Associations of Maternal Exposure to Dichlorodiphenyltrichloroethane and Pyrethroids With Birth Outcomes Among Participants in the Venda Health Examination of Mothers, Babies and Their Environment Residing in an Area Sprayed for Malaria Control

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Abstract

Although effective in controlling malaria, indoor residual spraying results in elevated exposure to insecticides such as dichlorodiphenyltrichloroethane (DDT) and pyrethroids. These chemicals cross the placenta, but no studies have examined their associations with birth outcomes in populations residing in indoor residual spraying areas. We investigated this question in the Venda Health Examination of Mothers, Babies and Their Environment (VHEMBE), a birth cohort study of 751 South African children born between 2012 and 2013. We measured maternal peripartum serum DDT and urine pyrethroid metabolite concentrations and collected data on birth weight, length, head circumference, and duration of gestation. We analyzed the data using marginal structural models with inverse-probability-of-treatment weights, generalized propensity scores, and standard conditional linear regression. Using all 3 analytical methods, *p,p′*-DDT, *o,p′*-DDT, and to a lesser extent *p,p′*-dichlorodiphenyldichloroethylene were related to elevated birth weight, birth length, and head circumference among girls. Changes in gestational duration did not mediate this relationship, suggesting that these exposures accelerate fetal growth, which is consistent with the known estrogenic properties of *o,p′*-DDT and *p,p′*-DDT. No associations with pyrethroid metabolites were found. Results suggest that prenatal exposure to DDT is related to elevated birth size. Further studies are needed to elucidate the implications of these findings.

Keywords: birth outcomes, birth weight, DDT, indoor residual spraying, insecticides, marginal structural models, pyrethroids, South Africa

1. Introduction

According to the World Health Organization (WHO), 212 million people were infected by malaria in 2015, resulting in almost half a million deaths, primarily among children under age 5 years.(1) Indoor residual spraying (IRS), the application of insecticides on the inside walls of residences, is commonly used for malaria control.(1, 2) Although this practice appears effective in reducing infection, it results in elevated exposure to insecticides with incompletely understood health consequences. In South Africa, pyrethroid insecticides and dichlorodiphenyl trichloroethane (DDT) are used for IRS.

Animal and *in vitro* studies suggest that pyrethroids and DDT may alter thyroid hormone homeostasis, which play a critical role in child growth and brain development, particularly during pregnancy and the neonatal period.(3) Several pyrethroids including deltamethrin, permethrin, cypermethrin, fenvalerate, and the non-specific metabolite 3-phenoxybenzoic acid (3-PBA) interact with the thyroid receptor (4, 5). No animal study has investigated the effect of prenatal exposure to pyrethroids on offspring thyroid function. However, postnatal intraperitoneal exposure to permethrin, deltamethrin, karate and talstar in rats and fenvalerate in mice was found to lower total triiodothyronine (T3) and total thyroxine (T4) and/or increase thyroid-stimulating hormone (TSH) blood concentrations (6-9). In contrast, rats intraperitoneally exposed to fenvalerate showed elevated total T3 and/or total T4, and no change in total T3, total T4 or TSH following oral exposure to lower doses of deltamethrin (10-12).

The only human study to investigate associations between prenatal exposure to pyrethroids and neonatal thyroid hormones found no associations between maternal urinary 3-PBA and TSH or free T4 concentration in 147 mother-child pairs from Tokyo, Japan but the

moderate sample size may have limited statistical power (13). Cross-sectional studies conducted in U.S. adults also found no associations between urinary 3-PBA concentrations and thyroid hormones (14, 15). However, an inverse relation was reported between urinary *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DCCA) and total triiodothyronine (T3) blood concentrations among U.S. males recruited from an infertility clinic.(15)

DDT reduces total T4 blood concentration and induces the microsomal enzyme uridinediphosphate glururonosyltransferase (UDP-GT) in rats (16). Since glucuronidation by UDP-GT is the rate-limiting step in T4 metabolism, this mechanism may underlie the reported hypothyroidic (lower T4 and, in some cases, elevated TSH) effect of DDT in rodents (16-19). While none of the three human studies investigating maternal or cord DDT/E blood concentrations and neonatal thyroid hormones found associations (20-22), two studies that measured thyroid hormone in cord blood reported inverse relations between DDT/E (also measured in cord blood) and free or total T4 (23, 24). However, studies conducted in adolescents(25) and adults,(26-29) including pregnant women,(21, 30-32) yielded conflicting results.

These prior studies were primarily conducted in high-income countries. However, vulnerable populations living in areas where IRS is conducted, where poverty, malnutrition, stress and poor health are prevalent, may be particularly susceptible to the adverse health effects of exposure to insecticides. Our objective was thus to evaluate whether prenatal exposure to pyrethroids, DDT or DDE is associated with altered thyroid hormone levels among neonates from Limpopo, South Africa, an area where pyrethroids and DDT are used annually to control malaria. We also aimed to identify factors that may modify these associations.

2. Methods

2.1. Study population

This analysis is based on data from the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), a birth cohort study that investigates the health effects of environmental exposures among pregnant women and children from the Vhembe district of Limpopo Province, South Africa. Women were approached between August 2012 and December 2013 shortly before or after giving birth at Tshilidzini Hospital in the town of Thohoyandou and were invited to participate in the VHEMBE study if they were at least 18 years of age, spoke Tshivenda (the most common language in the region), lived within 20 km of the hospital, planned to live in the area for the following two years, were not diagnosed with malaria during pregnancy, and gave birth to a live singleton. Of 920 eligible women, 152 refused to participate, 3 did not provide sufficient blood for DDT analysis, and 14 did not complete a baseline questionnaire. One week postpartum, we visited 722 of these participants at home (4 children died before the visit, 6 could not be scheduled and 19 dropped out) to conduct a home inspection and collect a dry blood spot from neonates via heel stick for the measurement of thyroid hormone levels. We collected 720 blood spots and obtained valid total T4 and TSH measures for 717 neonates. Eight women did not provide urine and one 3-PBA measurement did not meet quality control standards, leaving samples sizes ranging between 708 and 717. We found no significant differences between participants enrolled and those included in analyses for any of the variables considered in this analysis. All mothers gave informed consent prior to participation. The study was approved by the Institutional Review Boards of the University of California, Berkeley; McGill University; the University of Pretoria; the Limpopo Department of Health and Social Development; and Tshilidzini Hospital.

Data collection. Trained, bilingual (Tshivenda and English) research staff originating from the study area conducted structured interviews with mothers shortly after delivery. The interview included questions on socioeconomic characteristics, household assets, lifestyle, diet (via a food-frequency questionnaire validated in the study area),(33), stress (using a stressful life event scale adapted for the local population)(34, 35) and health and pregnancy history. Gestational age at birth was determined based on date of last menstrual period and maternal HIV status was ascertained from self-report or use of anti-retroviral drugs based on medical records. Total energy and iodine intakes were estimated by a South African expert nutritionist using the FoodFinder3 software (South Africa Medical Research Council/WAMTechnology, Stellenbosch, South Africa). Low daily energy intake was defined based on Institute of Medicine Guidelines for pregnant women (36, 37) following methods described in Huang et al.(38) We defined poverty as income below 386 Rands/person/month (about 30 USD) based on Statistics South Africa guidelines (39) and high stress if more than two stressful life events occurred during pregnancy (the median in this sample). In addition, we generated a wealth index via principal component analysis based on Demographics and Health Survey methodology for South Africa (40). We used data on asset ownership (15 items), livestock ownership (6 items), water source (8 items), number of household members, and cooking fuel (8 items) via maternal report; and toilet facilities (4 items), home floor and wall materials (20 items) via home inspections.(41) The questionnaire was developed in English, translated in Tshivenda and back-translated in English by native speakers in the translated language. Registered nurses abstracted medical records to obtain data on HIV status and delivery method (vaginal or cesarean).

Measurement of insecticides. Research staff collected maternal urine and blood samples before (N_{Blood}=568; N_{Urine}=444) or shortly after delivery (N_{Blood}=152; N_{Urine}=268) and

immediately processed and stored them at -80ºC until shipment to analytical laboratories. The Institut National de Santé Publique du Québec measured pyrethroid metabolite concentrations, including cis-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylicacid (*cis*-DBCA), *cis*-DCCA, *trans*-DCCA, 3-phenoxybenzoic acid (3PBA), and 4-fluoro-3-phenoxybenzoic acid (4F3PBA), in maternal urine by gas chromatography-mass spectrometry based on methods developed by Dewailly et al (42). Limits of detection were 0.0025 μg/L for *cis*-DBCA, 0.0045 μg/L for *cis*-DCCA, 0.0038 μg/L for *trans*-DCCA, 0.0047 μg/L for 3-PBA, and 0.005 μg/L for 4F3PBA. Pyrethroid concentrations were adjusted for dilution by dividing values by sample specific gravity, which was measured using a portable refractometer (Atago PAL-10S, Tokyo, Japan) at the time of sample collection. Creatinine concentration was also measured via spectrophotometry (DRI Creatinine-Detect Test, Thermo Scientific, Waltham, MA).

The Emory University Environmental Health Laboratory measured the *p,p'* and *o,p'* isomers of DDT and DDE as well as polychlorinated biphenyls (PCBs) 118, 138, 153 and 180 using high resolution gas chromatography-isotope dilution mass spectrometry (43). The limits of detection for *p,p'*-DDT, *p,p'*-DDE and *o,p'*-DDT were 0.01, 0.03 and 0.01 ng/mL serum, and the limits of quantification were 0.03, 0.09, and 0.03 ng/mL serum, respectively. DDT/E and PCB concentrations were lipid-corrected and expressed in ng/g lipid. Total lipids were estimated based on triglycerides and total cholesterol concentrations (44) measured using standard enzymatic methods (Roche Chemicals, Indianapolis, IN).

Thyroid hormone measurements. TSH and T4 sharply increase at birth and reduce gradually and stabilize over the next few days of life.(3) Capillary blood samples were thus taken from infants via heel stick at a median of 8 days postpartum (interquartile range: 7-10) and deposited on Whatman filter papers (GE Health Care Life Sciences, Maidstone, United

Kingdom). Samples were air-dried at ambient temperature and stored at -30^{o}C until shipped to the North-West University Newborn Screening Laboratory (Potchefstroom, South Africa) which measured total T4 and TSH using solid-phase, time-resolved sandwich fluoroimmunometric assays (DELFIA®, PerkinElmer Life and Analytical Sciences, Turku, Finland). The laboratory is certified by the U.S. Centers for Disease Control and Prevention (CDC) Newborn Screening Quality Assurance Program.(45) LODs and mean coefficients of variation are 1.5 µg/dL and 7.1% for total T4, and 2 µIU/mL and 7.9% for TSH. Reference intervals are 3.2-11.1 µg/dL for total T4 and 0.27-6.07 µIU/mL for TSH. Hormones were measured in duplicate and averaged.

2.6. Data analysis

Environmental exposures were $\log_{10}$-transformed to reduce the influence of outliers and TSH was $\log_{10}$-transformed to normalize residuals. Reported coefficients thus represent mean (total T4) or percent (TSH) change in outcomes for each 10-fold increase in exposure biomarker concentration. We used analysis of variance and Pearson's correlations for bivariate analyses. We used multiple linear or logistic regression models to estimate associations between biomarkers of exposure and thyroid hormone levels, which were expressed continuously or categorized based on reference ranges.

Directed Acyclic Graphs (DAGs) were used to identify potential confounders, which included maternal age (continuous), education (<12th grade, 12th grade, >12th grade), parity, marital status (married or living as married, not married) and total polychlorinated serum concentration (continuous); alcohol and drug consumption (any, none), smoking (ever, never), exposure to environmental tobacco smoke (ever, never), iodine intake (continuous) and HIV status (positive, negative) during pregnancy; household income per capita and wealth index

(continuous); child sex, gestational age at birth (continuous), age in days (continuous) and delivery method (vaginal, cesarean). We applied a Generalized Propensity Score (GPS) method for continuous exposure by estimating the conditional density of exposure given covariates and included the propensity score variable in models (46). This method allows for the control of a large number of potential confounders without unduly affecting statistical power. The GPS was estimated using the Super Learner algorithm, a loss-based machine-learning method that uses a weighted combination of prediction algorithms to return a function that minimizes cross-validated risk (47).

We ran several sensitivity analyses to evaluate the robustness of our results. First, we evaluated the linearity assumption by running Generalized Additive Models (GAMs) with a 3-degree of freedom cubic spline. Second, we ran models correcting pyrethroid metabolites for creatinine concentration (expressed in μg/g creatinine). Third, we re-ran all models by expressing biomarkers of exposure on a sample volume weight and including cholesterol and triglycerides (for DDT/E models) or specific gravity/creatinine (for pyrethroid models) as covariates in models. Fourth, we ran all models excluding possible outliers identified using the generalized extreme studentized deviate many-outlier procedure. Finally, we built models using a more traditional approach by adjusting only for variables that were associated with any of the exposure and outcomes at $p<0.20$. These models included maternal education; alcohol consumption and HIV status during pregnancy; household income per capita; and child sex, and delivery method. Results were not substantially affected by these different specifications. We show exposure biomarkers expressed linearly, corrected for lipids or specific gravity, including outliers and adjusted using the GPS.

We also investigated effect modification by child's sex, household poverty, and maternal stress, energy intake and HIV status during pregnancy by running separate models including one of these binary cross-product terms. In order to explore whether poverty may modify associations by affecting energy intake or stress, we also ran models including 3-way interaction terms (i.e. exposure*poverty*energy intake status or exposure*poverty*stress status). Missing values (<1.8%) were randomly imputed based on observed probability distributions. Biomarker concentrations between the LOD and the LOQ were assigned machine-read values; concentrations below the LOD were imputed based on a log-normal probability distribution whose parameters were determined via maximum likelihood estimation (48). All analyses were conducted using STATA, version 15 (StataCorp LP, College Station, TX, USA) or R, version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Population characteristics

Women included in this study were all Black/of African descent, and mostly young (mean age = 26.4 years), poor (61% < South African food poverty level) with low levels of education (86% $\leq 12^{th}$ grade education) and low energy intake (68%) during pregnancy (Table 1). About 14% were HIV-positive, and few reported consumption of alcohol (6%) or smoking (0.4%) during pregnancy.

About half (49%) of infants were girls, 13% were born preterm (< 37 weeks gestation), 8% had a low birth weight (< 2,500 g) and a quarter (25%) were small-for-gestational age (< 10^{th} percentile of weight-for-gestational age).

3.2 Concentrations of DDT/DDE, pyrethroid metabolites, and thyroid hormone

Table 2 shows the distribution of biomarkers of exposure to insecticides as well as their detection and quantification frequencies. *o,p'*-DDE and 4F3-PBA were quantified in only 16% and 8% of samples and were excluded from data analysis. Except for *p,p'*-DDT (98% detection) and *o,p'*-DDT (91% detection), the concentration of all other biomarkers of exposure were above the LOD. DDT/E (r=0.69 to 0.85, $p<0.05$) and pyrethroids (r=0.46 to 0.90, $p<0.05$) were intercorrelated within but not between (r=-0.03 to 0.05, $p>0.05$) chemical class. Maternal age, per capita household income and parity were positively associated with *cis*-DCCA, *trans*-DCCA and 3-PBA but not *cis*-DBCA. Male sex was associated with lower *p,p'*-DDT and *o,p'*-DDT, age at heel stick was associated with lower *o,p'*-DDT and vaginal delivery was associated with higher *cis*-DBCA. T4 and TSH were detected in all neonates. Mean T4 was 5.2 µg/dL (SD=1.5) and the geometric mean of TSH was 1.2 µIU/mL (GSD=1.9). Forty-seven (6.6%) children had low T4, two (0.3%) had high T4, none had low TSH and one (0.1%) had high TSH (>9 µIU/mL) blood concentrations. Per capita household income was positively associated with lower TSH, and child age at heel stick and maternal HIV seropositivity were associated with lower T4.

3.3 Associations between pyrethroids and thyroid hormone concentrations

All pyrethroid metabolites were positively associated with TSH; *trans*-DCCA and 3-PBA showed the strongest associations with an 11.0% (95%CI=1.2, 21.8) and 10.4% (95%CI=-1.0, 23.0) change for each 10-fold increase in biomarker concentration, respectively (Table 3). Associations were strongest among girls but interaction p-values were large (p_{int}=0.20-0.39). Overall associations between pyrethroid metabolites and T4 were negative but weak and

imprecise. However, as shown in Figure 1 and Table S1, associations with T4 were substantially stronger among children from poor households for all pyrethroid metabolites, and most particularly for 3-PBA (β=-0.32; 95%CI=-0.62, -0.03), *cis*-DCCA (β=-0.30; 95%CI=-0.59, -0.02 and *trans*-DCCA (β=-0.24; 95%CI=-0.49, 0.00). Figure 2 shows that, among children from poor households, associations between every maternal pyrethroid metabolites and neonatal T4 were more strongly negative for infants whose mothers experienced >2 stressful events during pregnancy than those who did not (e.g. β=-0.56; 95%CI=-1.07, -0.06 vs β=-0.21; 95%CI=-0.57, 0.216 for 3-PBA). However, there was limited statistical support for 3-way interaction (p_{int}= 0.23), possibly due to the limited power to detect such effects.

Associations with T4 were also stronger for all pyrethroid metabolites among children exposed to HIV with the strongest evidence for *cis*-DBCA (β=-0.36; 95%CI=-0.74, 0.01; p_{int}=0.08) but confidence intervals excluded the null (Tables S3 and S4). In addition, odds of low T4 were substantially elevated in relation to pyrethroid metabolites among children from poor households, with ORs ranging between 1.5 (95%CI=0.6, 4.1) for *cis*-DBCA and 2.9 (95%CI=0.7, 12.7) for 3-PBA, but evidence for effect modification was limited (p_{int} 0.20 to 0.48) and estimates were imprecise (Table S2). We found no effect modification by maternal energy intake or stress during pregnancy (data not shown).

3.4 Associations between DDT/E and thyroid hormone concentrations

DDT/E were not associated with TSH. Although some evidence of effect modification by poverty was present for associations with *p,p'*-DDT, estimates were weak and imprecise. However, *p,p'*-DDT (β=-0.20; 95%CI=-0.38, -0.02), *o,p'*-DDT (β=-0.25; 95%CI=-0.46, -0.05)

and, to a lesser extent *p,p'*-DDE (β=-0.17; 95%CI=-0.39, 0.05), were associated with reduced T4 among boys but not girls (p_{int} = 0.02 to 0.28). We found no substantial evidence of effect modification by maternal HIV status, stress or energy intake during pregnancy (data not shown).

4. Discussion

We report inverse associations between maternal *o,p'*-DDT and *p,p'*-DDT and neonatal T4 levels among boys. We also found inverse associations between maternal peripartum pyrethroid metabolite concentrations and T4 levels among neonates from poor households and positive associations with TSH overall. Studies conducted in Spain (n=387-453) and Sweden (n=198) found no association between maternal or cord DDT/E and neonatal TSH, free T4 or total T3 while inverse associations were reported between cord DDT/E and cord free or total T4 in Thailand (n=39) and Belgium (n=198). The only study examining exposure to pyrethroids found no association between maternal urinary 3-PBA during pregnancy and TSH or free T4 among 147 Japanese infants. Prior studies had smaller sample sizes than the current study and most were conducted in high-income countries.

Our results suggest that boys may be more susceptible to thyroid hormone disruption by DDT. Although the precise mechanism for this effect is unclear, DDT has been shown to differentially induce liver enzymes in male and female rats(49) and sexual dimorphism is commonly reported in studies of endocrine disruptors such as DDT.(50) While prior studies of prenatal exposure to DDT/E and thyroid hormones did not investigate effect modification by sex, a moderately-sized cross-sectional study conducted in adults found results that contrasted with

ours in that the sum of DDT and DDE concentrations was positively related with both T4 and T3 among 48 women but not 66 men.(51) However, effect modification was not formally tested.

Prior studies using VHEMBE data also suggest that insecticides may have sexually dimorphic effects. For instance, we found that prenatal exposure to DDT and DDE was associated with increased birth size and elevated BMI-for-age and weight-for-height at 1 and 2 years among girls only.(52, 53) In addition, maternal urinary pyrethroid metabolite concentrations were associated with altered expressive communication and motor development among girls but better motor skills in boys at age 2 based on the Bayley Scales of Infant Development III.(54) Although these results may suggest increased susceptibility to DDT/E among girls, we previously reported associations between prenatal exposure to bisphenol A and lower TSH among males neonates only in the California-based CHAMACOS study.(55)

Results from the present study also suggest that associations between maternal peripartum pyrethroid metabolite concentrations and lower T4 levels are modified by poverty. Although several studies found evidence of increased vulnerability to environmental exposures such as air pollution and lead among individuals of low socioeconomic status,(56-67) few studies have investigated the potential for economic hardship to potentiate the adverse effects of insecticides. We however recently reported associations between maternal DDT/E serum concentrations and higher rates of persistent fever among VHEMBE children from poor households but not among those from households with higher incomes.(38) We also found associations between DDT/E and higher fever rates among children of mothers who had low energy intake during pregnancy but not among those with sufficient energy intake, suggesting that undernutrition may underlie the potentiating effect of poverty. Although fasting is known to downregulate the hypothalamus-pituitary-thyroid (HPT) axis in humans by reducing TSH and/or

T4, likely to lower basal metabolism and save energy,(68-73) we found no evidence that low energy intake during pregnancy modified associations in the present study.

However, we found some evidence that stress during pregnancy may potentiate the inverse relation between maternal urinary pyrethroid metabolites and neonatal T4, particularly among children from poor households, suggesting that stress may represent a mechanism for the modifying effect of socioeconomic status. Biological plausibility for this hypothesis is supported by evidence demonstrating that stress suppresses the HPT axis in rodents.(74) Our results are also consistent with prior studies suggesting that stress and adversity increase the adverse health effects of contaminants. For instance, maternal stress during pregnancy based on a negative life events scale was found to potentiate the relation between maternal exposure to lead and IQ in Mexican children,(75) psychosocial stress increased the relation between $PM_{2.5}$ and blood pressure in Michigan adults (76) and stronger associations were found between prenatal exposure to organophosphate insecticides and lower IQ among California children experiencing adversity in early childhood than among those who did not.(77)

Our findings suggesting stronger associations among children from poorer households raise important questions of environmental justice. While discussion on this topic initially largely centered on the fact that vulnerable populations may experience higher exposure, our results contribute to a growing body of evidence that individuals of lower socioeconomic standing may be more susceptible to the harmful health effects of environmental exposures and support the need for policies that seek to protect vulnerable subpopulations. While such provisions are specifically integrated in environmental law in the U.S. (e.g. as part of the National Environmental Policy Act)(78) and to some extent in Europe (e.g. the Aarhus Convention),(79) low- and middle-income countries (LMICs), including African countries, lag behind in terms

codifying environmental justice into law. This is of particular concern given that the vast majority of the global environmental burden of disease, including 92% of pollution-related deaths, occurs in LMIC populations.(80)

Our results also have implications with regards to the funding of environmental health research. It has been estimated that, on average, 26% of the burden of disease is due to modifiable environmental factors in sub-Saharan countries(81) but, due to a lack of empirical data in African populations, these figures are primarily based on expert opinion or data generated in high-income countries. Given the potential for poverty to potentiate the impact of environmental exposures and the higher prevalence and severity of poverty in Africa relative to high-income countries, the environmental burden of disease may be substantially higher than these estimates suggest. A number of additional factors including different exposure sources, levels and mixtures which occur in widely different social, cultural and economic contexts may render African populations particularly vulnerable to toxic effects, highlighting the need to invest in research in Africa to increase our understanding of the health impact of contaminants so that proper action be taken to protect populations.

This study has several strengths. To our knowledge, this is the first study to investigate associations between prenatal exposure to DDT/E or pyrethroids and thyroid hormone levels among neonates from an area where IRS is conducted. This is also the first such study to investigate effect modification by sex, stress, HIV or poverty. Although the possibility of residual confounding cannot be dismissed, we considered a large number of potential confounders including multiple variables to account for socioeconomic status and adjusted for them via propensity score. Thyroid hormone levels increase sharply shortly after birth and decrease during the first few days of life.(3) In order to limit measurement error, we measured

thyroid hormones in duplicate at a median of 8 days postpartum and no earlier than 3 days. The very low rate of missing data and our use of experienced laboratories to measure biomarkers of exposure and thyroid hormones also constitute strengths. On the other hand, although we used food frequency questionnaires validated in the study population to estimate energy intake during pregnancy, these instruments have inherent limitations as they fully rely on accurate recall. In addition, the measurement of cortisol in serum or saliva may have provided a better indication of maternal stress than the stressful life events scale that we used but women were recruited at the time of delivery and so no samples were available earlier during gestation.

In summary, our results suggest that prenatal exposure to DDT/E is associated with lower total T4 among male neonates. Although thyroid hormones play an essential role in fetal brain development, we previously reported no association between maternal serum DDT/E and child neurodevelopment at age 1 and 2 years.(54) The developmental impact of the DDT/E and T4 associations that we report here is thus unclear. We also found that maternal exposure to pyrethroids is associated with higher TSH overall and lower neonatal T4 among children from poor households. Associations were found with 3-PBA, *trans*-DCCA and *cis*-DCCA but relations with *cis*-DBCA were weaker and imprecise and evidence for effect modification was limited. Given that *cis*-DBCA is a metabolite specific to deltamethrin, the main insecticide used for indoor residual spraying in the study area, results suggest that exposure through home or agricultural use may be of greater concern with regards to thyroid hormone disruption.

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REFERENCES

1. WHO. World Malaria Report. World Health Organization, 2016.
2. Maharaj R, Raman J, Morris N, et al. Epidemiology of malaria in South Africa: from control to elimination. *S Afr Med J* 2013;103(10 Pt 2):779-783.
3. Fisher DA, Brown RS. The maturation of thyroid function in the perinatal period and during childhood. In: Braveman LE, Cooper DS, eds. *Werner & Ingbar's The Thyroid: A fundamental and clinical text*. Philadelphia, PA: Lippincott, Williams & Wilkins, 2013:775-786.
4. Du G, Shen O, Sun H, et al. Assessing hormone receptor activities of pyrethroid insecticides and their metabolites in reporter gene assays. *Toxicological sciences : an official journal of the Society of Toxicology* 2010;116(1):58-66.
5. Ghisari M, Long M, Tabbo A, et al. Effects of currently used pesticides and their mixtures on the function of thyroid hormone and aryl hydrocarbon receptor in cell culture. *Toxicol Appl Pharmacol* 2015;284(3):292-303.

6. Akhtar N, Kayani SA, Ahmad MM, et al. Insecticide-induced changes in secretory activity of the thyroid gland in rats. *Journal of applied toxicology : JAT* 1996;16(5):397-400.

7. Maiti PK, Kar A. Dual role of testosterone in fenvalerate-treated mice with respect to thyroid function and lipid peroxidation. *Journal of applied toxicology : JAT* 1997;17(2):127-131.

8. Maiti PK, Kar A. Is triiodothyronine capable of ameliorating pyrethroid-induced thyroid dysfunction and lipid peroxidation? *Journal of applied toxicology : JAT* 1998;18(2):125-128.

9. Wang S, Shi N, Ji Z, et al. [Effects of pyrethroids on the concentrations of thyroid hormones in the rat serum and brain]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 2002;20(3):173-176.

10. Giray B, Caglayan A, Erkekoglu P, et al. Fenvalerate exposure alters thyroid hormone status in selenium- and/or iodine-deficient rats. *Biological trace element research* 2010;135(1-3):233-241.

11. Kaul PP, Rastogi A, Hans RK, et al. Fenvalerate-induced alterations in circulatory thyroid hormones and calcium stores in rat brain. *Toxicology letters* 1996;89(1):29-33.

12. Sekeroglu V, Sekeroglu ZA, Demirhan E. Effects of commercial formulations of deltamethrin and/or thiacloprid on thyroid hormone levels in rat serum. *Toxicology and industrial health* 2014;30(1):40-46.

13. Zhang J, Yoshinaga J, Hisada A, et al. Prenatal pyrethroid insecticide exposure and thyroid hormone levels and birth sizes of neonates. *The Science of the total environment* 2014;488-489:275-279.

14. Jain RB. Variability in the levels of 3-phenoxybenzoic acid by age, gender, and race/ethnicity for the period of 2001-2002 versus 2009-2010 and its association with thyroid function among general US population. *Environmental science and pollution research international* 2016;23(7):6934-6939.

15. Meeker JD, Barr DB, Hauser R. Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. *Reproductive toxicology (Elmsford, NY)* 2009;27(2):155-160.

16. Tebourbi O, Hallegue D, Yacoubi MT, et al. Subacute toxicity of p,p'-DDT on rat thyroid: Hormonal and histopathological changes. *Environmental toxicology and pharmacology* 2010;29(3):271-279.

17. O'Connor JC, Frame SR, Davis LG, et al. Detection of the environmental antiandrogen p,p-DDE in CD and long-evans rats using a tier I screening battery and a Hershberger assay. *Toxicological sciences : an official journal of the Society of Toxicology* 1999;51(1):44-53.

18. O'Connor JC, Frame SR, Ladics GS. Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. *Toxicological sciences : an official journal of the Society of Toxicology* 2002;69(1):92-108.

19. Yamada T, Kunimatsu T, Miyata K, et al. Enhanced rat Hershberger assay appears reliable for detection of not only (anti-)androgenic chemicals but also thyroid hormone modulators. *Toxicological sciences : an official journal of the Society of Toxicology* 2004;79(1):64-74.

20. Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, et al. Thyroid disruption at birth due to prenatal exposure to beta-hexachlorocyclohexane. *Environment international* 2008;34(6):737-740.

21. Darnerud PO, Lignell S, Glynn A, et al. POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. *Environment international* 2010;36(2):180-187.

22. Lopez-Espinosa MJ, Vizcaino E, Murcia M, et al. Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. *Journal of exposure science & environmental epidemiology* 2010;20(7):579-588.

23. Asawasinsopon R, Prapamontol T, Prakobvitayakit O, et al. The association between organochlorine and thyroid hormone levels in cord serum: a study from northern Thailand. *Environment international* 2006;32(4):554-559.

24. Maervoet J, Vermeir G, Covaci A, et al. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. *Environmental health perspectives* 2007;115(12):1780-1786.

25. Freire C, Koifman RJ, Sarcinelli PN, et al. Long-term exposure to organochlorine pesticides and thyroid status in adults in a heavily contaminated area in Brazil. *Environmental research* 2013;127:7-15.

26. Delport R, Bornman R, MacIntyre UE, et al. Changes in retinol-binding protein concentrations and thyroid homeostasis with nonoccupational exposure to DDT. *Environmental health perspectives* 2011;119(5):647-651.

27. Rylander L, Wallin E, Jonssson BA, et al. Associations between CB-153 and p,p'-DDE and hormone levels in serum in middle-aged and elderly men. *Chemosphere* 2006;65(3):375-381.

28. Teeyapant P, Ramchiun S, Polputpisatkul D, et al. Serum concentrations of organochlorine pesticides p,p'-DDE in adult Thai residents with background levels of exposure. *The Journal of toxicological sciences* 2014;39(1):121-127.

29. Turyk ME, Anderson HA, Persky VW. Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and DDE in adults. *Environmental health perspectives* 2007;115(8):1197-1203.

30. Chevrier J, Eskenazi B, Holland N, et al. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. *American journal of epidemiology* 2008;168(3):298-310.

31. Lopez-Espinosa MJ, Vizcaino E, Murcia M, et al. Association between thyroid hormone levels and 4,4'-DDE concentrations in pregnant women (Valencia, Spain). *Environmental research* 2009;109(4):479-485.

32. Takser L, Mergler D, Baldwin M, et al. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. *Environmental health perspectives* 2005;113(8):1039-1045.

33. MacIntyre UE, Venter CS, Vorster HH. A culture-sensitive quantitative food frequency questionnaire used in an African population: 1. Development and reproducibility. *Public Health Nutr* 2001;4(1):53-62.

34. Richter LM, Dev Griesel R, Barbarin O. Behavioral problems among preschool children in South Africa: A six-year longitudinal perspective from birth to age five. In: Singh N,

Leung J, Singh A, eds. *International perspectives on child and adolescent mental health Volume 1: Proceedings of the first international conference*. Amsterdam, The Netherlands: Elsevier Press, 2000:159-182.

35. Johnson J. Life events as stressors in childhood and adolescence. In: Lahey BB, Kazdin AE, eds. *Advances in clinical child psychology*. Boston, MA: Springer, 1982:219-253.

36. Rasmussen KM, Yaktine AL. Weight gain during pregnancy: Reexamining the guidelines. Washington, DC: Institute of Medicine and National Research Council; 2009. (https://www.ncbi.nlm.nih.gov/books/NBK32813/). (Accessed 15 October 2016).

37. Otten JJ, Hellwig JP, Meyers LD. Dietary reference intakes: The essential guide to nutrient requirements. Washington, DC: Institute of Medicine of the National Academies, 2006.

38. Huang J, Eskenazi B, Bornman R, et al. Maternal peripartum serum DDT/E and urinary pyrethroid metabolite concentrations and child infections at age 2 years in the VHEMBE birth cohort: Associations and modifiers. *Environmental health perspectives* 2018:In press.

39. Statistics South Africa. Poverty trends in South Africa: An examination of absolute poverty between 2006 and 2015. Pretoria, South Africa: Statistics South Africa; 2017. (http://www.statssa.gov.za/publications/Report-03-10-06/Report-03-10-062015.pdf). (Accessed 18 June 2018).

40. Demographics and Health Surveys. Wealth Index. United States Agency for International Development; 2017. (https://dhsprogram.com/topics/wealth-index/Wealth-Index-Construction.cfm). (Accessed April 9, 2018 2018).

41. Huang J, Eskenazi B, Bornman R, et al. Maternal peri-partum urinary pyrethroid metabolites are associated with thinner children at 3.5 years in the VHEMBE birth cohort (Limpopo, South Africa). *Environ Epidemiol* 2018:In press.

42. Dewailly E, Forde M, Robertson L, et al. Evaluation of pyrethroid exposures in pregnant women from 10 Caribbean countries. *Environment international* 2014;63:201-206.

43. Barr JR, Maggio VL, Barr DB, et al. New high-resolution mass spectrometric approach for the measurement of polychlorinated biphenyls and organochlorine pesticides in human serum. *J Chromatogr B Analyt Technol Biomed Life Sci* 2003;794(1):137-148.

44. Phillips DL, Pirkle JL, Burse VW, et al. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol* 1989;18(4):495-500.

45. Centers for Disease Control and Prevention. NSQAP: Newborn Screening Quality Assurance Program. U.S. Department of Health and Human Services; 2017. (https://www.cdc.gov/labstandards/nsqap.html). (Accessed 9 July 2018).

46. Hirano K, Imbens G. The propensity score with continuous treatment. In: Gelman A, Meng X-L, eds. *Applied bayesian modelling and causal inference from missing data*. New York, NY: Wiley, 2004:73-84.

47. Van der Laan MJ, Polley EC, Hubbard AE. Super Learner. *UC Berkeley Division of Biostatistics Working Paper Series* 2007:Working Paper 222. Available: http://biostats.bepress.com/ucbbiostat/paper222 [Online July 2007].

48. Lubin JH, Colt JS, Camann D, et al. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ Health Perspect* 2004;112(17):1691-1696.

49. Sierra-Santoyo A, Hernandez M, Albores A, et al. Sex-dependent regulation of hepatic cytochrome P-450 by DDT. *Toxicological sciences : an official journal of the Society of Toxicology* 2000;54(1):81-87.

50. Rosenfeld CS, Trainor BC. Environmental Health Factors and Sexually Dimorphic Differences in Behavioral Disruptions. *Curr Environ Health Rep* 2014;1(4):287-301.

51. Bloom MS, Jansing RL, Kannan K, et al. Thyroid hormones are associated with exposure to persistent organic pollutants in aging residents of upper Hudson River communities. *Int J Hyg Environ Health* 2014;217(4-5):473-482.

52. Chevrier J, Rauch S, Crause M, et al. Maternal Exposure to DDT and Pyrethroids and Birth Outcomes Among VHEMBE Birth Cohort Study Participants Residing in an Area Sprayed for Malaria Control. *American journal of epidemiology* 2018:In press.

53. Coker E, Chevrier J, Rauch S, et al. Association between prenatal exposure to multiple insecticides and child body weight and body composition in the VHEMBE South African birth cohort. *Environment international* 2018;113:122-132.

54. Eskenazi B, An S, Rauch SA, et al. Prenatal Exposure to DDT and Pyrethroids for Malaria Control and Child Neurodevelopment: The VHEMBE Cohort, South Africa. *Environmental health perspectives* 2018;126(4):047004.

55. Chevrier J, Gunier RB, Bradman A, et al. Maternal urinary bisphenol a during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study. *Environmental health perspectives* 2013;121(1):138-144.

56. Westergaard N, Gehring U, Slama R, et al. Ambient air pollution and low birth weight - are some women more vulnerable than others? *Environment international* 2017;104:146-154.

57. Barcelo MA, Saez M, Saurina C. Spatial variability in mortality inequalities, socioeconomic deprivation, and air pollution in small areas of the Barcelona Metropolitan Region, Spain. *The Science of the total environment* 2009;407(21):5501-5523.

58. Bravo MA, Son J, de Freitas CU, et al. Air pollution and mortality in Sao Paulo, Brazil: Effects of multiple pollutants and analysis of susceptible populations. *Journal of exposure science & environmental epidemiology* 2016;26(2):150-161.

59. Chi GC, Hajat A, Bird CE, et al. Individual and Neighborhood Socioeconomic Status and the Association between Air Pollution and Cardiovascular Disease. *Environmental health perspectives* 2016;124(12):1840-1847.

60. Dragano N, Hoffmann B, Moebus S, et al. Traffic exposure and subclinical cardiovascular disease: is the association modified by socioeconomic characteristics of individuals and neighbourhoods? Results from a multilevel study in an urban region. *Occupational and environmental medicine* 2009;66(9):628-635.

61. Malig BJ, Ostro BD. Coarse particles and mortality: evidence from a multi-city study in California. *Occupational and environmental medicine* 2009;66(12):832-839.

62. Ostro BD, Feng WY, Broadwin R, et al. The impact of components of fine particulate matter on cardiovascular mortality in susceptible subpopulations. *Occupational and environmental medicine* 2008;65(11):750-756.

63. Qiu H, Tian L, Ho KF, et al. Air pollution and mortality: effect modification by personal characteristics and specific cause of death in a case-only study. *Environ Pollut* 2015;199:192-197.

64. Son JY, Lee JT, Kim H, et al. Susceptibility to air pollution effects on mortality in Seoul, Korea: a case-crossover analysis of individual-level effect modifiers. *Journal of exposure science & environmental epidemiology* 2012;22(3):227-234.

65. Wong CM, Ou CQ, Chan KP, et al. The effects of air pollution on mortality in socially deprived urban areas in Hong Kong, China. *Environmental health perspectives* 2008;116(9):1189-1194.

66. Zeka A, Zanobetti A, Schwartz J. Individual-level modifiers of the effects of particulate matter on daily mortality. *American journal of epidemiology* 2006;163(9):849-859.

67. O'Lenick CR, Chang HH, Kramer MR, et al. Ozone and childhood respiratory disease in three US cities: evaluation of effect measure modification by neighborhood socioeconomic status using a Bayesian hierarchical approach. *Environ Health* 2017;16(1):36.

68. Boelen A, Wiersinga WM, Fliers E. Fasting-induced changes in the hypothalamus-pituitary-thyroid axis. *Thyroid* 2008;18(2):123-129.

69. Johnsen L, Kongsted AH, Nielsen MO. Prenatal undernutrition and postnatal overnutrition alter thyroid hormone axis function in sheep. *J Endocrinol* 2013;216(3):389-402.

70. Johnsen L, Lyckegaard NB, Khanal P, et al. Fetal over- and undernutrition differentially program thyroid axis adaptability in adult sheep. *Endocr Connect* 2018;7(5):777-790.

71. Passos MC, da Fonte Ramos C, Dutra SC, et al. Long-term effects of malnutrition during lactation on the thyroid function of offspring. *Horm Metab Res* 2002;34(1):40-43.

72. Fetoui H, Bouaziz H, Mahjoubi-Samet A, et al. Food restriction induced thyroid changes and their reversal after refeeding in female rats and their pups. *Acta Biol Hung* 2006;57(4):391-402.

73. Ramos CF, Teixeira CV, Passos MC, et al. Low-protein diet changes thyroid function in lactating rats. *Proc Soc Exp Biol Med* 2000;224(4):256-263.

74. Helmreich DL, Tylee D. Thyroid hormone regulation by stress and behavioral differences in adult male rats. *Horm Behav* 2011;60(3):284-291.

75. Tamayo y Ortiz M, Tellez-Rojo MM, Trejo-Valdivia B, et al. Maternal stress modifies the effect of exposure to lead during pregnancy and 24-month old children's neurodevelopment. *Environment international* 2017;98:191-197.

76. Hicken MT, Dvonch JT, Schulz AJ, et al. Fine particulate matter air pollution and blood pressure: the modifying role of psychosocial stress. *Environmental research* 2014;133:195-203.

77. Stein LJ, Gunier RB, Harley K, et al. Early childhood adversity potentiates the adverse association between prenatal organophosphate pesticide exposure and child IQ: The CHAMACOS cohort. *Neurotoxicology* 2016;56:180-187.

78. U.S. Environmental Protection Agency. Environmental justice and National Environmental Policy Act. Washington, DC: U.S. EPA; 2018. (https://www.epa.gov/environmentaljustice/environmental-justice-and-national-environmental-policy-act). (Accessed 20 July 2018).

79. United Nations Economic Commission for Europe. Convention on access to information, public participation in decision-making and access to justice in environmental matters. Aarhus, Denmark: UNECE, 1998.

80. Landrigan PJ, Fuller R, Acosta NJR, et al. The Lancet Commission on pollution and health. *Lancet* 2018;391(10119):462-512.

81. World Health Organization. Country profiles of environmental burden of disease by WHO regions. Geneva, Switzerland: WHO; 2009. (http://www.who.int/quantifying_ehimpacts/afrocountryprofiles2004-rev.pdf). (Accessed 7 August 2018).

Table 1. Demographic characteristics of VHEMBE study participants who completed the 1-week follow-up visit, Limpopo, South Africa, 2012-2013 (n=720)

Maternal characteristics		
Maternal age, mean ± standard deviation	26.4	± 6.2
Education, n (%)		
< 12th grade	399	(55.4)
Grade 12	217	(30.1)
> High school	104	(14.4)
Marital status, n (%)		
Married	342	(47.5)
Not married	378	(52.5)
Parity, n (%)		
0	312	(43.3)
1	192	(26.7)
2+	216	(30.0)
Alcohol consumption, n (%)		
Yes	41	(5.7)
No	679	(94.3)
Smoking, n (%)		
Yes	3	(0.4)
No	717	(99.6)
Environmental tobacco smoke exposure, n (%)		
Yes	276	(38.3)
No	444	(61.7)
HIV status, n (%)		
Positive	99	(13.8)
Negative	621	(86.2)
Low energy intake during pregnancy, n (%)		
Yes	490	(68.1)
No	230	(31.9)
Number of stressful live events, mean ± standard deviation	2	± 1.7
Child characteristics		
Sex, n (%)		
Boy	371	(51.5)
Girl	349	(48.5)
Preterm (<37 weeks), n (%)		
Yes	96	(13.3)
No	624	(86.7)
Low birth weight (<2500g), n (%)		
Yes	61	(8.5)

No	659	(91.5)
Delivery method		
Vaginal birth	555	(77.1)
Cesarean	165	(22.9)
Small for gestational age, n (%)		
Yes	177	(24.6)
No	543	(75.4)
Household characteristics		
Below food poverty level (R386/month per capita)		
Yes	439	(61.0)
No	281	(39.0)
Food security		
High	411	(57.1)
Low or Very low	309	(42.9)

Totals may not add to 720 due to missing data. Percentages may not add to 100% due to rounding

Table 2. Maternal peripartum serum DDT/E (ng/g, lipid-adjusted) and urinary pyrethroid metabolite (ug/L, specific-gravity adjusted) concentrations among VHEMBE study participants, Limpopo, South Africa, 2012-2013.

							Percentile					
	n	% Detected[a]	% Quantifiable[b]	Geometric Mean	± GSD	Min	10	25	50	75	90	Max
p,p'-DDT	720	98.1	90.6	69.3	± 6.7	<LOD	7.8	18.6	55.2	261.1	947.0	15027.6
p,p'-DDE	720	100.0	97.2	289.2	± 4.9	4.0	44.5	92.3	243.2	878.3	2612.1	26301.3
o,p'-DDT	720	90.4	43.3	9.0	± 4.7	<LOD	1.5	3.4	7.1	22.8	72.7	2029.3
o,p'-DDE	720	82.8	16.0	4.1	± 2.7	<LOD	<LOD	2.3	1.2	6.9	13.6	117.5
cis-DBCA	712	100	99.6	0.222	± 3.4	0.005	0.050	0.096	0.219	0.470	1.115	17.827
cis-DCCA	712	100	99.9	0.307	± 3.0	0.015	0.083	0.151	0.302	0.596	1.025	103.502
trans-DCCA	712	100	99.6	0.357	± 3.4	0.008	0.078	0.157	0.340	0.790	1.491	132.878
3-PBA	711	100	100	0.716	± 2.8	0.022	0.214	0.374	0.702	1.372	2.403	58.899
4F3PBA	687	12.7	8.0	N/A		<LOD	<LOD	<LOD	<LOD	<LOD	0.008	0.423

[a] Limits of detection are 0.01 ng/g serum for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; 0.03 ng/g serum for *p,p'*-DDE; and 0.0025 ug/L for *cis*-DBCA, 0.0045 ug/L for *cis*-DCCA, 0.0038 ug/L for *trans*-DCCA, 0.0047 ug/L for 3-PBA, and 0.005 ug/L for 4-F-3 PBA.

[b] Limits of quantification are 0.05 ng/g serum for *p,p'*-DDT, *o,p'*-DDT, and *o,p'*-DDE; and 0.15 ng/g for *p,p'*-DDE; 0.0082 ug/L for *cis*-DBCA, 0.015 ug/L for *cis*-DCCA, 0.013 ug/L for *trans*-DCCA, 0.016 ug/L for 3-PBA, and 0.011ug/L for 4-F-3 PBA.

Table 3. Associations between peripartum maternal serum DDT/E and urinary pyrethroid concentrations and neonatal thyroid hormone levels among VHEMBE study participants, Limpopo, South Africa, 2012-2013.[a]

	All	Boys	Girls	p_{int}[d]
TSH[b]				
p,p'-DDT	1.9% (-3.4, 7.4)	0.5% (-7.3, 9.0)	2.8% (-3.8, 9.8)	0.68
p,p'-DDE	-1.9% (-8.0, 4.5)	-1.1% (-9.5, 8.2)	-3.3% (-11.3, 5.4)	0.71
o,p'-DDT	2.1% (-4.2, 8.9)	1.0% (-8.2, 11.1)	2.6% (-5.4, 11.3)	0.80
cis-DBCA	8.2% (-1.2, 18.6)	3.7% (-8.9, 18.0)	14.1% (0.9, 29.0)*	0.29
cis-DCCA	9.1% (-1.7, 21.0)	5.2% (-9.8, 22.7)	13.8% (0.2, 29.2)*	0.43
trans-DCCA	11.0% (1.2, 21.8)*	8.8% (-5.0, 24.6)	13.8% (1.0, 28.2)*	0.62
3PBA	10.4% (-1.0, 23.0)	7.2% (-8.4, 25.5)	15.0% (-0.4, 32.9)	0.52
T4[c]				
p,p'-DDT	-0.10 (-0.25, 0.04)	-0.20 (-0.38, -0.02)*	-0.01 (-0.23, 0.21)	0.17
p,p'-DDE	-0.09 (-0.25, 0.07)	-0.17 (-0.39, 0.05)	0.00 (-0.23, 0.23)	0.28
o,p'-DDT	-0.07 (-0.23, 0.09)	-0.25 (-0.46, -0.05)*	0.12 (-0.12, 0.36)	0.02*
cis-DBCA	-0.03 (-0.25, 0.19)	-0.03 (-0.33, 0.26)	-0.01 (-0.35, 0.32)	0.92
cis-DCCA	-0.11 (-0.34, 0.12)	-0.16 (-0.47, 0.16)	-0.05 (-0.39, 0.29)	0.66
trans-DCCA	-0.08 (-0.29, 0.13)	-0.09 (-0.37, 0.20)	-0.07 (-0.37, 0.23)	0.95
3PBA	-0.10 (-0.34, 0.14)	-0.14 (-0.47, 0.18)	-0.03 (-0.39, 0.33)	0.65

[a] Models adjusted for propensity scores determined using the Super Learner algorithm.

[b] Estimates show percent change in TSH for each 10-fold increase in maternal peripartum serum DDT/E or pyrethroid metabolite concentration calculated as $(10^{\beta} - 1)*100$.

[c] Estimates show change in mean total T4 (µg/dL) for each 10-fold increase in maternal peripartum serum DDT/E or pyrethroid metabolite concentration.

[d] p_{int} = p-value for interaction term.

*$p<0.05$

Table 4. Associations between peripartum maternal serum DDT/E and urinary pyrethroid concentrations and odds of low total T4 among neonates participating in the VHEMBE study, Limpopo, South Africa, 2012-2013 [a]

	All	Boys	Girls	p_{int} [b]
p,p'-DDT	0.99 (0.67, 1.46)	1.35 (0.86, 2.14)	0.68 (0.38, 1.23)	0.05**
p,p'-DDE	1.44 (0.80, 2.58)	1.63 (0.84, 3.18)	1.25 (0.53, 2.96)	0.60
o,p'-DDT	1.16 (0.64, 2.09)	1.83 (0.85, 3.95)	0.70 (0.32, 1.53)	0.05**
cis-DBCA	1.24 (0.68, 2.24)	1.33 (0.59, 2.99)	1.14 (0.51, 2.54)	0.78
cis-DCCA	1.43 (0.75, 2.74)	1.30 (0.58, 2.94)	1.63 (0.61, 4.38)	0.72
trans-DCCA	1.45 (0.80, 2.65)	1.22 (0.56, 2.64)	1.84 (0.79, 4.27)	0.45
3PBA	1.64 (0.75, 3.58)	1.46 (0.54, 3.97)	1.94 (0.66, 5.74)	0.69

[a] Models adjusted for propensity scores determined using the Super Learner algorithm. Estimates show relative changes in the odds of low T4 for a 10-fold increase in DDT/E or pyrethroids.

[b] p_{int} = p-value for interaction term.
*$p<0.05$; **$p<0.10$

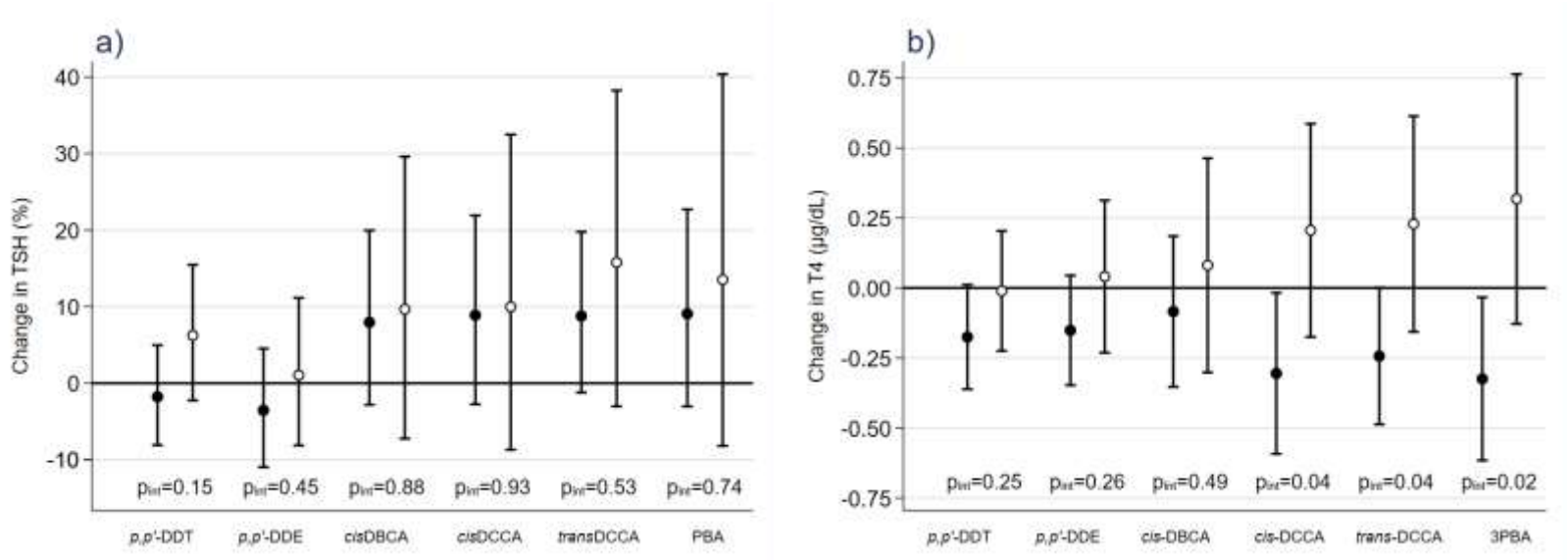

Figure 1. Associations between a) TSH and b) T4 with DDT/E and pyrethroids by poverty status. Error bars represent 95% confidence intervals. Solid circles represent children from households with income below the South African food poverty level and hollow circles represent households with incomes above the poverty threshold. Models adjusted for propensity scores determined using the Super Learner algorithm. p_{int} represents the p-value associated with the interaction term.

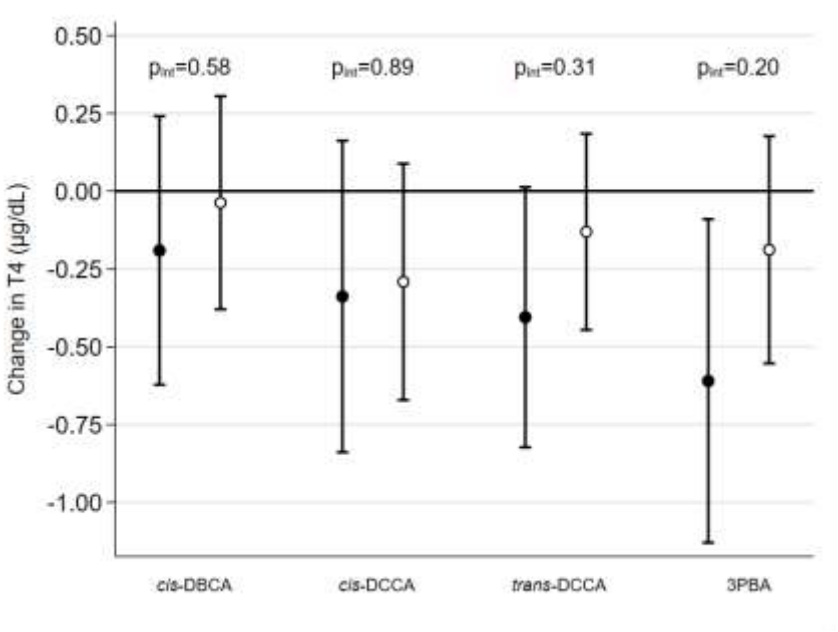

Figure 2. Associations between T4 and pyrethroids by stress level among households with income below the South African food poverty level. Error bars represent 95% confidence intervals. Solid circles represent children whose mothers experienced >2 stressful life events during pregnancy, and hollow circles represent mothers experiencing 2 or fewer stressors. p_{int} represents the p-value associated with the interaction term.

Supplementary Material

Table S1. Associations between maternal peripartum serum DDT/E and urinary pyrethroid concentrations and neonatal thyroid hormones by poverty status among VHEMBE study participants, Limpopo, South Africa, 2012-2013.[a]

	All	Poor	Not poor	p_{int}[d]
TSH[b]				
p,p'-DDT	1.9% (-3.4, 7.4)	-1.8% (-8.1, 5.0)	6.3% (-2.2, 15.5)	0.15
p,p'-DDE	-1.9% (-8.0, 4.5)	-3.5% (-11.0, 4.5)	1.0% (-8.1, 11.1)	0.45
o,p'-DDT	2.1% (-4.2, 8.9)	-0.9% (-8.3, 7.1)	7.3% (-3.7, 19.5)	0.24
cis-DBCA	8.2% (-1.2, 18.6)	8.0% (-2.8, 20.0)	9.6% (-7.2, 29.6)	0.88
cis-DCCA	9.1% (-1.7, 21.0)	8.9% (-2.7, 21.9)	10.0% (-8.7, 32.5)	0.93
trans-DCCA	11.0% (1.2, 21.8)*	8.8% (-1.2, 19.8)	15.8% (-3.0, 38.2)	0.53
3PBA	10.4% (-1.0, 23.0)	9.1% (-3.0, 22.7)	13.5% (-8.2, 40.4)	0.74
T4[c]				
p,p'-DDT	-0.10 (-0.25, 0.04)	-0.18 (-0.36, 0.01)	-0.01 (-0.22, 0.20)	0.25
p,p'-DDE	-0.09 (-0.25, 0.07)	-0.15 (-0.35, 0.04)	0.04 (-0.23, 0.31)	0.26
o,p'-DDT	-0.07 (-0.23, 0.09)	-0.13 (-0.32, 0.06)	0.03 (-0.25, 0.32)	0.35
cis-DBCA	-0.03 (-0.25, 0.19)	-0.08 (-0.35, 0.18)	0.08 (-0.30, 0.46)	0.49
cis-DCCA	-0.11 (-0.34, 0.12)	-0.30 (-0.59, -0.02)*	0.21 (-0.18, 0.59)	0.04*
trans-DCCA	-0.08 (-0.29, 0.13)	-0.24 (-0.49, 0.00)	0.23 (-0.16, 0.61)	0.04*
3PBA	-0.10 (-0.34, 0.14)	-0.32 (-0.62, -0.03)*	0.32 (-0.13, 0.76)	0.02*

[a] Models adjusted for propensity scores determined using the Super Learner algorithm.
[b] Estimates show percent change in TSH for each 10-fold increase in maternal peripartum serum DDT/E or pyrethroid metabolite concentration calculated as $(10^{\beta} - 1)*100$.
[c] Estimates show change in mean total T4 (µg/dL) for each 10-fold increase in maternal peripartum serum DDT/E or pyrethroid metabolite concentration.
[d] p_{int} = p-value for interaction term.
*$p<0.05$

Table S2. Associations between peripartum maternal serum DDT/E and urinary pyrethroid concentrations and odds of low neonatal total T4 by poverty status among VHEMBE study participants, Limpopo, South Africa, 2012-2013.[a]

	All	Poor	Not poor	p_{int}[b]
p,p'-DDT	0.99 (0.67, 1.46)	0.93 (0.51, 1.69)	1.10 (0.67, 1.81)	0.67
p,p'-DDE	1.44 (0.80, 2.58)	1.33 (0.64, 2.77)	1.62 (0.71, 3.71)	0.69
o,p'-DDT	1.16 (0.64, 2.09)	1.41 (0.62, 3.19)	0.99 (0.43, 2.25)	0.53
cis-DBCA	1.24 (0.68, 2.24)	1.53 (0.57, 4.10)	0.97 (0.46, 2.02)	0.48
cis-DCCA	1.43 (0.75, 2.74)	2.39 (0.80, 7.09)	0.92 (0.36, 2.35)	0.20
trans-DCCA	1.45 (0.80, 2.65)	1.99 (0.76, 5.19)	1.04 (0.47, 2.30)	0.30
3PBA	1.64 (0.75, 3.58)	2.87 (0.65, 12.67)	0.96 (0.32, 2.90)	0.28

[a] Models adjusted for propensity scores determined using the Super Learner algorithm. Estimates show relative changes in the odds of low T4 for a 10-fold increase in DDT/E or pyrethroids.

[b] p_{int} = p-value for interaction term.
*$p<0.05$

Table S3. Associations between maternal peripartum serum DDT/E and urinary pyrethroid concentrations and neonatal thyroid hormones by HIV status among VHEMBE study participants, Limpopo, South Africa, 2012-2013.[a]

	All	HIV-positive	HIV-negative	p_{int}
TSH[b]				
p,p'-DDT	1.9% (-3.4, 7.4)	4.4% (-9.6, 20.7)	1.6% (-4.0, 7.4)	0.72
p,p'-DDE	-1.9% (-8.0, 4.5)	-2.2% (-14.5, 11.8)	-1.8% (-8.4, 5.2)	0.95
o,p'-DDT	2.1% (-4.2, 8.9)	-0.9% (-13.5, 13.4)	2.7% (-4.2, 10.1)	0.63
cis-DBCA	8.2% (-1.2, 18.6)	-1.4% (-20.3, 22.1)	9.9% (-0.5, 21.5)	0.37
cis-DCCA	9.1% (-1.7, 21.0)	14.8% (-8.1, 43.2)	8.0% (-3.6, 21.1)	0.63
trans-DCCA	11.0% (1.2, 21.8)*	13.2% (-7.8, 39.0)	10.4% (-0.3, 22.2)	0.83
3PBA	10.4% (-1.0, 23.0)	10.3% (-10.7, 36.2)	10.0% (-2.8, 24.6)	0.99
T4[c]				
p,p'-DDT	-0.10 (-0.25, 0.04)	0.16 (-0.23, 0.55)	-0.14 (-0.29, 0.00)	0.15
p,p'-DDE	-0.09 (-0.25, 0.07)	0.07 (-0.36, 0.51)	-0.11 (-0.28, 0.06)	0.44
o,p'-DDT	-0.07 (-0.23, 0.09)	-0.31 (-0.77, 0.16)	-0.05 (-0.21, 0.12)	0.30
cis-DBCA	-0.03 (-0.25, 0.19)	-0.36 (-0.74, 0.01)	0.03 (-0.22, 0.28)	0.08**
cis-DCCA	-0.11 (-0.34, 0.12)	-0.28 (-0.96, 0.40)	-0.06 (-0.30, 0.17)	0.56
trans-DCCA	-0.08 (-0.29, 0.13)	-0.26 (-0.80, 0.28)	-0.03 (-0.24, 0.18)	0.43
3PBA	-0.10 (-0.34, 0.14)	-0.29 (-0.78, 0.20)	-0.02 (-0.28, 0.24)	0.34

[a] Models adjusted for propensity scores determined using the Super Learner algorithm.
[b] Estimates show percent change in TSH for each 10-fold increase in maternal peripartum serum DDT/E or pyrethroid metabolite concentration calculated as $(10^{\beta} - 1)*100$.
[c] Estimates show change in mean total T4 (µg/dL) for each 10-fold increase in maternal peripartum serum DDT/E or pyrethroid metabolite concentration.
[d] p_{int} = p-value for interaction term.
*p<0.05; **p<0.10

Table S4. Associations between peripartum maternal serum DDT/E and urinary pyrethroid concentrations and odds of low neonatal total T4 by HIV status among VHEMBE study participants, Limpopo, South Africa, 2012-2013.[a]

	All	HIV-positive	HIV-negative	p_{int}[b]
p,p'-DDT	0.99 (0.67, 1.46)	0.49 (0.15, 1.62)	1.24 (0.76, 2.01)	0.19
p,p'-DDE	1.44 (0.80, 2.58)	0.69 (0.16, 2.99)	1.93 (0.91, 4.10)	0.25
o,p'-DDT	1.16 (0.64, 2.09)	1.27 (0.47, 3.44)	1.20 (0.59, 2.41)	0.91
cis-DBCA	1.24 (0.68, 2.24)	0.89 (0.43, 1.87)	1.42 (0.64, 3.16)	0.40
cis-DCCA	1.43 (0.75, 2.74)	2.60 (0.84, 8.06)	1.10 (0.54, 2.23)	0.20
trans-DCCA	1.45 (0.80, 2.65)	1.81 (0.74, 4.42)	1.24 (0.63, 2.43)	0.49
3PBA	1.64 (0.75, 3.58)	1.69 (0.67, 4.22)	1.36 (0.52, 3.52)	0.74

[a] Models adjusted for propensity scores determined using the Super Learner algorithm. Estimates show relative changes in the odds of low T4 for a 10-fold increase in DDT/E or pyrethroids.

[b] p_{int} = p-value for interaction term.
[*] $p<0.05$