Associations between prenatal exposure to DDT and DDE and allergy symptoms and diagnoses in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), South Africa

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KEYWORDS

DDT, DDE, indoor residual spraying, insecticides, allergies, wheezing, asthma

CONFLICT OF INTEREST STATEMENT

Authors have no conflict of interest to declare.

Highlights

- DDT is used by 9 countries to control malaria via Indoor Residual Spraying (IRS).
- DDT interferes with the immune system but little data is available from IRS areas.
- Maternal serum DDT was associated with wheezing in South African children aged 3.5 years.
- Prenatal DDT exposure may be related to allergy symptoms among children from IRS areas.

ABBREVIATIONS

DDE: Dichlorodiphenyl dichloroethylene

DDT: Dichlorodiphenyl trichloroethane

IgE: Immunoglobulin E

IRS: Indoor residual spraying

ISAAC: International Study of Asthma and Allergies in Childhood

VHEMBE: Venda Health Examination of Mothers, Babies and their Environment

ABSTRACT

Dichlorodiphenyl trichloroethane (DDT) is an organochlorine insecticide that is banned internationally except for use as part of Indoor Residual Spraying (IRS) programs to control malaria. Although animal studies show that DDT and its breakdown product dichlorodiphenyl dichloroethylene (DDE) affect the immune system and may cause allergies, no studies have examined this question in populations where IRS is conducted. The aim of our study was to investigate whether prenatal exposure to DDT and DDE is associated with allergy symptoms and diagnosis among South African children living in an area where IRS is conducted. To accomplish this aim, we used data from the Venda Health Examination of Mothers, Babies, and their Environment (VHEMBE), an ongoing birth cohort study of 752 children born between 2012 and 2013 in the rural Vhembe district of Limpopo, South Africa. We measured maternal peripartum serum concentrations of DDT and DDE, and administered a questionnaire to the caregivers of 658 children when they were 3.5 years of age to collect information on allergy symptoms and diagnoses as well as potential confounders using validated instruments. Using multiple logistic regression models, we found positive association between DDT and DDE serum concentrations and most of the allergy symptoms and diagnoses. Maternal DDT (Odds Ratio [OR]= 1.5 per 10-fold increase, 95% Confidence interval, CI= 1.0, 2.3) and DDE (OR= 1.4, 95% CI= 0.8, 2.4) serum concentrations were most strongly associated with caregiver report of wheezing or whistling in the chest. Concentrations of DDT and/or DDE were also associated with increased odds of children's chests sounding wheezy during or after exercise, itchy rashes coming and going for at least six months, diagnosis of food allergy, and diagnosis of dust or dust mites allergy but confidence intervals crossed the null. Results suggest that prenatal exposure to DDT, and possibly DDE, is associated with elevated odds of wheezing among children.

1. INTRODUCTION

A total of 219 million people were infected with malaria worldwide in 2017, resulting in 435,000 deaths, 92% of which occurred in Africa (1). Indoor Residual Spraying (IRS), the use of insecticides on the interior walls of dwellings, is commonly used as a preventive measure, exposing 100 million people globally, including 64 million in Africa alone (2, 3). Dichlorodiphenyl trichloroethane (DDT), an organochlorine insecticide which was banned in the 1970s in most Western countries, is used for IRS by nine countries, eight of which are in Africa (1). In the Limpopo Province of South Africa, DDT has been used for malaria control since the 1940s (4). As a result, people living in this area are subjected to high levels of exposure to DDT and its main breakdown product, dichlorodiphenyl dichloroethylene (DDE) (2, 3, 5-13). Exposure to pregnant women is of particular concern, given that DDT and DDE cross the placental barrier and expose the developing fetus (14).

Human and animal data suggest that DDT and DDE can modulate the immune system and may increase the risk of allergies. This may occur through endocrine disruption as *p,p'*-DDT (the insecticidal component) and *o,p'*-DDT (a contaminant of the commercial mixture) are estrogenic while *p,p'*-DDE is anti-androgenic (15-20). Epidemiological studies strongly suggest that sex hormones, and in particular estrogens, play an important role in the etiology of allergies, particularly for asthma and allergic rhinitis (21-36). While a higher incidence of asthma, wheeze and rhinitis is observed in young boys, a gender switch occurs around puberty when the incidence becomes substantially higher among females (21, 26, 30, 35, 37-39). Asthma is also generally more severe in adult women than in men (23, 32, 35, 40), the risk of asthma and allergic rhinitis is higher among women experiencing early menarche (36, 41-46) and in multiparous women (47), and asthma symptoms are more severe during the premenstrual period

(48-50). In addition, while menopause appears to play a protective role for asthma (51, 52), postmenopausal hormonal supplementations and/or hormone replacement therapy have detrimental effects in older women (46, 51-55). Finally, pregnant women carrying female fetuses have higher risks of asthma (56-58).

A possible mechanism of action for the impact of DDT and DDE on asthma is through increased sensitization. For instance, a positive correlation was found between placental concentration of *p,p'*-DDE and cord serum Immunoglobulin E (IgE) level in a study conducted in two Slovak regions (59). Another study found strong associations between postnatal exposure to *p,p'*-DDE and increased IgE concentrations (>200 kU/l) among 7-10-year-old children in Germany (60).

Only two human studies have investigated association between prenatal exposure to DDT and allergies. No associations were found between concentrations of *p,p'*-DDT in cord blood or at four years of age and parental report of wheezing and doctor-diagnosed asthma among children aged 6.5 years from Menorca, Spain (61), and concentrations of *p,p'*-DDT in umbilical cord tissues and maternal report of atopic dermatitis among children aged 7 months from Japan (62). A larger number of studies have investigated prenatal exposure to *p,p'*-DDE and found associations with allergy symptoms and diagnoses, primarily among younger children. Three studies conducted in Spain reported that prenatal exposure to *p,p'*-DDE were associated with higher risks of parental report of wheezing and/or asthma at 12-14 months (63), four years (64, 65), and 6.5 years (61). However, associations between prenatal exposure to *p,p'*-DDE and parental report of wheezing and/or asthma were not observed at 6.5, 10 and 14 years in Menorca, Spain (65). Moreover, a Danish study found no association between maternal *p,p'*-DDE concentrations during pregnancy and doctor's or nurse's diagnosis of asthma in their children at

age 20 years (66). Taken together, these results suggest that exposure may affect wheezing and asthma in younger children only.

The only one study investigating associations between child serum DDT concentrations and allergy symptoms or diagnoses found no associations with doctor-diagnosed asthma among three to six-year-old Chinese children (67). Very few studies have investigated associations between postnatal exposure to DDE and allergy symptoms or diagnoses but most studies reported positive associations. Two Chinese studies found positive associations between postnatal *p,p'*-DDE and asthma risk and diagnosis among children aged three to six years, with *p,p'*-DDE being measured concurrently in children's serum and indoor dust, respectively (67, 68). A positive association was also reported between *p,p'*-DDE blood concentrations and odds of parental report of asthma among seven to eight-year-old German children (60).

However, none of the above studies were conducted in countries where DDT is applied for IRS and, to our knowledge, no prior study investigated associations between prenatal exposure to DDT or DDE and non-respiratory allergies. Populations from IRS areas experience high levels of exposure (2, 3, 5-13) and may be more vulnerable to the adverse effects of insecticides due to poverty, poor health, and malnutrition (69-78). The aim of the present study was thus to evaluate whether prenatal exposure to DDT and DDE is associated with elevated odds of allergy symptoms and diagnoses in South African children aged 3.5 years from an area where IRS is conducted annually.

2. METHODS

2.1 Study Participants

The current study used data from the Venda Health Examination of Mothers, Babies, and their Environment (VHEMBE), an ongoing birth cohort study taking place in the Vhembe district of Limpopo Province, South Africa. Women were enrolled in the VHEMBE study between August 2012 and December 2013 when they presented for delivery at Tshilidzini hospital in the town of Thohoyandou. Eligibility criteria included: being at least 18 years of age, speaking Tshivenda (the most commonly spoken language in the Vhembe district) at home, having contractions more than five minutes apart, living within 20 km of the hospital and planning to stay in the area for next two years, not being diagnosed with malaria during pregnancy and giving birth to a live and viable singleton. After the initial screening by study staff, 920 women were found to be eligible. Of these, 752 provided informed consent, completed a baseline questionnaire, and supplied an adequate blood sample for DDT and DDE quantification. Follow up assessments occurred one week as well as one, two and 3.5 years postpartum. Of the 667 children who were followed to age 3.5 years, 658 were accompanied by a primary caregiver who provided information about allergy symptoms and diagnoses.

Informed consent was obtained from all participants prior to data collection. The study was approved by the Institutional Review Boards at the University of California, Berkeley (Berkeley, California), McGill University (Montreal, Quebec, Canada), the University of Pretoria (Pretoria, South Africa), the Limpopo Department of Health and Social Development (Polokwane, South Africa), and the Ethics Committee of Tshilidzini Hospital (Thohoyandou, South Africa).

2.2 Data Collection Procedures

Delivery visit. Trained and bilingual (Tshivenda and English) local staff administered structured questionnaire-based interviews to study participants shortly after delivery and before leaving the hospital. Staff gathered information on maternal sociodemographic characteristics, pregnancy history, and personal habits, food intake, food security and stress during pregnancy. The questionnaire was designed in English, translated into Tshivenda, and back-translated into English by native speakers in the translated language. Food intake was assessed based on a locally-validated food frequency questionnaire (79) and energy intake was estimated by a South African expert nutritionist using the FoodFinder3 software (South Africa Medical Research Council/WAMTechnology, Stellenbosch, South Africa). Low energy intake was defined based on U.S. Institute of Medicine guidelines for pregnant women (80, 81) as described by Huang et al.(70) Recommended energy intake was estimated based on the assumption that mothers were physically active due to the typically arduous tasks of daily life in this population. Food security was ascertained with the U.S. National Center for Health Statistics' Household Food Security Survey (82) and stress was assessed based on a scale developed for the Soweto-based Birth to Twenty Study (83). Poverty was defined as having a household income below the South African mid-2013 food poverty level of 386 Rands/person/month (84). Medical records were abstracted by two registered nurses, who were blind to participants' exposure status. Gestational age at birth was determined based on date of last menstrual period as described in Chevrier et al.(85) and maternal HIV status was ascertained from self-report or use of anti-retroviral drugs based on medical records.

3.5-Year visit. At the 3.5-year follow-up interview, primary caregivers including biological mothers (n = 576), fathers (n = 8), or other caregivers (n = 74) were asked about the children's

allergy symptoms and diagnoses and exposure to allergens since the two-year interview. Questions about allergy symptoms were based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (see Supplementary Table 1) and included information about the prevalence of wheezing or whistling in the chest; chest sounding wheezy during/after exercise; dry cough at night; sneezing or runny or blocked nose; nose problems with itchy-watery eyes; hay fever; itchy rashes; and eczema (86). Questions about wheezing are meant to estimate asthma symptoms, those about nose problems and hay fever relate to allergic rhinoconjuctivitis and those about rashes concern eczema.

In addition, primary caregivers were asked whether the index child was diagnosed by a doctor or a nurse with asthma or allergies to any food, dust or dust mites, pollen, animals or medication. If an asthma diagnosis was reported, staff inquired whether any medicine was prescribed to treat the condition. Finally, we asked caregivers about exposure to allergens since the two-year interview, including the presence of any mold or mildew, cockroaches, rats, mice or other rodents, as well as cats or dogs in the house were the child lived.

2.3 Measurement of DDT and DDE

Maternal blood samples were collected prior to, or shortly after, delivery. Samples were immediately processed and stored at -80^{o}C until shipment on dry ice to analytical laboratories. The Emory University Environmental Health Laboratory (Atlanta, Georgia) measured *p,p'*-DDT and *p,p'*-DDE concentrations in serum using high-resolution gas chromatography-isotope dilution mass spectrometry (87). The limits of detection (LOD) for *p,p'*-DDT and *p,p'*-DDE were 0.01 and 0.03 ng/mL serum, and the limits of quantification (LOQ) were 0.05 and 0.15

ng/mL serum, respectively. Total lipid levels were estimated from triglycerides and total cholesterol measurements by standard enzymatic methods (Roche Chemicals, Indianapolis, Indiana), using the Phillips formula (88).

2.4 Statistical Analysis

We used machine-read values for DDT and DDE concentrations that fell between LOD and LOQ. Values below the LOD were randomly imputed based on a log-normal probability distribution whose parameters were estimated via maximum likelihood estimation (89). DDT and DDE concentrations were lipid-adjusted and expressed in ng/g lipid. To reduce the influence of outliers, serum concentrations of DDT and DDE were log-10 transformed. One-way analysis of variance (ANOVA) was used to examine bivariate associations between categorical variables and DDT or DDE concentrations, and Chi Square tests were used to evaluate relationships between categorical variables. Logistic regression was used to evaluate associations between DDT or DDE and allergy symptoms or diagnoses. Variables suspected to be causal determinants of asthma or allergies were considered as potential confounders and included maternal age (continuous), education (< grade 12, grade 12, > grade 12) and parity; maternal smoking (yes, no), exposure to environmental tobacco smoke (yes, no), alcohol consumption (yes, no), energy intake (sufficient, insufficient), HIV status (positive, negative), number of stressful life events (continuous) and poverty status during pregnancy (poor, not poor); presence of mold or mildew, dogs or cats, cockroaches, and rats, mice or other rodents in the house (yes, no); and child sex and duration of breastfeeding (< 18 months, > 18 months). Variables were included in final models if they were moderately ($p < 0.20$) associated with at least one of the exposures and one

of the outcomes under study. Final models were adjusted for maternal parity, poverty, child sex and report of mold or mildew in the house. We also estimated effect modification by child sex by including a cross-product term. Missing covariate values (<2.1%) were imputed at random based on observed probability distributions.

Associations were only estimated for allergy symptoms or diagnoses with at least 10 cases. These included reports of wheezing or whistling in the chest; sneezing, runny or blocked nose; nose problem with itchy-watery eyes; itchy rash coming and going for at least 6 months; and chest sound wheezy during/after exercise as well as diagnoses of food and dust allergies. To ensure that the method that we selected to correct for serum lipid concentration did not influence our results, all analyses were re-ran by expressing DDT or DDE on a serum volume basis and including triglycerides and total cholesterol concentrations as covariates in the models. Results from this analysis were similar, irrespective of the way the adjustments of lipids were performed (Supplementary Table 2). Analyses were conducted using STATA, version 13.0 (StataCorp LP, College Station, TX, USA).

3. RESULTS

3.1 Participant Characteristics

Table 1 shows participant demographic characteristics. At the time of delivery, most mothers (52.0%) were under 25 years of age and had a low level of education with 56.5% not having completed grade 12 and only 8.2% having pursued a diploma or further degree. Few smoked tobacco (n = 1) or consumed alcohol (n = 37) during pregnancy. Most women (55.9%) were parous before the birth of the study child. Poverty and low energy intake were common in this

population, with 60.6% living below the South African food poverty line and 68.9% having low energy intake based on Institute of Medicine guidelines. About 11.9% of women were HIV positive and 41.2% breastfed their child for more than 18 months.

Slightly more children were boys (51.2%) than girls (48.8%). While, at 7.8%, the prevalence of low birth weight approached that of high-income countries, the rate of preterm birth (12.6%) was high. Exposure to allergens was common among study children, with 79.3% having cockroaches, 43.3% having dogs or cats, 31.6% having rats, mice or other rodents, and 29.2% having mold or mildew in their house.

3.2 Exposure to DDT/E

Table 2 (in ng/g lipid) and Supplementary Table 3 (in pg/g serum) show the distribution of maternal peripartum biomarker concentrations. *p,p'*-DDT (98.2%) and *p,p'*-DDE (100%) were detected in almost all samples and had geometric means of 70.4 ng/g lipids (geometric standard deviation (GSD)=6.7) and 290.4 ng/g lipids (GSD=4.8), respectively. These levels are consistent with recent studies conducted in IRS areas (90, 91) but substantially higher than in non-IRS populations such as in Canada (median DDT<7.8 and DDE=127 ng/g lipid) or the United States (median DDT < 5.1 and DDE 131 ng/g lipid) (92, 93). Biomarkers were strongly intercorrelated ($r=0.85$, $p<0.05$).

3.3 Allergy Symptoms and Diagnoses

Table 3 summarizes children's allergy symptoms and diagnoses as reported by primary

caregivers. The most common allergy symptoms included sneezing or having a runny or blocked nose without a cold or the flu (12.8%); itchy rashes coming and going for at least 6 months (4.9%); wheezing or whistling in the chest (4.7%); and chest sounding wheezy during or after exercise (3.0%). Of those children who experienced wheezing, 20.0% (n=6) had their sleep disturbed for one or more nights per week, 20.0% (n=6) had their speech limited by wheezing and 13.3% had 4 or more attacks since the 2-year visit. As many as 33.7% of children experiencing a runny or blocked nose without a cold also had watery eyes. Only a small percentage of the caregivers reported children having hay fever (1.4%) and eczema (1.2%) at 3.5 years of age. Few children were reportedly diagnosed with allergies to food (3.0%), dust or dust mites (2.0%), pollen (1.2%) or animals (0.3%) and 1.7% were diagnosed with asthma. Table 4 shows univariate associations between outcomes and covariates. Children with low birth weights were less likely to experience sneezing or a runny or blocked nose; insufficient energy intake during pregnancy and poverty were associated with a lower risk of itchy rashes; stress during pregnancy was related to higher risks of wheeze during or after exercise; and mold and mildew in the house was associated with elevated risk of wheezing during or after exercise and of nose problems with itchy-watery eyes.

3.4 Association Between DDT and DDE and Allergy Symptoms and Diagnoses

p,p'-DDT and *p,p'*-DDE were positively associated with most of the allergy symptoms (Table 5). Maternal serum concentrations of *p,p'*-DDT (OR=1.5, 95% CI=1.0, 2.3) and *p,p'*-DDE (OR=1.4, 95% CI= 0.8, 2.4) were most strongly associated with wheezing or whistling in the chest. *p,p'*-DDT was also associated with having an itchy rash coming and going for at least

6 months (OR= 1.4, CI=0.9, 2.1). In addition, concentrations of *p,p'*-DDT (OR=1.3, 95% CI=0.8, 2.3) and *p,p'*-DDE (OR=1.5, 95% CI=0.8, 3.0) were associated with wheezing during or after exercise, but confidence intervals crossed the null. Similarly, there was some evidence that *p,p'*-DDT was associated with diagnosis of food allergy (OR=1.3, CI=0.7, 2.2), and *p,p'*-DDE with diagnosis of dust or dust mites allergy (OR=1.6, CI= 0.7, 3.7) but confidence intervals were wide. Finally, we found no associations between *p,p'*-DDT or *p,p'*-DDE and sneezing or having a runny or blocked nose without a cold, or having nose problems accompanied by itchy-watery eyes, suggesting no relation to allergic rhinoconjuctivitis. We found no evidence of effect modification by sex (data not shown) but statistical power may have been limited by the relatively small number of cases for some outcomes.

4. DISCUSSION

In this study, maternal peripartum serum concentrations of *p,p'*-DDT and *p,p'*-DDE were positively associated with most of the allergy symptoms and diagnoses that we investigated among 3.5-year-old South African children. Evidence was strongest for associations between maternal *p,p'*-DDT and caregiver report of wheezing or whistling in the chest. These results contrast with those of the only prior study of prenatal exposure to *p,p'*-DDT and wheezing and asthma, which found no association with maternal report of doctor diagnosis among children aged 6.5 years in Menorca, Spain (61). However, exposure levels were low and the study had a moderate sample size (n=241), which may have adversely affected statistical power. Differences in susceptibility to exposure and developmental timing of assessments between the present analysis and the Spanish study may also explain the different results. We also found some

evidence that prenatal exposure to *p,p'*-DDE may be associated with caregiver report of chest sounding wheezy during or after exercise and dust or dust mites allergy diagnosis but associations were imprecise, likely due to the small number of cases. These results are consistent with those of prior studies that found positive associations between prenatal exposure to *p,p'*-DDE and wheezing and/or asthma among children aged one to 6.5 years (61, 63-65).

Despite our finding of an association between *p,p'*-DDT and wheezing, at 4.7%, the prevalence of this condition remains low in the VHEMBE populations. Data among children with ages similar to that of VHEMBE children are scarce but, as a comparison, the 12-month prevalence of wheezing among six to seven-year-olds was reported to be 11.5% globally (94) and 11.2% in Polokwane, a city located in the same province as our study site (Limpopo) where IRS is not conducted (95). This, combined with the fact that populations from rural areas generally have a lower prevalence of wheezing and asthma than urban populations, suggests that factors other than insecticide exposure may play a larger etiological role. Yet, our findings may have important implications given the high burden of respiratory allergies in Africa. About 50 million Africans are asthmatic and the highest prevalence on the continent is found in South Africa (96). The prevalence of asthma and wheezing among children is on rise in many low-and-middle-income countries including South Africa (97), which ranks fifth in the world for asthma case fatality rates with 18.5 deaths per 100,000 asthmatics (96). In addition, more severe symptoms of respiratory allergies have been reported among black African children compared to children from high income countries, possibly due to limited access to health care, lower health care quality and insufficient income to purchase medication (98, 99). Consequently, any factor increasing respiratory allergy symptoms could have a notable impact on the health of African children.

It is also noteworthy that, though prior studies generally found associations between prenatal

exposure to *p,p'*-DDE and wheezing in young children, studies conducted in children older than 6.5 years generally found no association. This could be due to the fact that younger children are more susceptible to the impact of prenatal exposure. Because wheezing can also be caused by respiratory infections, which are more common in younger children, it is also possible that the association that we report may be due to an impact of exposure on infections. This hypothesis is supported by our prior finding that prenatal exposure to DDT and DDE was associated with elevated rates of persistent fever among VHEMBE children at age 2 (70).

Our study has several strengths. This is the first study to examine association between prenatal *p,p'*-DDT and *p,p'*-DDE exposure and allergy symptoms or diagnoses in children from an area where DDT is applied to control malaria. This is also the first study to investigate associations between maternal *p,p'*-DDT or *p,p'*-DDE and several allergy outcomes including skin rashes, food allergy and dust allergy. Retention rate was high in this study, with 88% of enrolled participants being followed from birth through 3.5 years of age. In addition, at 98.2%, the detection frequency for *p,p'*-DDT was higher than in prior studies, which allowed us to investigate associations with this compound. We also had detailed and nearly complete information on potential confounders including maternal demographics, lifestyle, diet, and HIV status during pregnancy as well as child exposure to allergens.

Our study also has some limitations. For instance, we had no information on postnatal exposure to *p,p'*-DDT or *p,p'*-DDE. However, in a subsample of VHEMBE participants (n=47), we found that the maternal serum concentration of these chemicals was highly correlated with levels measured in breast milk and child serum at 12 and 24 months (r= 0.87 to 0.98) (100), and so it may not be possible to tease out the effects of prenatal and postnatal exposure in this population. Moreover, outcomes were based on caregiver report, which may lead to misclassification.

However, because participants were blinded to the research questions, such misclassification would not be expected to be differential relative to exposure and thus should not explain the positive associations that we observed. Although wheezing may partly reflect respiratory infections in young children, the validity of our results is strengthened by the fact that outcomes were determined based on the ISAAC questionnaire, which was previously used and validated in multiple countries including South Africa (95, 101-105). Nonetheless, the possibility of a chance finding or that findings may be due to uncontrolled confounding cannot be dismissed. Finally, the relatively small number of cases for some outcomes may have limited statistical power and resulted in violation of the positivity assumption.

5. Conclusions

In summary, we found an association between maternal peripartum serum concentrations of *p,p'*-DDT and wheezing among 3.5-year-old South African children residing in an area where DDT is used annually for IRS to control malaria. This is the first study to report associations between prenatal exposure to *p,p'*-DDT and wheezing in children. Further research is needed to confirm our results and to determine whether associations persist at later ages.

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Table 1: Maternal peripartum serum *p,p'*-DDE and *p,p'*-DDT concentrations (ng/g lipid) by demographic characteristics among participants in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), Limpopo, South Africa (n=658)

			p,p'-DDE			*p,p'*-DDT		
Characteristic	n [a]	%[a]	GM[b]	GSD[c]	P-value	GM[b]	GSD[c]	P-value
Child Characteristics								
Sex					0.1			0.03
Boy	337	51.2	262.1	4.8		60.3	6.7	
Girl	321	48.8	323.4	4.8		82.9	6.7	
Preterm (<37 weeks)					0.9			0.4
No	575	87.4	291.5	4.8		72.1	6.7	
Yes	83	12.6	282.6	4.9		59.9	6.7	
Low birth weight (<2500g)					0.3			0.2
No	607	92.2	296.3	4.8		72.6	6.9	
Yes	51	7.8	228.7	4.5		49.5	4.6	
Maternal Characteristics								
Age (Years)					0.4			0.2
<25	342	52.0	303.1	5.0		67.8	7.1	
25-35	240	36.5	261.4	4.5		66.4	5.7	
>35	76	11.5	334.0	4.9		100.8	8.8	
Education					0.3			0.3
< 12th Grade	372	56.5	284.5	4.9		72.3	6.8	
Grade 12	192	29.2	332.3	4.8		79.3	6.7	
> Grade 12 or further studies started	40	6.1	203.9	4.6		45.7	6.3	
Diploma or further degree	54	8.2	268.8	4.3		53.1	6.6	
Exposure to environmental tobacco smoke					0.7			0.8
No	411	62.6	284.7	5.0		69.0	6.9	
Yes	245	37.4	298.4	4.5		72.0	6.5	
Smoking during pregnancy					0.5			1.0
No	657	99.9	289.9	4.8		70.4	6.7	
Yes	1	0.1	815.2	1.0		70.7	1.0	
Alcohol during pregnancy					0.3			0.5

No	621	94.4	286.3	4.9		69.6	6.7	
Yes	37	5.6	367.5	3.7		86.9	6.7	
Parity					0.01			0.8
0	290	44.1	353.0	5.2		68.9	7.4	
1	177	26.9	231.5	4.5		67.1	5.2	
≥2	191	29.0	266.3	4.5		76.2	7.2	
Energy Intake during pregnancy					0.2			0.5
Sufficient	201	31.1	254.7	4.5		64.7	5.9	
Insufficient	445	68.9	306.4	5.0		72.9	7.2	
HIV status					0.9			0.9
HIV Negative	577	88.1	289.5	4.9		70.7	6.9	
HIV Positive	78	11.9	293.1	4.4		68.5	5.9	
Number of Stress Sources					0.9			0.8
0-2	449	68.2	289.5	4.9		69.4	6.9	
>2 (3-9)	209	31.8	292.2	4.6		72.8	6.5	
Poverty status					0.5			0.1
Above poverty level	259	39.4	276.8	4.5		60.5	6.9	
Below poverty level	399	60.6	299.6	5.0		77.7	6.6	
Duration of breastfeeding					0.4			0.8
≤ 18 months	383	58.8	303.0	4.8		70.0	6.7	
> 18 months	268	41.2	275.8	4.8		72.7	6.7	
Duration of exclusive breastfeeding					0.6			0.6
≤2 months	371	56.6	282.9	4.8		67.8	7.0	
>2 months	284	43.4	300.7	4.9		73.4	6.4	
<u>Household Characteristics</u>								
Mold or mildew in the house					0.02			0.01
No	466	70.8	264.1	4.8		62.6	6.7	
Yes	192	29.2	365.7	4.6		93.9	6.7	
Dogs or cats in the house					0.6			0.6
No	373	56.7	281.6	4.6		68.3	6.8	
Yes	285	43.3	302.3	5.1		73.3	6.7	
Cockroaches in the house					0.2			0.2
No	136	20.7	332.5	4.1		83.9	6.7	

Yes	522	79.3	280.3	5.0		67.3	6.7	
Rats, mice or other rodents in the house					0.4			0.2
No	450	68.4	281.4	4.6		66.0	6.0	
Yes	208	31.6	310.8	5.2		81.1	8.4	

Abbreviations: DDT = dichlorodiphenyltrichloroethane, DDE = dichlorodiphenyldichloroethylene

[a] Totals may not add to 658 due to missing data. Percentages may not add to 100% due to rounding.

[b] GM = Geometric Mean

[c] GSD = Geometric Standard Deviation

Table 2: Maternal peripartum serum *p,p'*-DDT and *p,p'*-DDE (ng/g lipids) concentrations among participants in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), Limpopo, South Africa

	N	Detection Frequency (%)	Quantification Frequency (%)	GM[a]	GSD[b]	Min	Percentiles				Max
							25th	50th	75th	90th	
p,p'-DDT	658	98.2	90.6	70.4	6.7	<LOD[c]	18.6	57.2	266.1	1011.0	15027.6
p,p'-DDE	658	100	97.4	290.4	4.8	4.0	92.3	249.5	855.5	2673.6	22613.4

[a] GM = Geometric Mean
[b] GSD = Geometric Standard Deviation
[c] LOD = Limit of Detection

Table 3: Summary of allergy symptoms and diagnoses among 3.5 years old children in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), Limpopo, South Africa (n=658)

	N	%
Allergy Symptoms		
Wheezing or whistling in the chest		
Yes	31	4.7
No	627	95.3
If yes, number of attacks of wheezing		
1 to 3	26	86.7
4 to 12	1	3.3
More than 12	3	10.0
If yes, frequency of sleep disturbed by wheezing		
Never woken with wheezing	11	36.7
Less than one night per week	13	43.3
One or more nights per week	6	20.0
If yes, speech limited by wheezing		
Yes	6	20.0
No	24	80.0
Chest sounds wheezy during or after exercise		
Yes	20	3.0
No	636	96.7
Don't Know	2	0.3
Dry cough at night		
Yes	11	1.7
No	644	98.2
Don't Know	1	0.1
Sneezing, runny or blocked nose		
Yes	84	12.8
No	573	87.1
Don't Know	1	0.1
If yes, nose problem accompanied by itchy-watery eyes		
Yes	28	33.7
No	55	66.3

Hay fever		
Yes	9	1.4
No	646	98.3
Don't Know	2	0.3
Itchy rash coming and going for at least 6 months		
Yes	32	4.9
No	625	95.0
Don't Know	1	0.1
If yes, has rash cleared?		
Yes	27	84.4
No	5	15.6
Eczema		
Yes	8	1.2
No	650	98.8
Allergy Diagnoses		
Asthma		
Yes	11	1.7
No	647	98.3
If yes, asthma medicine prescribed		
Yes	6	60.0
No	4	40.0
Food allergy		
Yes	19	3.0
No	635	97.0
Dust or dust mite allergy		
Yes	13	2.0
No	644	98.0
Pollen allergy		
Yes	8	1.2
No	650	98.8
Animal allergy		
Yes	2	0.3
No	656	99.7

Medication allergy		
Yes	1	0.2
No	657	99.8

Note: Totals may not sum to 658 due to missing data.

Table 4: Allergy Symptoms and Diagnoses by demographic characteristics among participants in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), Limpopo, South Africa (n=658)

Characteristic	N	%	Allergy Symptoms: Wheezing or whistling in the chest	Chest sounds wheezy during or after exercise	Sneezing runny or blocked nose	Nose problem with itchy-watery eyes	Itchy rash coming and going for at least 6 months	Allergy Diagnosis: Food allergy	Dust or dust mite allergy
			N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Child Characteristics									
Sex									
Boy	337	51.2	19 (5.6)	12 (3.6)	42 (12.5)	16 (4.7)	12 (3.6)	6 (1.8)	5 (1.5)
Girl	321	48.8	12 (3.7)	8 (2.5)	42 (13.1)	12 (3.7)	20 (6.2)	13 (4.0)	8 (2.5)
Preterm (<37 weeks)									
No	575	87.4	25 (4.3)	17 (2.9)	75 (13.0)	22 (3.8)	25 (4.3)	17 (2.9)	11 (1.9)
Yes	83	12.6	6 (7.2)	3 (3.6)	9 (10.8)	6 (7.2)	7 (8.4)	2 (2.4)	2 (2.4)
Low birth weight (<2500g)									
No	607	92.2	27 (4.4)	18 (3.0)	82 (13.5)*	27 (4.4)	29 (4.8)	19 (3.1)	12 (2.0)
Yes	51	7.8	4 (7.8)	2 (3.9)	2 (3.9)	1 (2.0)	3 (5.9)	0 (0.0)	1 (2.0)
Breastfeeding duration									

≤ 18 months	383	58.8	19 (5.0)	12 (3.1)	53 (13.8)	18 (4.7)	21 (5.5)	8 (2.1)	11 (2.9)
> 18 months	268	41.2	11 (4.1)	7 (2.6)	31 (11.6)	10 (3.7)	11 (4.1)	11 (4.1)	2 (0.7)
Maternal Characteristics									
Age (Years)									
<25	342	52.0	17 (5.0)	11 (3.2)	47 (13.7)	12 (3.5)	17 (5.0)	10 (2.9)	5 (1.5)
25-35	240	36.5	11 (4.6)	7 (2.9)	26 (10.8)	12 (5.0)	12 (5.0)	8 (3.3)	7 (2.9)
>35	76	11.5	3 (3.9)	2 (2.6)	11 (14.5)	4 (5.3)	3 (3.9)	1 (1.3)	1 (1.3)
Education									
< Grade 12	372	56.5	21 (5.6)	9 (2.4)	44 (11.8)	14 (3.8)	15 (4.0)	10 (2.7)	6 (1.6)
Grade 12	192	29.2	9 (4.7)	9 (4.7)	26 (13.5)	10 (5.2)	11 (5.7)	7 (3.6)	5 (2.6)
> Grade 12	40	6.1	0 (0.0)	0 (0.0)	6 (15.0)	2 (5.0)	2 (5.0)	1 (2.5)	0 (0.0)
Diploma or further degree	54	8.2	1 (1.8)	2 (3.7)	8 (14.8)	2 (3.7)	4 (7.4)	1 (1.8)	2 (3.7)
Exposure to environmental tobacco smoke									
No	411	62.6	21 (5.1)	10 (2.4)	45 (10.9)	14 (3.4)	20 (4.9)	12 (2.9)	10 (2.4)
Yes	245	37.4	10 (4.1)	10 (4.1)	39 (15.9)	14 (5.7)	12 (4.9)	7 (2.8)	3 (1.2)
Smoking during pregnancy									
No	657	99.9	31 (4.7)	20 (3.0)	83 (12.6) *	27 (4.1)*	32 (4.9)	19 (2.9)	12 (1.8)*
Yes	1	0.1	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
Alcohol during pregnancy									
No	621	94.4	28 (4.5)	18 (2.9)	80 (12.9)	27 (4.3)	30 (4.8)	19 (3.0)	11 (1.8)

Yes	37	5.6	3 (8.1)	2 (5.4)	4 (10.8)	1 (2.7)	2 (5.4)	0 (0.0)	2 (5.4)
Parity									
0	290	44.1	15 (5.2)	10 (3.4)	40 (13.8)	14 (4.8)	14 (4.8)	9 (3.1)	6 (2.1)
1	177	26.9	4 (2.3)	3 (1.7)	20 (11.3)	3 (1.7)	9 (5.1)	3 (1.7)	3 (1.7)
≥2	191	29.0	12 (6.3)	7 (3.7)	24 (12.6)	11 (5.8)	9 (4.7)	7 (3.7)	4 (2.1)
Energy intake during pregnancy									
Sufficient	201	31.1	9 (4.5)	7 (3.5)	31 (15.4)	13 (6.5)	16 (8.0)*	7 (3.5)	4 (2.0)
Insufficient	445	68.9	22 (4.9)	13 (2.9)	52 (11.7)	15 (3.4)	16 (3.6)	12 (2.7)	9 (2.0)
HIV status									
HIV Negative	577	88.1	28 (4.8)	17 (2.9)	71 (12.3)	22 (3.8)	26 (4.5)	19 (3.3)	11 (1.9)
HIV Positive	78	11.9	3 (3.8)	3 (3.8)	13 (16.7)	6 (7.7)	6 (7.7)	0 (0.0)	2 (2.6)
Number of Stress Sources									
0-2	449	68.2	18 (4.0)	10 (2.2)	51 (11.3)	15 (3.3)	21 (4.7)	11 (2.4)	9 (2.0)
>2	209	31.8	13 (6.2)	10 (4.8)	33 (15.8)	13 (6.2)	11 (5.3)	8 (3.8)	4 (1.9)
Household Characteristics									
Income									
≥ poverty	259	39.4	8 (3.1)	6 (2.3)	34 (13.1)	10 (3.9)	18 (6.9)*	7 (2.7)	7 (2.7)
< poverty	399	60.6	23 (5.8)	14 (3.5)	50 (12.5)	18 (4.5)	14 (3.5)	12 (3.0)	6 (1.5)
Mold or mildew in the house									
No	466	70.8	19 (4.1)	10	53 (11.4)	13 (2.8)*	22 (4.7)	11 (2.4)	7 (1.5)

				(2.1)*					
Yes	192	29.2	12 (6.2)	10 (5.2)	31 (16.1)	15 (7.8)	10 (5.2)	8 (4.2)	6 (3.1)
Dogs or cats in the house									
No	373	56.7	15 (4.0)	11 (2.9)	43 (11.5)	17 (4.5)	19 (5.1)	11 (2.9)	7 (1.9)
Yes	285	43.3	16 (5.6)	9 (3.1)	41 (14.4)	11 (3.8)	13 (4.5)	8 (2.8)	6 (2.1)
Cockroaches in the house									
No	136	20.7	5 (3.7)	1 (0.7)	8 (5.9)*	3 (2.2)	8 (5.9)	4 (2.9)	5 (3.7)
Yes	522	79.3	26 (5.0)	19 (3.6)	76 (14.5)	25 (4.8)	24 (4.6)	15 (2.9)	8 (1.5)
Rats, mice or other rodents in the house									
No	450	68.4	20 (4.4)	11 (2.4)	56 (12.4)	18 (4.0)	24 (5.3)	10 (2.2)	8 (1.8)
Yes	208	31.6	11 (5.3)	9 (4.3)	28 (13.5)	10 (4.8)	8 (3.8)	9 (4.3)	5 (2.4)

* P-values <0.05

Note: Totals may not sum to 658 due to missing data

Table 5: Association between maternal peripartum serum *p,p'*-DDE and *p,p'*-DDT concentrations (ng/g lipids) and allergy symptoms and diagnoses among participants in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), Limpopo, South Africa (n=658)

		p,p'-DDE (log-10 transformed)		*p,p'*-DDT (log-10 transformed)	
	No of cases	Unadjusted OR[b] (95%CI[c])	Adjusted[a] OR[b] (95%CI[c])	Unadjusted OR[b] (95%CI[c])	Adjusted[a] OR[b] (95%CI[c])
Allergy Symptoms					
Wheezing or whistling in the chest	31	1.4 (0.8, 2.4)	1.4 (0.8, 2.4)	1.5 (1.0, 2.3)	1.5 (1.0, 2.3)
Chest sounds wheezy during or after exercise	20	1.6 (0.8, 3.0)	1.5 (0.8, 3.0)	1.4 (0.8, 2.4)	1.3 (0.8, 2.3)
Sneezing, runny or blocked nose	84	1.0 (0.7, 1.4)	1.0 (0.7, 1.3)	1.0 (0.8, 1.3)	1.0 (0.7, 1.3)
Nose problem with itchy-watery eyes	28	1.1 (0.6, 2.0)	1.1 (0.6, 1.9)	1.2 (0.8, 1.9)	1.1 (0.7, 1.8)
Itchy rash coming and going for at least 6 months	32	1.1 (0.7, 1.9)	1.1 (0.6, 1.9)	1.4 (0.9, 2.1)	1.4 (0.9, 2.1)
Allergy Diagnoses					
Food allergy	19	1.2 (0.6, 2.4)	1.1 (0.6, 2.3)	1.4 (0.8, 2.4)	1.3 (0.7, 2.2)
Dust or dust mites allergy	13	1.7 (0.8, 3.7)	1.6 (0.7, 3.7)	1.1 (0.6, 2.1)	1.1 (0.5, 2.1)

[a]Adjusted for child sex, parity, household poverty, and mold or mildew in the house.
[b]OR= Odds Ratio
[c]CI= Confidence Interval

Supplementary Tables

Supplementary Table 1: Questions adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire

1. “Since [CHILD NAME]’s last study visit, has [s/he] had wheezing or whistling in the chest?”

    If Yes-
    a. “Since [CHILD NAME]’s last study visit, how many attacks of wheezing has [s/he] had?”
    b. “Since [CHILD NAME]’s last study visit, how often, has [his/her] sleep been disturbed due to wheezing?”
    c. “Since [CHILD NAME]’s last study visit, has wheezing ever been severe enough to limit [his/her] speech to only one or two words at a time between breaths?”

2. “Since [CHILD NAME]’s last study visit, has [his/her] chest sounded wheezy during or after exercise?”

3. “Since [CHILD NAME]’s last study visit, has [s/he] had a dry cough at night, apart from a cough associated with a cold or chest infection?”

4. “Since [CHILD NAME]’s last study visit, has [s/he] had a problem with sneezing, or a runny, or blocked nose when [s/he] did not have a cold or the flu?”

    If Yes-
    a. “Since [CHILD NAME]’s last study visit, has this nose problem been accompanied by itchy-watery eyes?”
    b. “Since [CHILD NAME]’s last study visit, in which months did this nose problem occur?”
    c. “Since [CHILD NAME]’s last study visit, how much did this nose problem interfere with [his/her] daily activities?”

5. “Has [CHILD NAME] ever had hay fever?”

6 “Since [CHILD NAME]’s last study visit, has [s/he] had an itchy rash which was coming and going for at least 6 months in any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?”

    If Yes-

a. “Since [CHILD NAME]’s last study visit, has this rash cleared completely at any time?”

7. “Since [CHILD NAME]’s last study visit, has [s/he] had eczema?”

Supplementary Table 2: Association between maternal peripartum *p,p'*-DDE and *p,p'*-DDT serum concentrations (pg/g serum) and allergy symptoms and diagnoses among participants in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), Limpopo, South Africa (n = 658)

		p,p'-DDE (log-10 transformed)	*p,p'*-DDT (log-10 transformed)
	No of cases	Adjusted[a] OR[b] (95%CI[c])	Adjusted[a] OR[b] (95%CI[c])
Allergy Symptoms			
Wheezing or whistling in the chest	31	1.4 (0.8, 2.4)	1.5 (1.0, 2.3)
Chest sound wheezy during or after exercise	20	1.6 (0.8, 3.0)	1.4 (0.8, 2.3)
Sneezing, runny or blocked nose	84	1.0 (0.7, 1.3)	1.0 (0.7, 1.3)
Nose problem with itchy-watery eyes	28	1.1 (0.6, 1.9)	1.2 (0.7, 1.9)
Itchy rash coming and going for at least 6 months	32	1.1 (0.6, 1.9)	1.3 (0.9, 2.1)
Allergy Diagnoses			
Had doctor or nurse diagnosed with food allergy	19	1.2 (0.6, 2.3)	1.3 (0.7, 2.3)
Had doctor or nurse diagnosed with dust/dust mites allergy	13	1.5 (0.7, 3.5)	1.0 (0.5, 2.0)

[a]Adjusted for child sex, parity, household poverty, presence of mold or mildew in the house, triglyceride, and cholesterol.
[b]OR= Odds Ratio
[c]CI= Confidence Interval

Supplementary Table 3: Maternal peripartum serum *p,p'*-DDT and *p,p'*-DDE (pg/g serum) concentrations among participants in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), Limpopo, South Africa

	N	Detection Frequency (%)	Quantification Frequency (%)	GM[a]	GSD[b]	Min	Percentiles				Max
							25th	50th	75th	90th	
p,p'-DDT	658	98.2	90.6	490.8	6.7	1.0	135.1	391.2	1887.2	6436.3	84953.8
p,p'-DDE	658	100	97.4	2023.3	4.8	31.7	635.2	1661.2	6394.0	19303.6	177182.6

[a] GM = Geometric Mean
[b] GSD = Geometric Standard Deviation
[c] LOD = Limit of Detection