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Maternal Peripartum Serum DDT/E and Urinary Pyrethroid Metabolite Concentrations and Child Infections at 2 Years in the VHEMBE Birth Cohort

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Background: Indoor residual spraying (IRS) of insecticides, conducted in low- and middle-income countries to control malaria, may result in high exposure to dichlorodiphenyltrichloroethane (DDT), its breakdown product dichlorodiphenyldichloroethylene (DDE), or pyrethroids. Animal studies suggest *in utero* exposure to these chemicals may increase childhood infection frequency.

Objectives: We investigated associations between maternal DDT/E and pyrethroid metabolite concentration and child infection associations in an IRS setting in which susceptibility factors are common and infections are leading causes of child morbidity and mortality.

Methods: Using gas chromatography–mass spectrometry, we measured serum DDT/E and urinary pyrethroid metabolite concentrations in peripartum samples from 674 women participating in the Venda Health Examination of Mother, Babies and their Environment (VHEMBE) study. Counts of persistent child fevers, otitis media, and severe sore throat between 1 and 2 y of age were ascertained from maternal interviews. Associations between DDT/E and pyrethroid metabolite concentrations and infections were estimated using zero-inflated Poisson regression. We estimated relative excess risks due to interaction (RERI) with poverty, maternal energy intake, and maternal HIV status.

Results: Concentrations of DDT/E, particularly *p,p′*-DDE, were associated with higher rates of persistent fevers [IRR = 1.21 (95% CI: 1.01, 1.46)], for a 10-fold increase in *p,p′*-DDE). This association was stronger among children from households below versus above the South African food poverty line [IRR = 1.31 (95% CI: 1.08, 1.59) vs. IRR = 0.93 (95% CI: 0.69, 1.25), respectively] and for children whose mothers had insufficient versus sufficient caloric intake during pregnancy [IRR = 1.30 (95% CI: 1.07, 1.58) vs. IRR = 0.96 (95% CI: 0.72, 1.28), respectively].

Conclusions: *In utero* IRS insecticide exposure may increase childhood infection rates. This was particularly apparent among children from poorer households or whose mothers had low energy intake during pregnancy. https://doi.org/10.1289/EHP2657

Introduction

To control mosquito vectors, malaria control programs in 85 predominantly low- and middle-income countries apply insecticides directly to the inside walls of homes, a process known as indoor residual spraying (IRS). Consequently, approximately 106 million people may inadvertently become exposed to IRS insecticides (World Health Organization [WHO] 2016). Fetal exposure to insecticide used for IRS, including the pyrethroid deltamethrin and dichlorodiphenyltrichloroethane (DDT) and its breakdown product dichlorodiphenyldichloroethylene (DDE), have been shown in many animal and *in vitro* studies to alter immune function (Rehana and Rao 1992; Santoni et al. 1998; Banerjee 1999; Misumi et al. 2005; ATSDR 2002, 2003; Brander et al. 2016) and to specifically increase susceptibility to infection (Nuñez et al. 2002; Suwanchaichinda et al. 2005; Rehman et al. 2011). However, epidemiologic investigations of associations between fetal insecticide exposure and childhood infections have yielded conflicting evidence.

In a Spanish cohort, maternal antepartum *p,p′*-DDE blood concentrations were associated with higher risk of lower respiratory tract infection (LRTI) in the first year of life (Sunyer et al. 2010; Gascon et al. 2012). Similarly, in highly exposed Arctic populations, maternal *p,p′*-DDE concentrations were associated with elevated risks of gastrointestinal, ear (otitis media), and upper respiratory tract infections (URTI) in 1-y-olds (Dewailly et al. 2000; Dallaire et al. 2004), although dose–response trends were only detected with aggregated infections (Dallaire et al. 2004). Other studies have reported associations with lower risk of infections. For example, a German study reported that child (postnatal) *p,p′*-DDE concentrations were associated with lower risk of otitis media in 7- to 10-y-olds (Karmaus et al. 2001) and a Mexican study observed an association between higher prenatal *p,p′*-DDE concentration and lower risk of LRTI in 2-y-old boys (Cupul-Uicab et al. 2014). No studies have reported associations with DDT, possibly because of low detection frequencies (Sunyer et al. 2010). To our knowledge, no human studies have investigated associations between fetal pyrethroid exposure and child infections in settings where IRS is conducted.

Individuals living in IRS areas have been shown to have tissue concentrations of these chemicals orders of magnitude higher than in general populations (Gaspar et al. 2017; Aneck-Hahn et al. 2007; Whitworth et al. 2014). Understanding potential adverse side effects of IRS on childhood infections is critical because respiratory and gastrointestinal infections are leading causes of child morbidity and mortality in these settings. In our study area (Limpopo Province, South Africa), intestinal infections, influenza, and pneumonia together account for 26.2% and 23.6% of all deaths among infants under 1 y and children 1–14 y of age, respectively (Statistics South Africa 2017). Moreover, early adversity factors such as poverty (Appleton et al. 2016; Stein et al. 2016), malnutrition (Banerjee 1999; Bourke et al. 2016; Prescott 2016); and HIV (Ruck et al. 2016; Slogrove et al. 2017) are common among pregnant women in this setting and may increase children's susceptibility to any adverse impact of insecticides. This study aims to investigate whether prenatal exposure to IRS-related chemicals is associated with increased incidence of childhood infection and whether potential effects of IRS insecticides may be exacerbated by these susceptibility factors.

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Methods

Study Population and Recruitment

The current study was conducted in the context of the Venda Health Examination of Mothers, Babies, and their Environment (VHEMBE), a prospective birth cohort study investigating the effects of environmental exposures on the health and development of children born in the Vhembe district of Limpopo Province, South Africa, where IRS insecticides, primarily DDT and the pyrethroid deltamethrin, are applied during the malarial season. Between August 2012 and December 2013, women who presented for delivery at Tshilidzini Hospital in the town of Thohoyandou were screened for eligibility. Women were eligible if they were at least 18 y of age, spoke Tshivenda at home, lived within 20 km of the hospital and planned to remain in the area for the next 2 y, were free from malaria during the index pregnancy, had contractions at least 5 min apart, and delivered a live, viable singleton. Informed consent was obtained prior to data collection. 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.

Study staff screened 1,649 mothers, 920 of whom met eligibility criteria. Of the eligible women, 752 (82%) provided informed consent, completed a baseline delivery questionnaire, and provided a peripheral blood sample for DDT/E quantification. Mothers and babies were followed up for a home visit at 1 wk ($n = 723$, or 96% of initially enrolled; 29 mothers formally withdrew after delivery, had their index child die before 1 wk, or otherwise did not complete the visit), 1 y ($n = 700$ completed visits, or 93%; 13 children died prior to visit), and 2 y ($n = 685$ completed visits, or 91%; 7 died prior to visit) after delivery. Eleven children who missed their 1-y visit but were seen at 2 y were excluded because we would be unable to ascertain their infection rate between 1 and 2 y of age. Thus, the final sample size for this study was 674 (90% of initially enrolled) mother–child dyads. Dyads included in this analysis were less likely to be HIV positive, but were otherwise similar to those that were excluded with respect to all other characteristics used for our analyses (see Table S1).

Serum Collection and Analysis

Maternal peripheral blood was collected in red-top vacutainer tubes prior to, or immediately after, delivery. Samples were immediately processed and stored at −80°C until shipped on dry ice to Emory University's Environmental Health Laboratory (Atlanta, GA) for analyses. Gas chromatography–tandem mass spectrometry (GC-MS/MS) with isotope dilution quantification (Barr et al. 2003) was used to quantify *p,p′*-DDT, *p,p′*-DDE, *o,p′*-DDT, and *o,p′*-DDE. Limits of detection (LODs) were 0.01 ng/mL (*o,p′*-DDE, *o,p′*-DDT, and *p,p′*-DDT) and 0.03 ng/mL (*p,p′*-DDE). Corresponding limits of quantification (LOQs) were 0.05 ng/mL and 0.15 ng/mL. Concentrations of *o,p′*-DDE were only quantified in 16% ($n = 108$) of samples and were excluded from analyses. Total serum lipid concentrations were estimated using the Phillips formula (Bergonzi et al. 2009) from total triglyceride and cholesterol concentrations measured by standard enzymatic methods (Roche Chemicals).

Urine Collection and Analysis

Of the 674 women included in this study, 666 (99%) provided a sufficient urine sample for pyrethroid metabolite quantification, of which 413 (62%) provided a urine sample before and 253 (38%) after delivery. Urinary specific gravity was measured shortly after collection using a refractometer (Atago PAL-10S). Samples were immediately processed and stored at −80°C. Urine aliquots were shipped on dry ice to the Centre de Toxicologie du Québec (CTQ) of the Institut National de Santé Publique du Québec (INSPQ) in Quebec City, Canada, for analyses. Gas chromatography–mass spectrometry (Dewailly et al. 2014) was used to quantify the following five pyrethroid metabolites: 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA), *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DBCA), *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DCCA), *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*trans*-DCCA), and 3-phenoxybenzoic acid (3-PBA). LODs and LOQs were 0.005, 0.0025, 0.0045, 0.0038, and 0.0047 μg/L and 0.011, 0.0082, 0.015, 0.013, and 0.016 μg/L, respectively. Concentrations of 4-F-3-PBA were only quantified in 7.7% ($n = 51$) of samples and were excluded from analyses.

Covariate and Outcome Data

Maternal questionnaire data. Study staff administered questionnaires in the local Tshivenda language to mothers prior to hospital discharge to collect information on maternal sociodemographics, nutrition, health, and pregnancy history. The questionnaire was designed in English, translated to Tshivenda, and back-translated to English by native speakers in the translated language. We used maternal age, marital status, parity, food frequencies, and family income recorded in this questionnaire. Family income was classified as above or below the official South African mid-2013 food poverty level (Rand 386 per person per month; W. Ruch, written communication, Jan 2016). Maternal HIV status was ascertained from self-reported diagnosis or use of anti-retroviral drugs based on medical records that were abstracted by registered nurses. Total energy intake in kilojoules (kJ) was estimated by a South African expert nutritionist using FoodFinder 3 software (South Africa Medical Research Council/WAMTechnology CC) from a quantitative food frequency questionnaire designed and validated in a population residing in our study area (MacIntyre et al. 2001). At the 2-y follow-up interview, biological mothers ($n = 653$) or primary caretakers ($n = 21$) were asked about the number of persistent fevers lasting ≥4 d, number of ear infections, and number of severe sore throats the child had since the child's last visit (i.e., at 1 y of age), number of individuals living in the same residence as the child, and whether the mother spent any period during the year living away from the child. Additionally, interviewers were asked to rate the quality of the mother's responses to different questionnaire sections (1 = high quality to 4 = unsatisfactory). To estimate rates of infections (i.e., fever, ear infection, and sore throat rates), the number of days over which the infection events were counted were calculated for each child by taking the difference between the 2-y- and 1-y visit dates (median = 365 d).

Maternal energy intake sufficiency. Mothers were characterized as having insufficient energy intake if total daily energy intake was less than that recommended by the U.S. Institute of Medicine (IOM) for late pregnancy (IOM and NRC 2009). The recommended intake (in kilojoules) was estimated for each mother using the following equation: $4.184\,\text{kJ/cal} \times [452 + 354 - (6.91 \times \text{age in years}) + 1.27 \times (9.36 \times \text{weight in kg}) + 726 \times \text{height in meters}]$ (median = 12,055 kJ or 2,880 kcal). Two assumptions were made in these calculations: First, because physical activity was not directly measured, women were uniformly categorized as active due to common strenuous activities of daily living in the study area such as walking long distances for transportation and water collection and agricultural activities. Second, postpartum weight was used instead of antepartum weight (because the latter was measured in only 75% of study subjects). The median threshold would be 1,105 kJ lower categorizing women as "low active" (54 women reclassified) or 240 kJ higher based on antepartum weights (56 women with measures reclassified).

Child anthropometrics. Infant weight (to the nearest 2 g using a Tanita BD-815U scale), length (using a Seca 417 infantometer), and head circumference (using a measuring tape) were measured by nurses at delivery. At the 1- and 2-y study visits, study staff measured weight to the nearest 10 g using a digital scale (Tanita HD-351); recumbent length at 1 y of age (Seca 417) or standing height at 2 y of age using a stadiometer (Charder HM200P); and middle-upper arm circumference using a measuring tape. All anthropometric measures were taken in triplicate and averaged and U.S. National Health and Nutrition Examination Survey (NHANES) protocols were followed (National Center for Health Statistics 2011).

Statistical Analyses

For all exposures, we used machine-read values for concentrations between the LOD and LOQ and randomly imputed values below the LOD based on a log-normal probability distribution whose parameters were estimated by maximum likelihood estimation (Lubin et al. 2004). Next, serum DDT/E concentrations were corrected for total lipids (ng/g lipid) and urinary pyrethroid metabolite concentrations were corrected for specific gravity (μg/L) and both were log-transformed to minimize the influence of outliers. To estimate the association between chemical concentrations and count of infection outcomes at 2 y of age, we fitted multivariable, zero-inflated Poisson regression models with the number of days between visits as the offset. The following covariates were identified using a directed acyclic graph (DAG): continuous maternal age and parity; binary marital status, low energy intake, and HIV status at the time of delivery of the index child; binary household poverty; and child sex [see Figure S1; generated via DAGitty (version 2.3; Johannes Textor) Textor et al. 2011]. All analyses were performed using Stata SE (version 12.1; StataCorp).

Zero-inflation model fitting. Because our outcomes of interest, particularly otitis media, may be missed in young children, we assumed *a priori* that underestimation of events would be likely. Accordingly, we used certain measures from the 2-y follow-up interview related to the likelihood that the primary caretaker of the child may have failed to observe infection events to correct for this underreporting. During the 2-y follow-up interview, the primary caretaker was asked whether they lived with the child for the entire period since the child's last visit, that is, the period for which we were estimating infection rates. Caretakers who did not report living with the child "all of the time" would naturally have a greater chance of missing infection events: 13% of mothers reported this, primarily due to work or study in the city. Caretakers were also asked the number of individuals that regularly reside in the same household as the child. Those with more household members may affect the recall of number infections for the index child. Finally, interviewers were asked to rate the reliability of mother or caregiver's responses following the completion of the child health section where the number of infections are reported. Reliability was scored 1 for "high quality," 2 for "generally reliable," 3 for "questionable," 4 for "unreliable," and 5 for "not applicable." Scores 3, 4, and 5 were grouped together as poor quality. Poor quality is an indicator that mothers may not have accurate recall of the child's health, including infection events. These three variables (lived with child, household size, and reliability) were used to model the possibility that infection events were missed (number reported as zero) in regression models described below. To do this, we used the *zip* command in Stata to incorporate a model for the probability of falsely reported or excess zeroes (i.e., zero-inflation) to the standard Poisson regression. Excess zeroes were modeled by logistic regression with the following variables as independent variables: whether or not the respondent lived with the child throughout the second year; her age and parity; household size at the 2-y follow-up interview; and the interview reliability score. Models were fit in a complete data set generated by randomly imputing missing values (45 of 9,436 possible values = 0.5%) in 40 subjects (5.9%) with any missing values drawing from conditional probability distributions estimated from observed covariate distributions. This was done using multiple imputation by chained equations (White et al. 2011) and the *mi* set of commands in Stata and keeping the first imputed data set. Estimates using this approach were compared to those resulting from Poisson regression and multiple imputation (10 data sets).

To estimate the degree to which maternal poverty, low energy intake, or HIV status; child sex; or exclusive breastfeeding for <1 month may modify any effect of insecticide exposure on child infections, we included interaction terms between each potential modifier (dichotomous) and mean-centered exposure. Each effect modifier was evaluated in a separate model (i.e., only two-way interactions) and a relative excess risk due to the interaction (RERI) was calculated for each using the equation: $\mathrm{RERI} = \mathrm{RR}_{11} - \mathrm{RR}_{10} - \mathrm{RR}_{01} + 1$ (in which R_{11} = rate ratio for a 1-unit change in chemical concentration in the presence of the modifier; R_{10} = rate ratio for a 1-unit change in chemical concentration in the absence of the modifier; and R_{01} = rate ratio due to the modifier at mean concentration of the chemical; Richardson and Kaufman 2009). Confidence intervals (CIs) and *p*-values were derived by the delta method (Hosmer and Lemeshow 1992; Assmann et al. 1996) using the *nlcom* command. All analyses were performed using Stata SE 12.1 (StataCorp; College Station, Texas).

Sensitivity and Exploratory Analyses

Several quantitative sensitivity analyses were conducted to test assumptions made in variable construction and regression model analyses: We compared results obtained using zero-inflated versus standard Poisson regression models qualitatively and by Vuong test for nonnested models (Desmarais and Harden 2013). We additionally adjusted for number of children <5 y of age living in the household at 2 y of age. Because pyrethroids are rapidly metabolized and eliminated, we investigated whether associations differed by pyrethroid collection time (before or after delivery). Finally, we examined the influence of using a lower threshold for daily energy demands (i.e., by classifying women as "low active" rather than "active") on estimated associations.

DDT/E and pyrethroids have been associated with length of gestation and birth (Eskenazi et al. 2009) and child size (Jusko et al. 2006; Burns et al. 2012; Warner et al. 2013), themselves predictors of infection susceptibility (Duijts et al. 2009). We explored the degree to which birth weight, length, head circumference, or preterm birth may mediate any observed relationships using four-way decomposition analyses, allowing for exposure–mediator interaction (Vanderweele 2014). For the decompositions, the exposure effect was defined as increasing the exposure from the 25th percentile of concentration to the 75th percentile in a referent population of lower infection risk: female offspring born to mothers of average age and parity who are married, HIV-negative, living above the food poverty line, and with adequate daily caloric intake during late pregnancy. We were specifically interested in the pure indirect effect; that is, the potential effect of insecticides that is mediated by alterations in child growth but that is not due to interactions. This was estimated as the proportion of the total excess rate of the infectious outcome associated with increasing chemical concentration (from the 25th to 75th percentile) that was due specifically to changes in birth size or risk of preterm birth due to the chemical. This can be estimated under causal inference assumptions of consistency, no unmeasured exposure–outcome, exposure–mediator, exposure-induced, and mediator–outcome confounding, nonpositivity, and correct model specification (Vanderweele 2014).

Results

Participant Characteristics

All mothers were black South Africans and their mean age at delivery of the index child was 26.4 y. Most women were unmarried (53%), had less than a 12th grade education (56%), were multiparous (57%), and lived below the food poverty line (61%) (Table 1). Two-thirds of women (67%; $n = 454$) ate less than the IOM-recommended daily number of calories for late pregnancy and 13% were HIV positive. Most children (74%) were exclusively breastfed for at least 1 month and 11% for ≥6 months. During the second year of life (1–2 y of age), persistent fevers lasting ≥4 d were common, that is, they were reported in nearly 30% of children. Ear infections were less common (16%) and severe sore throats were rare (6%). No children had been diagnosed with malaria by 1 y of age, and one child by 2 y of age.

Maternal Exposure to DDT/E and Pyrethroids. As previously reported (Gaspar et al. 2017), *p,p′*-DDT, *o,p′*-DDT, and *p,p′*-DDE were detected in almost all serum samples in this population (Table 2). Urinary pyrethroid metabolites *cis*-DBCA, *cis*-DCCA, *trans*-DCCA, and 3-PBA were also detected in all samples (Table 2). Concentrations of DDT/E and pyrethroid metabolites were generally higher than those reported in contemporary U.S. populations: Median *p,p′*-DDE in VHEMBE was 254.4 ng/g lipid versus 113 ng/g lipid among pregnant women 18–40 y of age in the 1999–2000 National Health and Nutrition Examination Survey (NHANES; Bradman et al. 2007). Few pregnant women in NHANES had *o,p′*-DDT (20%) or *p,p′*-DDT (0.2%) above the LOD of 10.6 ng/g lipid (Bradman et al. 2007). The 75th percentile of pyrethroid metabolites ranged from 0.48 to 1.38 in VHEMBE vs. 0.15 to 0.5 in NHANES (Castorina et al. 2010). The distributions of urine pyrethroid metabolite concentrations were similar in samples collected before and after delivery (see Figure S2).

We found that higher concentrations of DDT/E were associated with higher rates of persistent fevers and severe sore throats, particularly *p,p′*-DDE; associations between DDT/E and otitis media were mixed (Table 3). A 10-fold increase in maternal serum *p,p′*-DDE concentration was associated with a 21% higher rate of persistent fevers [incidence rate ratio(IRR) = 1.21 (95% CI: 1.01, 1.46)]. DDT/E appeared to be associated with higher rates of severe sore throat [IRR = 1.53 (95% CI: 0.77, 3.03), IRR = 1.58 (95% CI: 0.80, 3.14), and IRR = 1.78 (95% CI: 0.82, 3.84) for *o,p′*-DDT, *p,p′*-DDT, and *p,p′*-DDE, respectively]; however, due to the small number of events, estimates were imprecise. Findings were similar in sensitivity analyses with adjustments for number of children (see Table S2), using uncorrected exposure concentrations and adjusting for lipid concentrations (see Table S3), and using multiply imputed Poisson regression (see Table S4). Additionally, classifying women as "low active" rather than "active" for the purposes of estimating daily energy requirements did not alter observed associations between DDT/E and infection outcomes (see Table S5).

There was limited evidence that pyrethroid metabolite concentrations were associated with the studied outcomes (Table 3). When stratified by collection timing, *cis*-DCCA, *trans*-DCCA, and 3-PBA concentrations were positively associated with higher rates of persistent fevers only among mothers whose urine was collected prior to delivery (i.e., most proximal to home exposures), whereas the opposite was observed for *cis*-DBCA (see Table S6). However, evidence for effect modification was generally weak ($p = 0.08$ to 0.59; see Table S6). For ear infections, higher concentrations were associated with higher rates only among the postdelivery subsample (see Table S6). For severe sore throats, inverse associations were observed in both strata; however, CIs were very wide in the postdelivery stratum. Overall, interpretation of true differences by collection timing was limited by reduced stratified sample sizes (see Table S6). Given the limited evidence of associations between pyrethroid metabolites and outcomes, we focused on DDT/E-persistent fever associations in subsequent analyses.

In interaction models, we observed that DDT/E were positively associated with persistent fevers among children from households below, but not above, the South African food poverty

Table 1. Characteristics of Venda Health Examination of Mothers, Babies, and their Environment (VHEMBE) study participants (Vhembe District, Limpopo, South Africa; $n = 674$).

Characteristic	*n* (%) or mean ± SD
Maternal characteristics at delivery	
Age (y)	26.4 ± 6.3
Education	
<Grade 12	376 (56)
Grade 12	200 (30)
>Grade 12	98 (15)
Parity (number of previous children)	
0	288 (43)
1	184 (27)
2	108 (16)
≥3	94 (14)
Marital status	
Married	320 (47)
Unmarried	354 (53)
History of high blood pressure or preeclampsia[a]	
Yes	86 (13)
No	588 (87)
Energy intake status[b,c]	
Low/insufficient	454 (67)
Sufficient	209 (31)
HIV status[b]	
Positive	86 (13)
Negative	586 (87)
Family characteristics at birth	
Number of people living with index child	5.2 ± 2.8
Poverty status[b,d]	
Below poverty line	409 (61)
Above poverty line	262 (39)
Child characteristics	
Sex	
Female	325 (48)
Male	349 (52)
Preterm (<37 wk gestation)	
No	589 (87)
Yes	85 (13)
Low birth weight (<3,500 g)	
No	623 (92)
Yes	51 (8)
Exclusively breastfed	
<1 month	174 (26)
≥1 month, but <6 months	427 (63)
≥6 months	73 (11)
Any persistent fever (lasting ≥4 d)[b]	
No	476 (71)
Yes	197 (29)
Any ear infection[b]	
No	563 (84)
Yes	109 (16)
Any severe sore throat[b]	
No	631 (94)
Yes	40 (6)

Note: SD, standard deviation.
[a]Any high blood pressure, preeclampsia, or use of blood pressure medications in current or previous pregnancies, by self-report or medical records.
[b]Individuals missing values for these variables (count in parentheses): energy intake (11), HIV status (2), poverty level (3), persistent fevers (1), ear infections (2), and severe sore throat (3).
[c]Based on whether the individual's total energy intake falls below the IOM and NRC (2009) recommendation for women in late pregnancy based on age, height, weight, and activity level (mean recommended intake in this population = 12,145 kJ; range = 10,163 to 14,711 kJ).
[d]Mid-2013 food poverty threshold = Rand 386 per person per month (W. Ruch, written communication, Jan 2016).

Table 2. Maternal lipid-corrected concentrations of dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) in serum samples (ng/g lipid) and specific gravity–corrected concentrations of pyrethroid metabolites in urine samples (μg/L).

Analyte	n	Detected (%)[a]	Quantified (%)[b]	Geometric mean[c]	Min	10th %ile	25th %ile	Median	75th %ile	90th %ile	Max
Serum DDT/E											
o,p′-DDT	674	90.5	44.5	9.18	<LOD	1.5	3.5	7.5	23.3	74.8	2029.3
p,p′-DDE	674	100	97.3	292.95	3.98	45.3	92.3	254.4	878.6	2709.8	26301.3
p,p′-DDT	674	98.1	90.5	70.04	<LOD	7.7	18.6	56.9	261.3	994.8	15027.6
Urinary pyrethroid metabolites											
cis-DBCA	666	100	99.5	0.22	0.005	0.05	0.10	0.22	0.48	1.12	17.8
cis-DCCA	666	100	99.8	0.31	0.015	0.08	0.15	0.30	0.60	1.05	103.5
trans-DCCA	666	100	99.5	0.36	0.008	0.08	0.16	0.34	0.80	1.63	132.9
3-PBA	665	100	100	0.72	0.022	0.21	0.38	0.70	1.38	2.40	58.9

Note: %ile, percentile; DBCA, 3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; DCCA, 3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; LOD, limit of detection; LOQ, limit of quantification; Max, maximum; Min, minimum; PBA, phenoxybenzoic acid.
[a]LODs: 0.01 ng/mL (*o,p′*-DDT, and *p,p′*-DDT); 0.03 ng/mL (*p,p′*-DDE); 0.0025 μg/L (*cis*-DBCA); 0.0045 μg/L (*cis*-DCCA); 0.0038 μg/L (*trans*-DCCA); and 0.0047 μg/L (3-PBA).
[b]LOQs: 0.05 ng/mL (*o,p′*-DDT, and *p,p′*-DDT); 0.15 ng/mL (*p,p′*-DDE); 0.0082 μg/L (*cis*-DBCA); 0.015 μg/L (*cis*-DCCA); 0.013 μg/L (*trans*-DCCA); and 0.016 μg/L (3-PBA).
[c]Geometric means for DDT/E include values below the LOD imputed at random based on log-normal probability distributions whose parameters were determined by maximum likelihood estimation.

line. Similarly, positive associations were observed among children of mothers with insufficient, but not adequate, caloric intake during late pregnancy. There were no consistent patterns across strata defined by maternal HIV status, exclusive breastfeeding duration, or child sex (Table 4). Using formal tests of additive interactions, the rate ratio associated with increasing serum *p,p′*-DDE concentrations in mothers who were from poorer households was 43% higher [RERI = 0.43 (95% CI: 0.03, 0.84); Table 4] than expected based on observed associations with *p,p′*-DDE in those above the food poverty line [$IRR_{10} = 0.93$ (95% CI: 0.69, 1.25)] and food poverty status alone [$IRR_{01} = 1.16$ (95% CI: 0.91, 1.48)]. Associations with *o,p′*-DDT and *p,p′*-DDT showed similar patterns (Table 4). Finally, exploratory mediation analyses provided little evidence that birth characteristics mediated exposure–outcome relationships (see Table S7).

Discussion

A major motivation for this study was to investigate whether common maternal and familial factors found in IRS settings may increase susceptibility to the health effects of insecticides. Overall, we found that higher maternal serum DDT/E, and particularly *p,p′*-DDE, concentrations were associated with higher rates of persistent fever in their children during their second year of life. Moreover, we found consistent evidence that associations between chemical concentrations and rate of infectious outcomes were stronger among children of mothers living below the poverty line or having insufficient antepartum energy intake. In fact, among children without those susceptibility factors, the associations were weak or absent. Given the high prevalence of these comorbidities in this population (61% and 68%, respectively) and the fact that these factors were rare in previous studies, further replication of our analysis in other low- and middle-income settings is critical: If these are causal susceptibility factors, findings from prior studies, many of which were conducted in Western countries where the prevalence of such susceptibility factors is lower, may not be applicable to populations in which IRS is currently practiced (i.e., where the prevalence of such factors is higher).

Extensive animal evidence suggests several plausible pathways for developmental immunotoxic effects of DDT/E; however, human studies are scarce. In animal studies, postnatal exposure to DDT/E and pyrethroids has been shown to inhibit macrophage activity, response to bacterial infection (Nuñez et al. 2002), and leukocyte proliferation and to increase apoptosis (Misumi et al. 2005). Fetal exposure may specifically impair thymocyte proliferation (Rehana and Rao 1992) and alter Th1/Th2 balance (Santoni et al. 1998). The positive associations between *p,p′*-DDE and fevers, ear infections, and sore throat in this study corroborate past findings in a population of Inuit children exposed to organochlorines *in utero* through maternal diet (Dallaire et al. 2004). Dallaire et al. (2004) found IRRs for all infections, otitis media, and upper respiratory infections at 1 y of age of 1.13 (95% CI: 0.95, 1.34), 1.02 (95% CI: 0.76, 1.35), and 1.30 (95% CI: 0.96, 1.78) when comparing highest to lowest quartile (>472 vs. <183 ng/g lipid) of prenatal *p,p′*-

Table 3. Associations between insecticide biomarker concentrations and rate of persistent fevers, ear infections, and severe sore throats in the second year of life.

	Persistent fevers (lasting ≥4 days)		Ear infections		Severe sore throat	
Exposure	IRR	(95% CI)	IRR	(95% CI)	IRR	(95% CI)
DDT/E ($n = 674$)						
o,p′-DDT	1.10	(0.94, 1.30)	0.83	(0.60, 1.15)	1.53	(0.77, 3.03)
p,p′-DDE	1.21	(1.01, 1.46)	1.03	(0.75, 1.41)	1.78	(0.82, 3.84)
p,p′-DDT	1.14	(0.99, 1.32)	1.06	(0.80, 1.40)	1.58	(0.80, 3.14)
Pyrethroid metabolites ($n = 666$)						
cis-DBCA	1.07	(0.82, 1.39)	0.70	(0.45, 1.08)	0.63	(0.21, 1.87)
cis-DCCA	1.09	(0.82, 1.45)	0.84	(0.50, 1.41)	0.80	(0.14, 4.50)
trans-DCCA	1.11	(0.87, 1.42)	0.84	(0.57, 1.25)	0.86	(0.29, 2.52)
3-PBA	1.06	(0.80, 1.40)	0.71	(0.42, 1.20)	0.68	(0.19, 2.42)

Note: IRRs are given per 10-fold higher pesticide concentration. These were estimated by zero-inflated Poisson regression adjusted for maternal age, marital status, and parity at birth of index child, as well as low energy intake (daily intake <IOM recommendations for late pregnancy, ~12,000 kJ), income below the mid-2013 South African food poverty threshold (monthly income <Rand 386 per household member), HIV status, and child sex. An offset (in days between last child visit and date of outcome assessment) was used to account for the difference in observation time between subjects. The inflation (i.e., excess zeroes) due to underreporting or lack of observation were modeled using maternal age, parity, whether the mother regularly lived with the child at the 2-y visit, number of individuals living in the same home as the index child at the 2-y visit, and the interviewer-rated score of the mother's quality of responses to child health questions. CI, confidence interval; IRR, incidence rate ratio.

Table 4. Effect measure modification of the association between serum DDT/E and rate of persistent fevers, by maternal poverty, low energy intake, HIV status, and exclusive breastfeeding duration and child sex ($n = 674$).

Effect modifier	*o,p′*-DDT		*p,p′*-DDE		*p,p′*-DDT	
Contrast	IRR	(95% CI)	IRR	(95% CI)	IRR	(95% CI)
Household poverty status[a]						
↓ poverty line vs. ↑ poverty line (IRR_{01})	1.18	(0.93, 1.51)	1.16	(0.91, 1.48)	1.18	(0.92, 1.50)
↑ insecticide among ↑ poverty line (IRR_{10})	1.00	(0.73, 1.35)	0.93	(0.69, 1.25)	0.92	(0.73, 1.16)
↑ insecticide among ↓ poverty line (IRR_{11} vs. IRR_{01})	1.07	(0.88, 1.30)	1.31	(1.08, 1.59)	1.18	(1.00, 1.39)
↑ insecticide and ↓ poverty line (IRR_{11})	1.27	(0.89, 1.65)	1.52	(1.08, 1.96)	1.39	(1.00, 1.77)
RERI ($= IRR_{11} - IRR_{10} - IRR_{01} + 1$)	0.09	(−0.31, 0.48)	0.43	(0.03, 0.84)	0.29	(−0.02, 0.60)
Maternal low daily energy intake[b]						
Insufficient vs. sufficient (IRR_{01})	0.88	(0.69, 1.12)	0.85	(0.67, 1.08)	0.87	(0.69, 1.10)
↑ insecticide among sufficient (IRR_{10})	0.81	(0.59, 1.10)	0.96	(0.72, 1.28)	0.89	(0.70, 1.13)
↑ insecticide among insufficient (IRR_{11} vs. IRR_{01})	1.17	(0.96, 1.43)	1.30	(1.07, 1.58)	1.18	(1.01, 1.39)
↑ insecticide and insufficient (IRR_{11})	1.03	(0.73, 1.33)	1.11	(0.80, 1.42)	1.03	(0.75, 1.31)
RERI	0.34	(0.02, 0.66)	0.29	(0.06, 0.65)	0.27	(−0.002, 0.55)
Maternal HIV status						
Negative vs. positive (IRR_{01})	0.63	(0.41, 0.96)	0.62	(0.41, 0.96)	0.63	(0.41, 0.96)
↑ insecticide among negative (IRR_{10})	1.05	(0.89, 1.25)	1.18	(1.00, 1.39)	1.08	(0.94, 1.24)
↑ insecticide among positive (IRR_{11} vs. IRR_{01})	0.97	(0.53, 1.78)	1.21	(0.64, 2.27)	1.19	(0.70, 2.02)
↑ insecticide and positive (IRR_{11})	0.61	(0.14, 1.09)	0.75	(0.22, 1.28)	0.74	(0.27, 1.21)
RERI	−0.07	(−0.48, 0.34)	−0.05	(−0.56, 0.46)	0.04	(−0.37, 0.46)
Exclusive breastfeeding						
<1 month vs. ≥1 month (IRR_{01})	1.10	(0.83, 1.45)	1.09	(0.83, 1.44)	1.08	(0.81, 1.43)
↑ insecticide among ≥1 month (IRR_{10})	1.19	(0.96, 1.46)	1.24	(0.99, 1.55)	1.09	(0.93, 1.28)
↑ insecticide among <1 month (IRR_{11} vs. IRR_{01})	0.96	(0.74, 1.18)	1.15	(0.82, 1.48)	1.24	(0.88, 1.61)
↑ insecticide and <1 month (IRR_{11})	1.06	(0.65, 1.46)	1.25	(0.75, 1.76)	1.34	(0.83, 1.86)
RERI	−0.23	(−0.56, 0.10)	−0.08	(−0.51, 0.36)	0.18	(−0.22, 0.57)
Child Sex						
Female vs. male (IRR_{01})	0.93	(0.70, 1.22)	0.93	(0.72, 1.22)	0.92	(0.70, 1.22)
↑ insecticide among male (IRR_{10})	1.05	(0.87, 1.27)	1.28	(1.03, 1.59)	1.12	(0.94, 1.32)
↑ insecticide among female (IRR_{11} vs. IRR_{01})	1.17	(0.88, 1.46)	1.14	(0.82, 1.47)	1.17	(0.91, 1.42)
↑ insecticide and female (IRR_{11})	1.08	(0.73, 1.43)	1.07	(0.70, 1.44)	1.08	(0.74, 1.42)
RERI	0.11	(−0.20, 0.41)	−0.15	(−0.53, 0.23)	0.04	(−0.24, 0.32)

Note: IRRs are contrasts between levels of the binary effect modifier (e.g., below vs. above poverty line; first row under each subheading; IRR_{01}) or per 10-fold higher pesticide concentration (each of the other rows; IRR_{10}, $IRR_{11vs.01}$ IRR_{11}). These were estimated by zero-inflated Poisson regression adjusted for maternal age, marital status, and parity at birth of index child, as well as low energy intake (daily intake below the IOM and NRC (2009) recommendations for late pregnancy, ~12,000 kJ), income below the mid-2013 South African food poverty threshold (monthly income <Rand 386 per household member), HIV status, and child sex. Additionally, for each effect modifier, including a product term for the respective modifier (e.g., poverty status) and DDT/E concentration. An offset (in days between last child visit and date of outcome assessment) was used to account for the difference in observation time between subjects. The inflation (i.e., excess zeroes) due to underreporting or lack of observation were modeled using maternal age, parity, whether the mother regularly lived with the child at the 2-y visit, number of individuals living in the same home as the index child at the 2-y visit, and the interviewer-rated score of the mother's quality of responses to child health questions. CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; IRR, incidence rate ratio; RERI, relative excess risk due to interaction.

[a]Mid-2013 South African food poverty line = Rand 386 per household member per month.

[b]IOM and NRC (2009) caloric intake recommendations for late pregnancy ~12,000 kJ.

DDE exposure using standard Poisson models. Our findings also corroborate other studies showing positive associations between prenatal DDT/E exposure and child infections. In a Spanish birth cohort, Sunyer et al. (2010) found that each log-unit higher maternal serum DDE concentrations during the first trimester of pregnancy was associated with a 25% higher risk of LRTIs in 14-month-old offspring [RR = 1.25 (95% CI: 1.01, 1.55)]. In a similar, but larger, cohort, Gascon et al. (2012) reported a positive association between maternal first- and second-trimester DDE serum concentrations and LRTI risk [RR = 1.11 per log-unit (95% CI: 1.00, 1.22)]. Conversely, in a Mexican population residing in an area in which DDT was historically applied for malaria control, Cupul-Uicab et al. (2014) did not find strong evidence for association between maternal DDE levels at delivery and LRTI risk in 21-month-old boys. However, we did not evaluate associations with LRTI directly, limiting the comparability with our findings.

A few limitations of our study are worth highlighting. Notably, child infections are difficult to properly capture, given that they may result in subtle symptoms and may be omitted by self-report. We attempted to address this challenge by several means, including restricting outcomes to those of greater severity and duration (i.e., high fever for ≥4 d) and explicitly modeling the potential for missing events. Nonetheless, mismeasurement of the outcome, even in a nondifferential fashion, may have reduced our ability to detect associations. Future studies could consider alternative, objective biomarkers of infection history to validate self-reports. Due to their rapid elimination, urinary pyrethroid metabolite concentrations in samples collected after delivery may reflect exposure in the hospital rather than longer-term (i.e., in-home) exposure. We found *cis*-DCCA, *trans*-DCCA, and 3-PBA to be positively associated with persistent fevers only among predelivery samples, that is most proximal to home exposure, whereas the reverse was true for *cis*-DBCA (see Table S6). However, findings stratified by collection timing were imprecise due to small stratified sample sizes and event counts (see Table S6). This was particularly true for the postpartum stratum ($n = 253$), which had only 30 subjects with any ear infections and 7 with any sore throat events. Differential weight gain, and therefore effective fetal dose, may play a role in explaining observed associations. Because prepregnancy weight is not customarily measured or recorded in our study population, we had no data on pregnancy weight gain. However, adjusting for antepartum weight did not substantially change our estimates (see Table S8).

In addition to novelty as the first study we are aware of which has investigated associations between DDT/E and pyrethroids and child infection in an IRS setting, several strengths of the study population and approach are worth highlighting. First, the VHEMBE study had very high year-to-year retention, with over 91% of all enrolled subjects participating in the 2-y visit (and 94% of all children still alive at 2 y of age). Moreover, strong data collection and quality control protocols meant that very few values (<1%) of variables

included in models were missing among study participants. The study also benefited from state-of-the-art serum DDT/E and urinary pyrethroid metabolite quantification and extensive data collection from questionnaire, medical records, and staff-collected anthropometry. To our knowledge, this study is the first to investigate associations between maternal perinatal pyrethroid metabolites and child infections. With respect to data analyses, results were robust to numerous sensitivity analyses to missing data, loss to follow-up, and variable mismeasurement that were conducted to assess the influence of modeling choices and assumptions on our findings.

The potential long-term effects of *in utero* exposure to DDT/E in low- and middle-income settings where IRS is practiced, including their interaction with maternal and familial factors, such as socioeconomic status (Appleton et al. 2016; Stein et al. 2016; Cakmak et al. 2016), malnutrition (Banerjee 1999; Prescott 2016), and HIV (Ruck et al. 2016; Slogrove et al. 2017) remain poorly studied despite indications that they may increase susceptibility to environmental chemicals. Such interactions are now recognized to be critically important to understanding child health in low- and middle- income countries (Goldstein et al. 2017; DeBoer et al. 2012) because their distributions and effects will differ from high income countries. This study represents one of the first to investigate the interactions between several relevant maternal exposures, including poverty and low energy intake, on the effect of intrauterine insecticide exposure on child health. Specifically, we found that associations with *p,p′*-DDT/E are stronger, and possibly only present, in the context of poverty and maternal low energy intake. Moreover, we found little evidence for associations between pyrethroid metabolites and rates of childhood infections. Finally, our findings lend additional support to the importance of structural or social deprivation to environmentally related child disease burdens.

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