DDT/E ($r = −0.03$ to 0.07).

Child Weight Trajectories Estimated Using SITAR

SITAR models containing all three random effects (size, tempo, and intensity) did not converge. The best-fitting SITAR models for the cohort overall, as well as when stratified by child sex, were all based on log-transformed weight, three degrees of freedom in the population average spline, and random effect parameters for size and tempo (eTables 1.1–3; https://links.lww.com/EDE/B914). Since the overall and sex-specific models had similar fit (based on variance explained), correlations between random effects, and shape of the weight trajectory (eFigure 1.1; https://links.lww.com/EDE/B914), we selected the overall model as the final model for parsimony. The fitted weight trajectories (weight vs. age and weight velocity vs. age) are shown in Figure. The estimated average age at peak weight velocity was just under 1 month (26.6 days, SD = 12.2).

TABLE 2. - Distribution of Maternal Peripartum Serum DDT/E (ng/g Lipid) and Urinary Pyrethroid Metabolites (µg/L, Specific Gravity Corrected) Concentrations Among VHEMBE Study Participants, Limpopo, South Africa (n = 751)

						Percentiles			
	n	**%>LOD**	**%>LOQ**	**GM (SD)**	**Min**	**25**	**50**	**75**	**Max**
DDT/E									
o,p'-DDT	751	91%	43%	8.9 (4.6)	0.1	3.4	7.1	22.7	2,029.3
p,p'-DDT	751	98%	91%	69.6 (6.7)	0.1	19.0	55.3	261.0	15,027.6
p,p'-DDE	751	100%	97%	287.9 (4.8)	4.0	91.8	242.2	878.9	26,301.3
Pyrethroid metabolites									
cis-DBCA	738	100%	100%	0.35 (3.02)	0.02	0.16	0.33	0.74	13.39
cis-DCCA	738	100%	100%	0.48 (2.55)	0.05	0.26	0.46	0.79	209.49
trans-DCCA	738	100%	100%	0.55 (3.07)	0.03	0.26	0.53	1.05	268.95
3-PBA	737	100%	100%	1.12 (2.38)	0.10	0.66	1.05	1.84	102.38

GM, geometric mean.

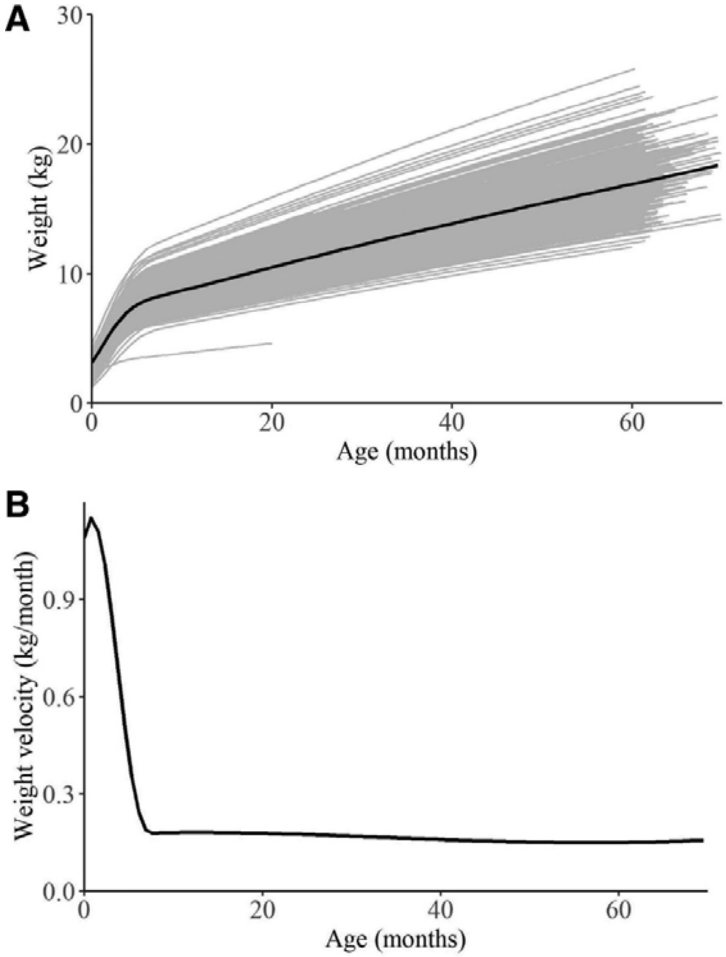

FIGURE.: SITAR-modeled weight trajectories of VHEMBE children from birth to 5 years, based on all available weight measurements (study visits and medical records). A, Predicted population average (black line) and child-specific weight trajectories (gray lines), kg versus month. B, Predicted population average weight velocity, kg/month versus month.

Inverse Probability of Treatment Weights

The distribution and diagnostics of the IPTW for each exposure are shown in eAppendix 3; https://links.lww.com/EDE/B914. The mean of the stabilized IPTW was 1.00 and no extreme weights were observed (eTable 3.1; https://links.lww.com/EDE/B914). For each IPTW, all correlations between exposure and continuous covariates were below 0.1 (eFigure 3.2; https://links.lww.com/EDE/B914) and all standardized differences were below 0.2 (eFigure 3.3; https://links.lww.com/EDE/B914), indicating that balance was achieved.[54]

Effects of Prenatal Insecticide Exposure on Child Weight Trajectory Parameters

We found that higher maternal concentrations of multiple pyrethroid metabolites were associated with smaller size and tempo parameters, corresponding to lower average weight from birth to 5 years and earlier age at peak weight velocity, respectively; however, all estimates were imprecise (Table 3). For instance, a 10-fold increase in *cis*-DBCA was associated with 13 g smaller size (95% CI = −29, 2.5) and 1.8-day earlier tempo (95% CI = −3.7, 0.1).

TABLE 3. - Effects of a 10-fold Increase in Maternal Peripartum DDT/E (ng/g Lipid) or Pyrethroid Metabolite (μg/L) Concentrations on Birth to 5-year Weigh t Trajectory Parameters Among Children Participating in the VHEMBE Study, Limpopo, South Africa

	n	Size (g), β (95% CI)	Tempo (days), β (95% CI)
o,p′-DDT	751	1.5 (−10, 13)	−0.4 (−1.7, 1.0)
p,p′-DDT	751	2.9 (−6.5, 12)	−0.4 (−1.5, 0.6)
p,p′-DDE	751	6.5(−5.1, 18)	−0.3 (−1.6, 1.0)
cis-DBCA	738	−13 (−29, 2.5)	−1.8 (−3.7, 0.1)
cis-DCCA	738	−7.6 (−27, 11)	−1.9 (−4.0, 0.3)
trans-DCCA	738	−7.2 (−21, 6.7)	−1.4 (−3.2, 0.4)
3-PBA	737	−0.2 (−19, 18)	−2.2 (−4.8, 0.5)

Estimated using marginal structural models.

However, in analyses examining effect modification by sex, we found that 10-fold higher maternal concentrations of *trans*-DCCA were associated with 21g (95% CI = −40, −1.6) smaller size among boys only (*P*-value for interaction, P_{inter} = 0.07). Other pyrethroid metabolites, including *cis*-DCCA and *cis*-DBCA were also associated with smaller size among boys but estimates were imprecise and evidence of effect modification by sex was limited (p_{inter} = 0.13 for *cis*-DCCA and 0.41 for *cis*-DBCA; Table 4). Associations between pyrethroids and size among girls were weak, with estimates ranging from −6.7 g (95% CI = −30, 17) for *cis*-DBCA to 10 g (95% CI = −19, 40) for 3-PBA.

We did not observe associations between DDT/E and size or tempo parameters, overall or by sex (Tables 3 and 4). Moreover, we did not observe effect modification by food poverty, food insecurity, or maternal energy intake during pregnancy for any associations between maternal insecticide concentrations and these child weight trajectory parameters (eTables 4.1–4.3; https://links.lww.com/EDE/B914).

DISCUSSION

Main Findings and Interpretation

This study aimed to estimate the effect of prenatal exposure to DDT, DDE, and pyrethroid insecticides on child weight trajectories from birth to age 5 years among South African children from an area where indoor residual spraying is conducted annually to control malaria. Our results suggest that the pyrethroid metabolite *trans*-DCCA, and to a lesser extent *cis*-DBCA and *cis*-DCCA, are associated with lower average weight among boys between birth and 5 years of age. These findings are consistent with previous VHEMBE studies based on weight measured at 1, 2, and 3.5 years,[10–12] though no associations were observed with birthweight.[9] Similarly, two Chinese birth cohorts did not observe associations between *cis*- or *trans*-DCCA and weight at birth (n = 454)[13] or 1 year (n = 497).[15] This suggests that the potential effect of pyrethroid metabolites on child growth may not be evident at birth, and the lower exposures and statistical power in the latter study may explain the null finding at 1 year. The only study to investigate a dynamic growth metric did not find an association between self-reported use of pyrethroid-containing products and weight change during the first month,[28] however, nondifferential exposure misclassification may have contributed to this null finding. Among the few animal studies, exposure to cypermethrin, an insecticide used for indoor residual spraying in the VHEMBE area that metabolizes into *cis/trans*-DCCA

TABLE 4. - Effects of a 10-Fold Increase in Maternal Peripartum DDT/E (ng/g Lipid) or Pyrethroid Metabolite (μg/L) Concentrations on Birth to 5-Year Weight Trajectory Parameters, by Child Sex

	n	Boys β (95% CI)	Girls β (95% CI)	*P*, Interaction
Size (g)				
o,p′-DDT	751	−6.7 (−22, 8.8)	12 (−7.2, 31)	0.15
p,p′-DDT	751	−4.4 (−18, 9.0)	11 (−4.0, 25)	0.15
p,p′-DDE	751	1.1 (−15, 17)	12 (−6.5, 31)	0.39
cis-DBCA	738	−20 (−42, 1.2)	−6.7 (−30, 17)	0.41
cis-DCCA	738	−23 (−51, 5.1)	9.3 (−19, 37)	0.13
trans-DCCA	738	−21 (−40, −1.6)	9.2 (−14, 33)	0.07[b]
3-PBA	737	−12 (−39, 15)	10 (−19, 40)	0.31
Tempo (days)				
o,p′-DDT	751	0.4 (−1.4, 2.2)	−1.1 (−3.2, 1.0)	0.31
p,p′-DDT	751	−0.1 (−1.6, 1.4)	−0.9 (−2.4, 0.7)	0.50
p,p′-DDE	751	0.8 (−0.9, 2.5)	−1.4 (−3.5, 0.6)	0.12
cis-DBCA	738	−1.7 (−4.4, 1.1)	−1.8 (−4.6, 1.0)	0.95
cis-DCCA	738	−2.5 (−5.2, 0.3)	−1.2 (−4.7, 2.2)	0.59
trans-DCCA	738	−2.0 (−4.1, 0.2)	−0.7 (−3.7, 2.3)	0.50
3-PBA	737	−1.7 (−5.1, 1.7)	−2.7 (−6.9, 1.5)	0.72

Estimated using marginal structural models.

[a]95% CI excludes the null.

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

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

and 3-PBA, resulted in lower weight from childhood to adulthood among rat offspring.[63] However, two studies of prenatal exposure to deltamethrin, which is also used in indoor residual spraying and metabolizes into *cis*-DBCA and 3-PBA, found no effect on mouse offspring weight at birth[64] nor in adulthood.[65]

Our results also suggest that prenatal exposure to pyrethroid insecticides may be associated with earlier age at peak weight velocity, though estimates were imprecise. The timing of peak weight velocity identified by the SITAR model is consistent with minipuberty, a period of HPG axis activation occurring in the first few months of life characterized by surges in gonadotropins and sex hormones that drives a growth spurt.[3] Thus, if pyrethroid exposure does affect growth and the timing of peak weight velocity, one possible mechanism would be through disruption of this endocrine axis. Several experimental studies have shown that exposure to pyrethroids lowers testosterone levels in mice,[66–69] though two studies reported increases.[63,69] Furthermore, there is some animal and human evidence that pyrethroids disrupt the timing of puberty, which is another period of HPG activation.[7,70–72] The potential link between gestational exposure to pyrethroids, disruption of the HPG axis, and growth merits further investigation.

We did not find evidence that prenatal exposure to DDT or DDE was associated with postnatal growth within the first 5 years, which is consistent with the literature investigating weight at single time points. The literature concerning dynamic growth metrics is mixed, and not directly comparable due to differences in outcome measures. One study in an agricultural region of California (n = 249) estimated latent growth patterns, reporting an association between maternal serum DDT and stable and then increasing body mass index after age 5 among boys which was no longer present after confounder adjustment.[29] Larger studies of interval-based metrics have reported associations between DDE and weight gain in the first 6 months (three pooled Spanish birth cohorts, n = 1,285)[31] and 24 months (five pooled European birth cohorts, n = 1,791),[33] while a Greek birth cohort of similar size to VHEMBE (n = 689) with much lower DDE exposure did not observe associations with weight gain from birth to 6 months.[32]

Limitations and Strengths

A limitation of this analysis is the measurement of exposure at a single timepoint to represent gestational exposure to pyrethroids, which have a biologic half-life measured in hours or days.[73] However, intraclass correlation coefficients for repeated spot urine measurements of pyrethroid metabolites have been found to vary from 0.21 in the United States to 0.85 in Poland, suggesting that the reliability of a single measurement may vary from one population to another.[74,75] Furthermore, the pyrethroids used for indoor residual spraying have been designed to be stable in the environment, and remain effective throughout the rainy season for up to 10 months, aided by protection indoors from direct sunlight and external elements which would otherwise lead to their rapid degradation[76]; therefore, elevated exposure to inhabitants may persist for months from repeated contact with contaminated surfaces, bedding, furniture, and stored food. Moreover, indicators of regular pesticide use, such as storing pesticide containers on the homestead and self-reported use of pesticides in the yard, were associated with higher pyrethroid metabolite concentrations among VHEMBE mothers,[5] suggesting that a single measurement may be representative of longer-term exposure in the VHEMBE population.

Although numerous studies have investigated environmental chemical influences on child weight measured at single time points, very few have examined child growth trajectories. This is, to the best of our knowledge, the first study of prenatal environmental chemical influences on child growth trajectories estimated by SITAR, a method which overcomes limitations of other measures. For example, interval-based metrics can vary widely in the timepoints selected (e.g., birth to 6 vs 12 months), the measure being compared (e.g., absolute vs. standardized weight), and whether it is expressed in absolute versus relative terms and continuously versus categorically[36]; grouping-based methods result in loss of information due to categorization, and the number and interpretation of the observed growth patterns is subjective.[23] SITAR parameters have a straightforward biologic interpretation, comparing the weight (size parameter) as well as the timing (tempo) and velocity (intensity) of the growth spurt to the average child,[37] while accounting for nonlinear growth patterns and allowing for flexibility in the timing and number of measurements for each child. Also, compared with parametric mixed-effects growth models, spline-based SITAR can lead to better model fit.[34,35]

Another methodological advantage of this study is the use of IPTW, which allowed us to verify that all measured potential confounders identified a priori were balanced across exposure quartiles, which presents an important advantage over multivariable regression adjustment used in most of the existing literature. We also verified the balance of postexposure covariates not included in the generalized propensity score such as preterm birth, household food poverty at follow-up visits and child's dietary diversity and intake of fruit, vegetables, meat, and fish at 3.5 years (Figure, eFigures 3.2 and 3.3; https://links.lww.com/EDE/B914). Extensive questionnaires allowed us to control for a wide variety of potential confounders.

CONCLUSIONS

This study demonstrated a novel application of SITAR growth trajectory modeling to examine the influence of environmental chemicals on growth. Our results suggest that prenatal exposure to pyrethroid insecticides suppresses growth among boys, which may reflect disruption of androgens critical to physical development in early infancy. Evidence was strongest for *trans*-DCCA, a metabolite of several pyrethroids including cypermethrin, which is used for indoor residual spraying as well as in commercial insecticides. Implications of our findings may thus apply to contexts beyond that of indoor residual spraying. The worldwide use of pyrethroids in agricultural and domestic applications has been increasing as the main replacement for banned organophosphate pesticides, reaching a global market value of 3 billion USD.[77] However, given the sparsity of the existing literature, further studies are needed to confirm our findings, especially among populations in which indoor residual spraying occurs and in other settings with high exposure.

ACKNOWLEDGMENTS

We gratefully acknowledge the dedicated and hard-working VHEMBE field staff and the VHEMBE participants for making this study possible. We also thank Stephen Rauch for his invaluable data management work.

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