



Sex and poverty modify associations between maternal peripartum concentrations of DDT/E and pyrethroid metabolites and thyroid hormone levels in neonates participating in the VHEMBE study, South Africa



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ABSTRACT

Indoor Residual Spraying (IRS), the application of insecticides on the inside walls of dwellings, is used by 84 countries for malaria control. Although effective in preventing malaria, this practice results in elevated insecticide exposure to > 100 million people, most of whom are Africans. Pyrethroid insecticides and dichlorodiphenyl trichloroethane (DDT) are currently used for IRS. Animal and in vitro studies suggest that pyrethroids and DDT interfere with thyroid hormone homeostasis but human studies are inconsistent and no prior study has investigated this question in a population residing in an area where IRS is conducted. Our objective was thus to evaluate whether prenatal exposure to pyrethroids, DDT or DDT's breakdown product dichlorodiphenyl dichloroethylene (DDE) is associated with altered thyroid hormone levels among neonates from Limpopo, South Africa, where pyrethroids and DDT are used annually to control malaria. We measured serum DDT/E and urinary pyrethroid metabolite concentrations in maternal peripartum samples from 717 women participating in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), a birth cohort study conducted in Limpopo's Vhembe district. We measured total thyroxine (T4) and thyroid-stimulating hormone (TSH) in dried blood spots collected via heel stick. We found that all pyrethroid metabolites were positively associated with TSH; *trans*-DCCA and 3-PBA showed the strongest associations with a 12.3% (95%CI = 3.0, 22.3) and 14.0% (95%CI = 0.5, 30.2) change for each 10-fold increase in biomarker concentration, respectively. These associations were substantially stronger among children from households below the South African food poverty line. DDT and DDE were associated with lower total T4 among boys only ($\beta = -0.27 \mu\text{g/dL}$ per 10-fold increase; 95%CI = $-0.47, -0.04$). Results suggest that prenatal exposure to DDT, DDE and pyrethroid insecticides is associated with changes in neonatal thyroid hormones consistent with hypothyroidism/hypothyroxinemia and that sex and poverty modify associations. Further research is needed to confirm these findings and examine whether they have implications for child development.

Abbreviations: 3-PBA, 3-phenoxybenzoic acid; 4-F-3-PBA, 4-fluoro-3-phenoxybenzoic acid; *cis*-DBCA, *cis*-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; *cis*-DCCA, *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; BMI, Body mass index; DDT, Dichlorodiphenyl trichloroethane; DDE, Dichlorodiphenyl dichloroethylene; GPS, Generalized propensity score; HIV, Human immunodeficiency virus; IRS, Indoor residual spraying; LMICs, Low- and middle-income countries; LOD, Limit of detection; OR, Odds ratio; PCBs, Polychlorinated biphenyls; T3, Triiodothyronine; T4, Thyroxine; *trans*-DCCA, *trans*-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid; TSH, Thyroid-stimulating hormone; UDP-GT, Uridinediphosphate glucuronosyltransferase; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment

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1. Introduction

According to the World Health Organization, 219 million people were infected by malaria in 2017, resulting in 430,000 deaths, primarily among African children under age 5 years (World Health Organization, 2018). Indoor residual spraying (IRS), the application of insecticides on the inside walls of residences, is used by 84 countries for malaria control (World Health Organization, 2018; Maharaj et al., 2013). Although this practice appears effective in reducing infection, it results in elevated exposure to insecticides with incompletely understood health consequences (Gaspar et al., 2017; Rauch et al., 2018). 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 hormones homeostasis, which play a critical role in child growth and brain development, particularly during pregnancy and the neonatal period (Fisher and Brown, 2013). Several pyrethroids including deltamethrin, permethrin, cypermethrin, and fenvalerate as well as the non-specific pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA) interact with the thyroid receptor (Du et al., 2010; Ghisari et al., 2015). No animal study has investigated the effect of prenatal exposure to pyrethroids on offspring thyroid function. However, post-natal intraperitoneal exposure to permethrin, deltamethrin, karate (a λ -cyhalothrin formulation) and talstar (a bifenthrin formulation) 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 (Akhtar et al., 1996; Maiti and Kar, 1997; Maiti and Kar, 1998; Wang et al., 2002). 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 (Giray et al., 2010; Kaul et al., 1996; Sekeroglu et al., 2014).

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 concentrations in 147 mother-child pairs from Tokyo, Japan, but the moderate sample size may have limited statistical power (Zhang et al., 2014). Cross-sectional studies conducted in U.S. adults also found no associations between urinary 3-PBA concentrations and thyroid hormones (Jain, 2016; Meeker et al., 2009). However, an inverse relation was reported between urinary *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*-DCCA) and total T3 blood concentrations among U.S. males recruited from an infertility clinic (Meeker et al., 2009).

DDT reduces total T4 blood concentration and induces the microsomal enzyme uridinediphosphate glucuronosyltransferase (UDP-GT) in rats (Tebourbi et al., 2010). 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 (Tebourbi et al., 2010; O'Connor et al., 1999; O'Connor et al., 2002; Yamada et al., 2004). While none of the three human studies investigating maternal blood concentrations of DDT, or DDT's breakdown product dichlorodiphenyl dichloroethylene (DDE), and neonatal thyroid hormones found associations (Alvarez-Pedrerol et al., 2008; Darnerud et al., 2010; Lopez-Espinosa et al., 2010), two studies reported inverse relations between cord DDT/E and cord free or total T4 concentrations (Asawasinsopon et al., 2006; Maervoet et al., 2007). However, studies conducted in adolescents (Freire et al., 2013) and adults (Darnerud et al., 2010; Delpont et al., 2011; Rylander et al., 2006; Teeyapant et al., 2014; Turyk et al., 2007; Chevrier et al., 2008; Lopez-Espinosa et al., 2009; Takser et al., 2005) yielded conflicting results.

These prior studies were primarily conducted in high-income countries. However, vulnerable populations living in areas where IRS is conducted, and where poverty, malnutrition 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 whether child sex and household poverty 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 women-child dyads from the Vhembe district of Limpopo Province, South Africa. Women were approached between August 2012 and December 2013 at Tshilidzini Hospital in the town of Thohoyandou, shortly before or after giving birth, and were invited to participate in the VHEMBE study if they were at least 18 years of age, had contractions > 5 min apart, 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.

2.2. 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 socio-economic characteristics, household assets, lifestyle, diet (via a food-frequency questionnaire validated in the study area) (MacIntyre et al., 2001), stress (using a stressful life event scale adapted for the local population) (Richter et al., 2000; Johnson, 1982) 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 U.S. Institute of Medicine Guidelines for pregnant women (Rasmussen and Yaktine, 2009; Otten et al., 2006) following methods described in Huang et al. (2018a). We defined poverty as income below 386 Rands/person/month based on Statistics South Africa guidelines (Statistics South Africa, 2017) 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 (Demographics and Health Surveys, 2017). 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 (Huang et al., 2018b). 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 section).

2.3. Measurement of insecticides

Research staff collected maternal urine and blood samples before ($N_{\text{Blood}} = 568$; $N_{\text{Urine}} = 444$) or shortly after delivery ($N_{\text{Blood}} = 152$; $N_{\text{Urine}} = 268$) and immediately processed and stored them at -80°C 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-carboxylic acid (*cis*-DBCA), *cis*-DCCA, *trans*-DCCA, 3-phenoxybenzoic acid (3-PBA), and 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA), in maternal urine by gas chromatography–mass spectrometry based on methods developed by Dewailly et al. (2014). Limits of detection were 0.0025 $\mu\text{g/L}$ for *cis*-DBCA, 0.0045 $\mu\text{g/L}$ for *cis*-DCCA, 0.0038 $\mu\text{g/L}$ for *trans*-DCCA, 0.0047 $\mu\text{g/L}$ for 3-PBA, and 0.005 $\mu\text{g/L}$ for 4-F-3-PBA. Corresponding limits of quantification were 0.0082, 0.015, 0.013, 0.016 $\mu\text{g/L}$. Pyrethroid concentrations were corrected for dilution based on sample specific gravity, which was measured using a portable refractometer (Atago PAL-10S, Tokyo, Japan). 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 (Barr et al., 2003). PCBs were considered as potential confounders due to prior reports of associations with neonatal thyroid hormones (Chevrier et al., 2007). Limits of detection were 0.01 ng/mL serum for *p,p'*-DDT, *o,p'*-DDT and *o,p'*-DDE, and 0.03 ng/mL serum for *p,p'*-DDE. Corresponding limits of quantification were 0.03 and 0.09 ng/mL serum. 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 (Phillips et al., 1989) as measured using standard enzymatic methods (Roche Chemicals, Indianapolis, IN).

2.4. Thyroid hormone measurements

TSH and T4 sharply increase at birth and then reduce gradually and stabilize over the next few days of life (Fisher and Brown, 2013). 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°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 fluorimmunoassay (DELFLIA®, 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 (Centers for Disease Control and Prevention, 2017). Limits of detection (LODs) and mean coefficients of variation are 1.5 $\mu\text{g/dL}$ and 7.1% for total T4, and 2 $\mu\text{IU/mL}$ and 7.9% for TSH, respectively. Reference intervals are 3.2–11.1 $\mu\text{g/dL}$ for total T4 and 0.27–6.07 $\mu\text{IU/mL}$ for TSH. Hormones were measured in duplicate and averaged.

2.5. Data analysis

Biomarkers of exposure were \log_{10} -transformed to reduce the influence of outliers and TSH was \log_{10} -transformed to normalize residuals. Reported associations thus represent mean (total T4) or percent (TSH) change in outcomes for each 10-fold increase in exposure biomarker concentration. Biomarker concentrations between the LOD and the limit of quantification 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 (Lubin et al., 2004). We used analysis of variance and Pearson's correlations for bivariate analyses. We estimated the causal effect of exposure to IRS insecticides on neonatal thyroid hormone levels (expressed continuously or categorized based on reference ranges) based on marginal structural models with inverse probability-of-treatment weights. We applied stabilized weights which were estimated using the Super Learner algorithm, a loss-based supervised learning method that computes a weighted combination of prediction algorithms to return a function that minimizes cross-validated risk (Van der Laan et al., 2007).

Potential confounders were identified via Directed Acyclic Graphs and included maternal age (continuous), education (< 12 th grade, 12th grade, > 12 th grade), 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); and child sex and age in days at the time of heel stick (continuous). Variables such as birth weight, gestational age at birth and delivery method were not considered because they may be situated on the causal pathway between exposures and thyroid hormones. Missing covariate values ($< 1.8\%$) were randomly imputed based on observed probability distributions.

We ran several sensitivity analyses to evaluate the robustness of our results. First, we evaluated the linearity assumption by running Generalized Additive Models with a 3-degree of freedom cubic spline. Second, we ran models correcting pyrethroid metabolites for creatinine concentration (expressed in $\mu\text{g/g}$ 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 (Rosner, 1983). Fifth, because pyrethroid metabolite concentrations may represent in part exposure in the hospital, we re-ran analyses stratified on whether urine samples were collected before or after delivery. Sixth, 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 (Hirano and Imbens, 2004). The GPS was estimated using the Super Learner algorithm and a cubic spline was applied to the propensity score. 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. Results were not substantially affected by any of these different specifications. We show results based on marginal structural models with exposure biomarkers expressed linearly, corrected for lipids for DDT and DDE or specific gravity for pyrethroids, and including outliers.

We also investigated effect modification by child's sex and household poverty by running separate models including one of these binary cross-product terms. Although prior research in this and other populations suggests that poverty may affect susceptibility to the adverse effects of insecticides and other environmental exposures (Huang et al.,

Table 1
Demographic characteristics of VHEMBE study participants with valid neonatal TSH or T4 measurements, Limpopo, South Africa, 2012–2013 (n = 720).

Maternal characteristics		
Maternal age, mean \pm standard deviation	26.4	\pm 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	2	(0.3)
No	718	(99.7)
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 \pm standard deviation	2	\pm 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 (< 2500 g), n (%)		
Yes	61	(8.5)
No	659	(91.5)
Delivery method, n (%)		
Vaginal birth	555	(77.1)
Cesarean	165	(22.9)
Small-for-gestational age (< 10th percentile), n (%)		
Yes	177	(24.6)
No	543	(75.4)
Household characteristics		
Below food poverty level (R386/month per capita), n (%)		
Yes	439	(61.0)
No	281	(39.0)
Food security, n (%)		
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.

2018a; Westergaard et al., 2017; Barcelo et al., 2009; Bravo et al., 2016; Chi et al., 2016; Dragano et al., 2009; Malig and Ostro, 2009; Ostro et al., 2008; Qiu et al., 2015; Son et al., 2012; Wong et al., 2008; Zeka et al., 2006; O'Lenick et al., 2017), the mechanisms for this effect modification have not been identified. Thus, as a secondary analysis we also examined whether maternal stress and low energy intake during pregnancy modified associations. 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 12th grade education) and low energy intake (68% < U.S. Institute of Medicine guidelines) during pregnancy (Table 1). About 14% were HIV-positive, and few reported consumption of alcohol (6%) or smoking (0.3%) during pregnancy. About half (49%) of infants were girls, 13% were born preterm (< 37 weeks gestation), 8% had a low birth weight (< 2500 g), and 25% were small-for-gestational age (< 10th percentile of weight-for-gestational age).

3.2. Concentrations of DDT/E, pyrethroid metabolites, and thyroid hormones

Table 2 shows the distribution of biomarkers of exposure to insecticides as well as their detection and quantification frequencies. *o,p'*-DDE and 4-F-3-PBA were quantified in only 16% and 8% of samples, respectively, and were excluded from data analysis. Except for *p,p'*-DDT (98% detection) and *o,p'*-DDT (90% 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 ($r = 0.31$ to 0.88 , $p < 0.05$) were intercorrelated within but not between ($r = -0.03$ to 0.04 , $p > 0.05$) chemical class. Maternal age 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 (data not shown). T4 and TSH were detected in all neonates. Mean T4 was 5.2 $\mu\text{g}/\text{dL}$ (standard deviation = 1.5) and the geometric mean of TSH was 1.2 $\mu\text{U}/\text{mL}$ (geometric standard deviation = 1.9). 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. 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 blood concentrations. We examined associations with low T4 but not with other categories due to insufficient number of cases.

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 a 12.3% (95%CI = 3.0, 23.3) and 14.0% (95%CI = 0.5, 30.2) change for each 10-fold increase in biomarker concentrations, respectively (Table 3). Associations between pyrethroid metabolites and T4 were generally negative but weak and imprecise. However, as shown in Fig. 1 and Table S1, associations with T4 were substantially stronger among children from poor households for all pyrethroid metabolites, and most particularly for *cis*-DCCA ($\beta = -0.39$; 95%CI = -0.74 , -0.06), *trans*-DCCA ($\beta = -0.28$; 95%CI = -0.50 , 0.00) and 3-PBA ($\beta = -0.33$; 95%CI = -0.70 , 0.05) with interaction *p*-values below 0.04. In addition, odds of low T4 were elevated in relation to pyrethroid metabolites among children from poor households, with odds ratios (ORs) ranging between 1.5 (95%CI = 0.6, 3.8) for *cis*-DBCA and 5.2 (95%CI = 1.5, 17.3) for 3-PBA, and evidence of interaction ($p_{\text{int}} = 0.02$ to 0.08) for *cis*-DCCA, and 3-PBA (Table 4).

3.4. Associations between DDT/E and thyroid hormone concentrations

DDT/E were not associated with TSH (Table 3). Associations with T4 were also weak overall and varied between -0.11 $\mu\text{g}/\text{dL}$ (95%CI = -0.24 , 0.03) and -0.08 $\mu\text{g}/\text{dL}$ (95%CI = -0.24 , 0.08) per 10-fold increase in *p,p'*-DDT and *p,p'*-DDE, respectively. However, investigation of effect modification by sex revealed that *o,p'*-DDT was associated with reduced T4 among boys ($\beta = -0.27$; 95%CI = -0.47 , -0.04) but not among girls ($\beta = 0.10$; 95%CI = -0.13 , 0.32) ($p_{\text{int}} = 0.02$). *p,p'*-DDT was also inversely associated with T4 among boys ($\beta = -0.20$; 95%CI = -0.38 , -0.03) but statistical evidence for

Table 2

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

	n	% detected ^a	% quantifiable ^b	GM ^c	± GSD ^d	Percentile						
						Min	10	25	50	75	90	Max
<i>p,p'</i> -DDT	720	98.1	90.6	69.3	± 6.7	< LOD	7.8	18.6	55.2	261.1	947.0	15,027.6
<i>p,p'</i> -DDE	720	100.0	97.2	289.2	± 4.9	4.0	44.5	92.3	243.2	878.3	2612.1	26,301.3
<i>o,p'</i> -DDT	720	90.4	43.3	9.0	± 4.7	< LOD	1.5	3.4	7.1	22.8	72.7	2029.3
<i>o,p'</i> -DDE	720	82.8	16.0	4.1	± 2.7	< LOD	< LOD	2.3	1.2	6.9	13.6	117.5
<i>cis</i> -DBCA	712	100	99.6	0.346	± 3.048	0.017	0.084	0.158	0.318	0.736	1.481	13.393
<i>cis</i> -DCCA	712	100	99.9	0.479	± 2.560	0.051	0.152	0.261	0.462	0.789	1.477	209.488
<i>trans</i> -DCCA	712	100	99.6	0.557	± 3.074	0.032	0.141	0.266	0.535	1.050	2.350	268.945
3-PBA	711	100	100	1.118	± 2.392	0.103	0.399	0.656	1.050	1.851	3.192	102.383
4-F-3-PBA	687	12.7	8.0	– ^e	– ^e	< LOD	< LOD	< LOD	< LOD	< LOD	0.017	0.517

^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 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 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 µg/L for *cis*-DBCA, 0.015 µg/L for *cis*-DCCA, 0.013 µg/L for *trans*-DCCA, 0.016 µg/L for 3-PBA, and 0.011 µg/L for 4-F-3 PBA.

^c Geometric mean.

^d Geometric standard deviation.

^e Geometric mean and standard deviation not computed due to the low detection frequency.

effect modification was more limited ($p_{int} = 0.15$). In addition, we observed a positive association between the odds of low T4 and *p,p'*-DDT (OR = 1.4; 95%CI = 0.9, 2.3) and *o,p'*-DDT (OR = 1.6; 95%CI = 0.9, 2.8) among boys ($p_{int} = 0.04$ and 0.03, respectively) but confidence intervals crossed the null (Table S2).

Results for DDT/E and pyrethroids did not change markedly using either standard regressions (Tables S3–S6) or propensity score models (Tables S7–S10), or in other sensitivity analyses (not shown). We found no substantial evidence of effect modification by stress or energy intake during pregnancy (data not shown).

4. Discussion

We report associations between maternal peripartum pyrethroid metabolite concentrations and neonatal TSH overall and inverse associations with T4 levels among neonates from poor households. We also found inverse associations between maternal DDT concentrations and

T4 among boys, particularly for *o,p'*-DDT. 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 (Sierra-Santoyo et al., 2000) and sexual dimorphism is commonly reported in studies of endocrine disruptors such as DDT (Rosenfeld and Trainor, 2014). While prior studies of prenatal exposure to DDT/E and thyroid hormones did not investigate effect modification by sex, a moderately-sized cross-sectional

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				
<i>p,p'</i> -DDT	1.4% (–3.7, 6.4)	0.5% (–7.7, 9.1)	1.8% (–4.8, 9.0)	0.810
<i>p,p'</i> -DDE	–0.2% (–6.5, 6.3)	–0.2% (–8.8, 9.5)	–1.1% (–9.3, 7.4)	0.938
<i>o,p'</i> -DDT	3.0% (–3.2, 9.5)	1.9% (–7.0, 11.9)	3.2% (–5.1, 12.2)	0.812
<i>cis</i> -DBCA	10.6% (0.5, 20.9)*	4.5% (–9.2, 19.6)	18.2% (2.1, 36.0)*	0.228
<i>cis</i> -DCCA	11.7% (–0.4, 24.0)	6.4% (–9.4, 23.6)	17.8% (0.6, 37.8)*	0.338
<i>trans</i> -DCCA	12.3% (3.0, 23.3)*	9.5% (–2.7, 24.2)	16.0% (0.7, 33.7)*	0.55
3-PBA	14.0% (0.5, 30.2)*	8.5% (–8.3, 33.9)	22.2% (1.6, 44.6)*	0.376
T4^c				
<i>p,p'</i> -DDT	–0.11 (–0.24, 0.03)	–0.20 (–0.38, –0.03)*	–0.01 (–0.21, 0.19)	0.152
<i>p,p'</i> -DDE	–0.08 (–0.24, 0.08)	–0.17 (–0.38, 0.07)	0.02 (–0.19, 0.24)	0.214
<i>o,p'</i> -DDT	–0.09 (–0.24, 0.07)	–0.27 (–0.47, –0.04)*	0.10 (–0.13, 0.32)	0.016†
<i>cis</i> -DBCA	0.05 (–0.20, 0.28)	0.06 (–0.30, 0.40)	0.07 (–0.28, 0.43)	0.922
<i>cis</i> -DCCA	–0.16 (–0.42, 0.12)	–0.16 (–0.52, 0.22)	–0.16 (–0.54, 0.25)	0.994
<i>trans</i> -DCCA	–0.10 (–0.32, 0.13)	–0.08 (–0.38, 0.24)	–0.12 (–0.46, 0.23)	0.852
3-PBA	–0.05 (–0.32, 0.24)	–0.03 (–0.40, 0.39)	–0.03 (–0.45, 0.39)	0.986

^a Marginal structural models based on 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 $(10^{\beta} - 1) \times 100$.

^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_{int} < 0.1.

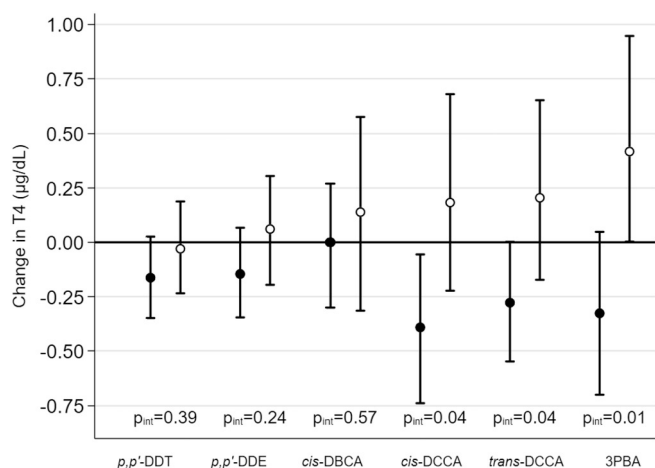


Fig. 1. Associations between T4 and DDT/E and pyrethroids by poverty status. Error bars represent 95% confidence intervals. Solid circles represent children from households with income below the South African food poverty level and hollow circles represent households with incomes above the poverty threshold. Marginal structural models are based on stabilized weights determined using the Super Learner algorithm. p_{int} represents the p-value associated with the interaction term.

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
<i>p,p'</i> -DDT	0.97 (0.63, 1.47)	1.43 (0.88, 2.32)	0.67 (0.34, 1.16)	0.038 [†]
<i>p,p'</i> -DDE	1.28 (0.83, 1.92)	1.49 (0.82, 2.62)	1.08 (0.60, 1.88)	0.414
<i>o,p'</i> -DDT	1.10 (0.71, 1.63)	1.61 (0.88, 2.84)	0.69 (0.37, 1.10)	0.034 [†]
<i>cis</i> -DBCA	1.20 (0.65, 2.25)	1.36 (0.56, 3.35)	1.06 (0.39, 2.53)	0.722
<i>cis</i> -DCCA	2.04 (0.81, 4.24)	1.65 (0.45, 4.02)	2.72 (0.71, 8.32)	0.532
<i>trans</i> -DCCA	1.84 (0.89, 3.48)	1.43 (0.51, 3.03)	2.63 (0.85, 7.10)	0.368
3-PBA	1.94 (0.82, 4.28)	1.34 (0.48, 3.31)	3.11 (0.75, 11.69)	0.352

^a Marginal structural models based on 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.

[†] $p_{int} < 0.1$

study conducted in adults found results that contrasted with ours in that the sum of DDT and DDE concentrations was positively related to both T4 and T3 among 48 women, but not among 66 men (Bloom et al., 2014). 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 body mass index (BMI)-for-age and weight-for-height at 1 and 2 years among girls only (Coker et al., 2018; Chevrier et al., 2019). In addition, maternal urinary pyrethroid metabolite concentrations were associated with poorer 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 (Eskenza et al., 2018). We also found inverse associations between maternal urinary pyrethroid metabolites and measures of body composition among boys only between 1 and 3.5 years of age (Huang et al., 2018b; Coker et al., 2018).

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 (Westergaard et al., 2017; Barcelo et al., 2009; Bravo et al., 2016; Chi et al., 2016; Dragano et al., 2009; Malig and Ostro, 2009; Ostro et al.,

2008; Qiu et al., 2015; Son et al., 2012; Wong et al., 2008; Zeka et al., 2006; O'Lenick et al., 2017), few studies have investigated the potential for economic hardship to potentiate the adverse effects of insecticides. However, we 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 (Huang et al., 2018a). 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 axis in humans by reducing TSH and/or T4, likely to lower basal metabolism and save energy (Boelen et al., 2008; Johnsen et al., 2013; Johnsen et al., 2018; Passos et al., 2002; Fetoui et al., 2006; Ramos et al., 2000), we found no evidence that low energy intake during pregnancy modified associations in the present study.

Stress may represent another potential mechanism for the modifying effect of socioeconomic status. However, despite evidence that stress suppresses the hypothalamus-pituitary-thyroid axis in rodents (Helmreich and Tylee, 2011) and prior human studies suggesting that stress and adversity potentiate the adverse health effects of contaminants such as lead (Tamayo y Ortiz et al., 2017), PM_{2.5} (Hicken et al., 2014) and organophosphate insecticides (Stein et al., 2016), we found no evidence of effect modification in the VHEMBE population.

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 (Westergaard et al., 2017; Barcelo et al., 2009; Bravo et al., 2016; Chi et al., 2016; Dragano et al., 2009; Malig and Ostro, 2009; Ostro et al., 2008; Qiu et al., 2015; Son et al., 2012; Wong et al., 2008; Zeka et al., 2006; O'Lenick et al., 2017) and support the need for policies that seek to protect vulnerable sub-populations. While such provisions are specifically integrated in environmental law in the United States (e.g. as part of the National Environmental Policy Act) (U.S. Environmental Protection Agency, 2018) and to some extent in Europe (e.g. the Aarhus Convention) (United Nations Economic Commission for Europe, 1998), low- and middle-income countries (LMICs), including African countries, lag behind in terms of 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 (Landrigan et al., 2018). It has been estimated that, on average, 26% of the burden of disease is due to modifiable environmental factors in sub-Saharan countries (World Health Organization, 2009) 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 conduct research in Africa and other LMICs.

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, poverty, stress or maternal undernutrition. 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 in marginal structural models. Thyroid hormone levels increase sharply after birth and decrease during the first few days of life (Fisher and Brown, 2013). 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.

5. Conclusion

In summary, our results suggest that prenatal exposure to DDT/E is associated with lower total T4 among male neonates. Thyroid hormones play an essential role in fetal brain development. Although we previously reported some evidence that maternal serum DDT concentrations were inversely associated with motor scores on the Bayley Scales of Infant Development III at ages 1 and 2 years, associations were detected among girls only (Eskenazi et al., 2018). The developmental impact of the DDT and T4 association that we identified here among males 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, results suggest that exposure to other pyrethroid insecticides may be of greater concern with regards to thyroid hormone disruption.

Declaration of Competing Interest

Authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.104958>.

References

- Akhtar, N., Kayani, S.A., Ahmad, M.M., et al., 1996. Insecticide-induced changes in secretory activity of the thyroid gland in rats. *Journal of applied toxicology: JAT* 16 (5), 397–400.
- Alvarez-Pedrol, M., Ribas-Fito, N., Torrent, M., et al., 2008. Thyroid disruption at birth due to prenatal exposure to beta-hexachlorocyclohexane. *Environ. Int.* 34 (6), 737–740.
- Asawasinsopon, R., Prapamontol, T., Prakobvitayakit, O., et al., 2006. The association between organochlorine and thyroid hormone levels in cord serum: a study from northern Thailand. *Environ. Int.* 32 (4), 554–559.
- Barcelo, M.A., Saez, M., Saurina, C., 2009. Spatial variability in mortality inequalities, socioeconomic deprivation, and air pollution in small areas of the Barcelona Metropolitan Region, Spain. *Sci. Total Environ.* 407 (21), 5501–5523.
- Barr, J.R., Maggio, V.L., Barr, D.B., et al., 2003. 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* 794 (1), 137–148.
- Bloom, M.S., Jansing, R.L., Kannan, K., et al., 2014. Thyroid hormones are associated with exposure to persistent organic pollutants in aging residents of upper Hudson River communities. *Int. J. Hyg. Environ. Health* 217 (4–5), 473–482.
- Boelen, A., Wiersinga, W.M., Fliers, E., 2008. Fasting-induced changes in the hypothalamus-pituitary-thyroid axis. *Thyroid* 18 (2), 123–129.
- Bravo, M.A., Son, J., de Freitas, C.U., et al., 2016. Air pollution and mortality in Sao Paulo, Brazil: effects of multiple pollutants and analysis of susceptible populations. *Journal of exposure science & environmental epidemiology* 26 (2), 150–161.
- Centers for Disease Control and Prevention, 2017. NSQP: Newborn Screening Quality Assurance Program. U.S. Department of Health and Human Services. <https://www.cdc.gov/labstandards/nsqp.html>, Accessed date: 9 July 2018.
- Chevrier, J., Eskenazi, B., Bradman, A., et al., 2007. Associations between prenatal exposure to polychlorinated biphenyls and neonatal thyroid-stimulating hormone levels in a Mexican-American population, Salinas Valley, California. *Environ. Health Perspect.* 115 (10), 1490–1496.
- Chevrier, J., Eskenazi, B., Holland, N., et al., 2008. Effects of exposure to polychlorinated biphenyls and organochlorine pesticides on thyroid function during pregnancy. *Am. J. Epidemiol.* 168 (3), 298–310.
- Chevrier, J., Rauch, S., Crause, M., et al., 2019. 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. *Am. J. Epidemiol.* 188 (1), 130–140.
- Chi, G.C., Hajat, A., Bird, C.E., et al., 2016. Individual and neighborhood socioeconomic status and the association between air pollution and cardiovascular disease. *Environ. Health Perspect.* 124 (12), 1840–1847.
- Coker, E., Chevrier, J., Rauch, S., et al., 2018. Association between prenatal exposure to multiple insecticides and child body weight and body composition in the VHEMBE South African birth cohort. *Environ. Int.* 113, 122–132.
- Darnerud, P.O., Lignell, S., Glynn, A., et al., 2010. POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. *Environ. Int.* 36 (2), 180–187.
- Delport, R., Bornman, R., MacIntyre, U.E., et al., 2011. Changes in retinol-binding protein concentrations and thyroid homeostasis with nonoccupational exposure to DDT. *Environ. Health Perspect.* 119 (5), 647–651.
- Demographics and Health Surveys, 2017. Wealth Index. United States Agency for International Development. <https://dhsprogram.com/topics/wealth-index/Wealth-Index-Construction.cfm>, Accessed date: April 2018.
- Dewailly, E., Forde, M., Robertson, L., et al., 2014. Evaluation of pyrethroid exposures in pregnant women from 10 Caribbean countries. *Environ. Int.* 63, 201–206.
- Dragano, N., Hoffmann, B., Moebus, S., et al., 2009. 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. *Occup. Environ. Med.* 66 (9), 628–635.
- Du, G., Shen, O., Sun, H., et al., 2010. Assessing hormone receptor activities of pyrethroid insecticides and their metabolites in reporter gene assays. *Toxicological sciences: an official journal of the Society of Toxicology* 116 (1), 58–66.
- Eskenazi, B., An, S., Rauch, S.A., et al., 2018. Prenatal exposure to DDT and pyrethroids for malaria control and child neurodevelopment: the VHEMBE cohort, South Africa. *Environ. Health Perspect.* 126 (4), 047004.
- Fetoui, H., Bouaziz, H., Mahjoubi-Samet, A., et al., 2006. Food restriction induced thyroid changes and their reversal after refeeding in female rats and their pups. *Acta Biol. Hung.* 57 (4), 391–402.
- Fisher, D.A., Brown, R.S., 2013. The maturation of thyroid function in the perinatal period and during childhood. In: Braveman, L.E., Cooper, D.S. (Eds.), *Werner & Ingbar's the Thyroid: A Fundamental and Clinical Text*. Lippincott, Williams & Wilkins, Philadelphia, PA, pp. 775–786.
- Freire, C., Koifman, R.J., Sarcinelli, P.N., et al., 2013. Long-term exposure to organochlorine pesticides and thyroid status in adults in a heavily contaminated area in Brazil. *Environ. Res.* 127, 7–15.
- Gaspar, F.W., Chevrier, J., Quiros-Alcala, L., et al., 2017. Levels and determinants of DDT and DDE exposure in the VHEMBE cohort. *Environ. Health Perspect.* 125 (7), 077006.
- Ghisari, M., Long, M., Tabbo, A., et al., 2015. Effects of currently used pesticides and their mixtures on the function of thyroid hormone and aryl hydrocarbon receptor in cell culture. *Toxicol. Appl. Pharmacol.* 284 (3), 292–303.
- Giray, B., Caglayan, A., Erkekoglu, P., et al., 2010. Fenvalerate exposure alters thyroid hormone status in selenium- and/or iodine-deficient rats. *Biol. Trace Elem. Res.* 135 (1–3), 233–241.
- Helmreich, D.L., Tylee, D., 2011. Thyroid hormone regulation by stress and behavioral differences in adult male rats. *Horm. Behav.* 60 (3), 284–291.
- Hicken, M.T., Dvonch, J.T., Schulz, A.J., et al., 2014. Fine particulate matter air pollution and blood pressure: the modifying role of psychosocial stress. *Environ. Res.* 133, 195–203.
- Hirano, K., Imbens, G., 2004. The propensity score with continuous treatment. In: Gelman, A., Meng, X.-L. (Eds.), *Applied Bayesian Modelling and Causal Inference from Missing Data*. Wiley, New York, NY, pp. 73–84.
- Huang, J., Eskenazi, B., Bornman, R., et al., 2018a. Maternal peripartum serum DDT/E and urinary pyrethroid metabolite concentrations and child infections at 2 years in the VHEMBE birth cohort. *Environ. Health Perspect.* 126 (6), 067006.
- Huang, J., Eskenazi, B., Bornman, R., et al., 2018b. 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* 2, e026.
- Jain, R.B., 2016. 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. *Environ. Sci. Pollut. Res. Int.* 23 (7), 6934–6939.
- Johnsen, L., Kongsted, A.H., Nielsen, M.O., 2013. Prenatal undernutrition and postnatal overnutrition alter thyroid hormone axis function in sheep. *J. Endocrinol.* 216 (3), 389–402.
- Johnsen, L., Lyckegaard, N.B., Khanal, P., et al., 2018. Fetal over- and undernutrition differentially program thyroid axis adaptability in adult sheep. *Endocr Connect* 7 (5), 777–790.
- Johnson, J., 1982. Life events as stressors in childhood and adolescence. In: Lahey, B.B., Kazdin, A.E. (Eds.), *Advances in Clinical Child Psychology*. Springer, Boston, MA, pp. 219–253.
- Kaul, P.P., Rastogi, A., Hans, R.K., et al., 1996. Fenvalerate-induced alterations in circulatory thyroid hormones and calcium stores in rat brain. *Toxicol. Lett.* 89 (1), 29–33.
- Landrigan, P.J., Fuller, R., Acosta, N.J.R., et al., 2018. The lancet commission on pollution and health. *Lancet* 391 (10119), 462–512.
- Lopez-Espinosa, M.J., Vizcaino, E., Murcia, M., et al., 2009. Association between thyroid hormone levels and 4,4'-DDE concentrations in pregnant women (Valencia, Spain). *Environ. Res.* 109 (4), 479–485.
- Lopez-Espinosa, M.J., Vizcaino, E., Murcia, M., et al., 2010. Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. *Journal of exposure science & environmental epidemiology* 20 (7), 579–588.
- Lubin, J.H., Colt, J.S., Camann, D., et al., 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ. Health Perspect.* 112 (17), 1691–1696.
- MacIntyre, U.E., Venter, C.S., Vorster, H.H., 2001. A culture-sensitive quantitative food frequency questionnaire used in an African population: 1. Development and reproducibility. *Public Health Nutr.* 4 (1), 53–62.
- Maervoet, J., Vermeir, G., Covaci, A., et al., 2007. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. *Environ. Health Perspect.* 115 (12), 1780–1786.
- Maharaj, R., Raman, J., Morris, N., et al., 2013. Epidemiology of malaria in South Africa: from control to elimination. *S. Afr. Med. J.* 103 (10 Pt 2), 779–783.
- Maiti, P.K., Kar, A., 1997. Dual role of testosterone in fenvalerate-treated mice with respect to thyroid function and lipid peroxidation. *Journal of applied toxicology: JAT* 17 (2), 127–131.
- Maiti, P.K., Kar, A., 1998. Is triiodothyronine capable of ameliorating pyrethroid-induced thyroid dysfunction and lipid peroxidation? *Journal of applied toxicology: JAT* 18 (2), 125–128.
- Malig, B.J., Ostro, B.D., 2009. Coarse particles and mortality: evidence from a multi-city study in California. *Occup. Environ. Med.* 66 (12), 832–839.
- Meeker, J.D., Barr, D.B., Hauser, R., 2009. Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. *Reprod. Toxicol.* 27 (2), 155–160.
- O'Connor, J.C., Frame, S.R., Davis, L.G., et al., 1999. 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* 51 (1), 44–53.
- O'Connor, J.C., Frame, S.R., Ladics, G.S., 2002. Evaluation of a 15-day screening assay using intact male rats for identifying antiandrogens. *Toxicological sciences: an official journal of the Society of Toxicology* 69 (1), 92–108.
- O'Lenick, C.R., Chang, H.H., Kramer, M.R., et al., 2017. 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* 16 (1), 36.
- Ostro, B.D., Feng, W.Y., Broadwin, R., et al., 2008. The impact of components of fine particulate matter on cardiovascular mortality in susceptible subpopulations. *Occup. Environ. Med.* 65 (11), 750–756.
- Otten, J.J., Hellwig, J.P., Meyers, L.D., 2006. *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. Institute of Medicine of the National Academies, Washington, DC.
- Passos, M.C., da Fonte Ramos, C., Dutra, S.C., et al., 2002. Long-term effects of malnutrition during lactation on the thyroid function of offspring. *Horm. Metab. Res.* 34 (1), 40–43.
- Phillips, D.L., Pirkle, J.L., Burse, V.W., et al., 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch. Environ. Contam. Toxicol.* 18 (4), 495–500.
- Qiu, H., Tian, L., Ho, K.F., et al., 2015. Air pollution and mortality: effect modification by personal characteristics and specific cause of death in a case-only study. *Environ. Pollut.* 199, 192–197.
- Ramos, C.F., Teixeira, C.V., Passos, M.C., et al., 2000. Low-protein diet changes thyroid function in lactating rats. *Proc. Soc. Exp. Biol. Med.* 224 (4), 256–263.
- Rasmussen, K.M., Yaktine, A.L., 2009. *Weight Gain during Pregnancy: Reexamining the Guidelines*. Institute of Medicine and National Research Council, Washington, DC. <https://www.ncbi.nlm.nih.gov/books/NBK32813/>, Accessed date: 15 October 2016.
- Rauch, S., Bradman, A., Coker, E., et al., 2018. Determinants of exposure to pyrethroid insecticides in the VHEMBC cohort, South Africa. *Environ Sci Technol* 52 (21), 12108–12121.
- Richter, L.M., Dev Griesel, R., Barbarin, O., 2000. 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. Proceedings of the First International Conference*, vol. 1. Elsevier Press, Amsterdam, The Netherlands, pp. 159–182.
- Rosenfeld, C.S., Trainor, B.C., 2014. Environmental health factors and sexually dimorphic differences in behavioral disruptions. *Curr Environ Health Rep* 1 (4), 287–301.
- Rosner, B., 1983. Percentage points for a generalized ESD many-outlier procedure. *Technometrics* 25 (2), 165–172.
- Rylander, L., Wallin, E., Jonsson, B.A., et al., 2006. Associations between CB-153 and p,p'-DDE and hormone levels in serum in middle-aged and elderly men. *Chemosphere* 65 (3), 375–381.
- Sekeroglu, V., Sekeroglu, Z.A., Demirhan, E., 2014. Effects of commercial formulations of deltamethrin and/or thiacloprid on thyroid hormone levels in rat serum. *Toxicol. Ind. Health* 30 (1), 40–46.
- Sierra-Santoyo, A., Hernandez, M., Albores, A., et al., 2000. Sex-dependent regulation of hepatic cytochrome P-450 by DDT. *Toxicological sciences: an official journal of the Society of Toxicology* 54 (1), 81–87.
- Son, J.Y., Lee, J.T., Kim, H., et al., 2012. 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* 22 (3), 227–234.
- Statistics South Africa, 2017. *Poverty Trends in South Africa: An Examination of Absolute Poverty between 2006 and 2015*. Statistics South Africa, Pretoria, South Africa. <http://www.statssa.gov.za/publications/Report-03-10-06/Report-03-10-062015.pdf>, Accessed date: 18 June 2018.
- Stein, L.J., Gunier, R.B., Harley, K., et al., 2016. Early childhood adversity potentiates the adverse association between prenatal organophosphate pesticide exposure and child IQ: the CHAMACOS cohort. *Neurotoxicology* 56, 180–187.
- Takser, L., Mergler, D., Baldwin, M., et al., 2005. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. *Environ. Health Perspect.* 113 (8), 1039–1045.
- Tebourbi, O., Hallegue, D., Yacoubi, M.T., et al., 2010. Subacute toxicity of p,p'-DDT on rat thyroid: hormonal and histopathological changes. *Environ. Toxicol. Pharmacol.* 29 (3), 271–279.
- Teeyapant, P., Ramchian, S., Polputpisatkul, D., et al., 2014. Serum concentrations of organochlorine pesticides p,p'-DDE in adult Thai residents with background levels of exposure. *J. Toxicol. Sci.* 39 (1), 121–127.
- Turyk, M.E., Anderson, H.A., Persky, V.W., 2007. Relationships of thyroid hormones with polychlorinated biphenyls, dioxins, furans, and DDE in adults. *Environ. Health Perspect.* 115 (8), 1197–1203.
- U.S. Environmental Protection Agency, 2018. *Environmental Justice and National Environmental Policy Act*. U.S. EPA, Washington, DC. <https://www.epa.gov/environmentaljustice/environmental-justice-and-national-environmental-policy-act> (Accessed 20 July 2018).
- United Nations Economic Commission for Europe, 1998. *Convention on Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters*. UNECE, Aarhus, Denmark.
- Van der Laan, M.J., Polley, E.C., Hubbard, A.E., 2007. *Super Learner*. UC Berkeley Division of Biostatistics Working Paper Series. Working Paper 222. Available: <http://biostats.bepress.com/ucbbiostat/paper222> (Online July 2007).
- Wang, S., Shi, N., Ji, Z., et al., 2002. 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* 20 (3), 173–176.
- Westergaard, N., Gehring, U., Slama, R., et al., 2017. Ambient air pollution and low birth weight - are some women more vulnerable than others? *Environ. Int.* 104, 146–154.
- Wong, C.M., Ou, C.Q., Chan, K.P., et al., 2008. The effects of air pollution on mortality in socially deprived urban areas in Hong Kong, China. *Environ. Health Perspect.* 116 (9), 1189–1194.
- World Health Organization, 2009. *Country Profiles of Environmental Burden of Disease by WHO Regions*. WHO, Geneva, Switzerland. http://www.who.int/quantifying_ehimpacts/afrcountryprofiles2004-rev.pdf, Accessed date: 7 August 2018.
- World Health Organization, 2018. *World Malaria Report 2018*. WHO, Geneva Switzerland.
- Yamada, T., Kunimatsu, T., Miyata, K., et al., 2004. 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* 79 (1), 64–74.
- Zeka, A., Zanobetti, A., Schwartz, J., 2006. Individual-level modifiers of the effects of particulate matter on daily mortality. *Am. J. Epidemiol.* 163 (9), 849–859.
- Zhang, J., Yoshinaga, J., Hisada, A., et al., 2014. Prenatal pyrethroid insecticide exposure and thyroid hormone levels and birth sizes of neonates. *Sci. Total Environ.* 488–489, 275–279.
- Tamayo y Ortiz, M., Tellez-Rojo, M.M., Trejo-Valdivia, B., et al., 2017. Maternal stress modifies the effect of exposure to lead during pregnancy and 24-month old children's neurodevelopment. *Environ. Int.* 98, 191–197.