Maternal exposure to pyrethroid insecticides during pregnancy and respiratory allergy symptoms among children participating in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE)

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Abstract

Background: Pyrethroid insecticides use for indoor residual spraying (IRS) in malaria-endemic areas results in high levels of exposure to local populations. Pyrethroids may cause asthma and respiratory allergies but no prior study has investigated this question in an IRS area.

Methods: We measured maternal urinary concentrations of pyrethroid metabolites (*cis*-DBCA, *cis*-DCCA, *trans*-DCCA, 3-PBA) in samples collected at delivery from 751 mothers participating in the Venda Health Examination of Mothers, Babies, and their Environment (VHEMBE), a birth cohort study based in Limpopo, South Africa. At 3.5-year and 5-year follow-up visits, caregivers of 647 and 620 children, respectively, were queried about children's respiratory allergy symptoms based on validated instruments. We applied marginal structural models for repeated outcomes to estimate associations between biomarker concentrations and asthma diagnosis as well as respiratory allergy symptoms at ages 3.5 and 5 years.

Results: We found that a10-fold increase in maternal urinary *cis*-DCCA, *trans*-DCCA and 3-PBA concentrations were associated with more than a doubling in the risk of doctor-diagnosed asthma (*cis*-DCCA: RR=2.1, 95% CI = 1.3, 3.3; *trans*-DCCA: RR=2.1, 95% CI = 1.1, 3.9; 3-PBA: RR=2.4, 95% CI = 1.0, 5.8) and an about 80% increase in the risk of wheezing or whistling in the chest (*cis*-DCCA: RR=1.8, 95% CI = 1.1, 3.0; *trans*-DCCA: RR=1.7, 95% CI = 1.1, 2.6; 3-PBA: RR=1.8, 95% CI = 1.0, 3.3) and suspected asthma (*cis*-DCCA: RR=1.8, 95% CI = 1.1, 3.1; *trans*-DCCA: RR=1.8, 95% CI = 1.1, 2.8). We also observed that higher concentrations of *cis*-DBCA and 3-PBA were related to increases in the risks of dry cough at night (RR=3.5, 95% CI = 1.3, 9.5) and seasonal rhinoconjunctivitis (RR=2.0, 95% CI = 1.1, 3.9), respectively.

Conclusion: Maternal exposure to pyrethroids may increase the risk of asthma and other respiratory allergy symptoms among preschool children from an IRS area.

Keywords

Indoor residual spraying; Insecticides; Pyrethroids; Asthma; Respiratory allergies; Wheezing

Abbreviations

3-PBA, 3-phenoxybenzoic acid; 4-F-3-PBA, 4-fluoro-3-phenoxybenzoic acid; CCCEH, Columbia Center for Children's Environmental Health; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; CI, Confidence interval; DAG, Directed acyclic graph; GPS, Generalized propensity score; HOME, Health Outcomes and Measures of the Environment; ICC, Intraclass Correlation Coefficient; IFN, Interferon; IgE, Immunoglobulin; INSPQ, Institut National de Santé Publique du Québec; IOM, Institute of Medicine; IPCW, Inverse probability of censoring weights; IPTW, Inverse probability of treatment weights; IRS, Indoor Residual Spraying; ISAAC, International Study of Asthma and Allergies in Childhood; LOD, Limit of detection; LOQ, Limit of Quantification; Min, Minimum; Max, Maximum; OR, Odds Ratio; p_{inter},P-value for interaction; RR, Risk Ratio; r_s,Spearman's correlation coefficient; SD, Standard deviation; SG, Specific Gravity; TH2, T-helper 2; VHEMBE, Venda Health Examination of Mothers, Babies and their Environment

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1. Introduction

About 334 million people worldwide suffer from asthma [1], the most prevalent chronic respiratory disease in children and adolescents globally [1-3]. Asthma is a complex airway disease characterized by its multifaceted nature, displaying a diverse range of clinical variations and triggers. Although the prevalence of asthma and respiratory allergies in children has been reported to be stable or decreasing over the last decades in developed countries, increasing trends have been reported in low- and middle- income countries including South Africa [4-7], where reports of wheezing, increased from 16% to 21% between 1995 and 2017 [8] compared to a global prevalence of 14% [1, 9]. Despite substantial reductions in asthma mortality globally over the past decade, South Africa has the fifth largest case mortality rate in the world with an estimated 18.5 deaths per 100,000 asthma cases (2,315 deaths in 2017) [10]. Prenatal exposure to insecticides may contribute to such trends, most particularly in malaria-endemic areas where indoor residual spraying (IRS) – the use of insecticides on interior walls of dwellings – results in high exposure [11-13].

Pyrethroids are one of the most commonly used insecticides for IRS as well as in agriculture and retail products [11]. In South Africa's Limpopo province, a malaria-endemic region, DDT or the pyrethroids deltamethrin or cypermethrin are applied annually on the interior walls and eaves of homes at the start of the rainy season (October to April). Pyrethroids, such as lambda-cyhalothrin, have been shown to cause asthma or respiratory allergy symptoms such as chest congestion and severe coughing in rats when injected intraperitoneally [14]. These insecticides may cause such symptoms by interfering with estrogens [15, 16], which play an important role in the etiology of allergies [17, 18].

While we are aware of only five studies that investigated associations between postnatal exposure to pyrethroids and asthma or respiratory allergy symptoms [19-23], fewer studies have

explored relations with prenatal exposure. A study conducted among New York African-American and Dominican mother-child pairs participating in the Columbia Center for Children's Environmental Health (CCCEH) birth cohort (n=338) found that *cis*- (OR=1.30 per log unit; 95% Confidence Interval [95% CI] = 1.03, 1.56) and *trans*- (OR=1.20 per log unit, 95% CI = 0.93, 1.43) permethrin levels measured in mothers' personal air samples during pregnancy were associated with elevated odds of cough in children at 2 to 5 years of age [24]. Positive associations with wheeze (*cis*-permethrin: OR=1.10; 95% CI = 0.86, 1.33; *trans*-permethrin: OR=1.10; 95% CI = 0.88, 1.40) were reported in the same study, though confidence intervals included the null [24]. In another study conducted among 224 mother-child pairs from the CCCEH cohort, positive but imprecise trends were also found between permethrin personal air concentrations and cough (OR=1.50; 95% CI = 0.80, 3.00), whereas null associations were reported with asthma (OR = 1.00; 95% CI = 0.50, 2.00) among children aged 5 to 6 years [22]. Similarly, in a Costa Rican study (n=303), maternal urinary pyrethroid metabolite (3-PBA; 3-phenoxybenzoic acid) concentrations were associated with increased odds of cough (OR=1.15; 95% CI = 0.67, 1.96) and wheeze (OR=1.22; 95% CI = 0.64, 2.27) but lower odds of asthma (OR = 0.60; 95% CI = 0.23, 1.38) among 5-year old children; however, confidence intervals crossed the null. In the same study, maternal urinary pyrethroid metabolite (DCCA; 3-(2,2-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid) concentrations were related to lower risks of asthma (OR=0.21; 95% CI = 0.05, 0.62) [23]. Finally, null associations were reported between maternal 3-PBA and wheeze among all 8-year old U.S. children participating in the Health Outcomes and Measures of the Environment (HOME) birth cohort (n=367) [25].

Prior studies had moderate sample sizes, thereby limiting their statistical power and did not consider time-varying confounders such as exposure to allergens. Two out of the four studies

measured pyrethroids using air samplers and thus could not account for dietary and dermal exposure. These studies also had relatively low detection frequencies ($\sim 40\%$). In addition, no prior study has been conducted in populations residing in an area where IRS is conducted where poverty, malnutrition and poor health may increase susceptibility to the toxic effects of insecticides. While we previously assessed associations with DDT [26], the objective of this study was therefore to determine whether exposure to maternal urinary pyrethroid metabolites is associated with increased risk of asthma and respiratory allergies among South African children residing in a malaria-endemic area where IRS is currently practiced, while taking time-varying confounding into account.

2. Methods

2.1 Study population

Pregnant women were recruited in the VHEMBE study when they presented for delivery between August 2012 and December 2013 at Tshilidzini hospital in the Vhembe district of Limpopo Province, South Africa. Eligible women were at least 18 years old, spoke Tshivenda at home, lived within 20 km of the hospital, had no intention of moving outside of the area in the following 2 years, were not infected with malaria during pregnancy, had contractions at least 5 minutes apart and delivered a live singleton infant. Study staff approached 1,649 mothers, 920 of whom met eligibility criteria. Of the eligible women, 751 provided informed consent and completed a baseline questionnaire, and 738 women provided urine samples for pyrethroid metabolites quantification. Additional contacts included a home visit 1 week postpartum and field office visits at 1, 2, 3.5 and 5 years. We followed 647 and 620 children through to ages 3.5 and 5 years, respectively, and a total of 657 children attended at least one of these visits.

A small number of caregivers did not know if children had experienced some outcomes at the 3.5-year (wheezing during exercise, n=1; hay fever, n=1) and 5-year visits (wheezing, n=1; wheezing during exercise, n=1; dry cough at night = 1; sneezing, runny or blocked nose, n=1; nose problems with itchy-watery eyes, n=1; hay fever, n=2; doctor-diagnosed asthma, n=1 and doctor's prescription for asthma medication, n=1) leaving sample sizes ranging between 646 and 647 at 3.5 years) and 618 and 619 (at 5 years) for the analyses. Ethics approval for the VHEMBE study was obtained from McGill University (Montreal, Quebec, Canada), the University of Pretoria (Pretoria, Gauteng, South Africa), Tshilidzini Hospital (Thohoyandou, Limpopo, South Africa), the Limpopo Department of Health and Social Development (Polokwane, Limpopo, South Africa), and the University of California, Berkeley (Berkeley, California, USA).

2.2 Urine collection and analysis

Urine samples were collected from women at delivery and were immediately processed and stored at -80 °C until shipment on dry ice to the Institut National de Santé Publique du Québec (INSPQ) (Quebec City, Quebec, Canada) for analysis [27]. Pyrethroid metabolites, including *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DBCA), *cis*-3-(2,2-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DCCA), *trans*-3-(2,2-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*trans*-DCCA), 3-phenoxybenzoic acid (3-PBA) and 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA) were measured in urine using gas chromatography-tandem mass spectrometry. Limits of detection were 0.0025 µg/L for *cis*-DBCA, 0.0045 µg/L for *cis*-DCCA, 0.0038 µg/L for *trans*-DCCA, 0.0047 µg/L for 3-PBA and 0.005 µg/L for 4-F-3-PBA. Urine specific gravity was measured with a portable refractometer (Atago PAL-10S; Tokyo, Japan) and creatinine was measured using commercial immunoassays. Pyrethroid metabolite

concentrations (C_{meas}) were corrected (C_{corr}) for urine dilution via specific gravity (SG), based on the formula from Levine and Fahy (1945) [28]: C_{corr} (expressed in µg/L) = $C_{meas} \times (1.024-1)/(SG-1)$. Creatinine-adjusted concentrations (expressed in µg/g) were obtained by dividing pyrethroid metabolite concentrations by the creatinine concentration. One 3-PBA measurement did not meet quality control standards and was discarded. 4-F-3-PBA was excluded from further analyses due to low quantification frequencies (8%). For the other analytes, concentrations below the limits of detection (LOD) were imputed at random based on log-normal probability distributions whose parameters were estimated via maximum likelihood [29]. Values between the LOD and limit of quantification (LOQ) were assigned the machine-read values.

2.3 Data collection

Questionnaire-based interviews were conducted by trained, bilingual (Tshivenda and English) staff with mothers at delivery and primary caregivers (defined as mothers, fathers or any other relative who lived with the child during the entire period for which we were investigating respiratory allergies) at each follow-up visit to collect data about potential confounders and health outcomes.

Covariates. At the time of delivery, mothers reported their age, marital status, mode of delivery, total household income, and total household size. Household poverty status was derived by dividing the household income by the number of people supported by the income and dichotomized into below or above South Africa's mid-2013 food poverty threshold of 386 Rands per month per person (about 30-35 CAD) [30]. Mother's food intake was assessed based on a quantitative food frequency questionnaire validated in the local population [31] and total daily

energy intake in kilojoules (kJ) was estimated by a South African expert nutritionist using the FoodFinder3 software (South Africa Medical Research Council/WAMTechnology CC). Insufficient energy intake during pregnancy was defined as being below the Institute of Medicine (IOM)-recommended total daily caloric intake for high-activity mothers in their third trimester [32, 33]. Maternal HIV status during pregnancy was ascertained from self-report or information about use of anti-retroviral drugs obtained from medical records abstracted by two registered nurses.

Using data obtained from the delivery questionnaire and the 1-week home visit (questionnaire and staff observations), a family wealth index was constructed based on South Africa Demographic and Health Survey methodology, in order to capture socioeconomic status in this region where much of the economy is informal [34, 35]. Duration of breastfeeding was calculated based on responses from questionnaires administered at 1 week and 1, 2 and 3.5 years.

Time-varying data about exposure to allergens in the preceding 18 months, including the presence of any mold or mildew, cockroaches, rats, mice or other rodents, cats or dogs and exposure to environmental tobacco smoke at home where the child had lived were obtained from questionnaires conducted at the 3.5-year and 5-year follow-up visits.

Respiratory allergy symptoms and asthma diagnoses. At the 3.5-year and 5-year follow-up visits, primary caregivers, including mothers (n=565 at 3.5-year and n=530 at 5-year) or other family members (n=82 at 3.5 years and n=90 at 5 years), were queried about children's respiratory allergy symptoms in the prior 18 months using the International Study of Asthma and Allergies in

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Childhood (ISAAC) questionnaire, which was conducted in many different countries including South Africa [36, 37]. While pulmonary function tests may generally provide more accurate data, studies have shown that the reliability and feasibility of these tests are limited among preschool-aged children, such as those included in the present study [38, 39]. The ISAAC questionnaire has been validated in multiple countries, relying on medical or clinical diagnoses and respiratory evaluations. It has proven to be a globally reproducible and reliable tool for mapping and comparing allergic diseases in children and adolescents in low-, middle- and high- income countries [40-43]. It consistently demonstrated strong criterion validity and internal consistency [40-43]. In both adults and children, questionnaires showed high agreement with respiratory physician diagnoses regarding asthma symptoms in the past 12 months. The positive and negative predictive values for physician diagnosis of asthma in children were 0.61 and 0.94 respectively. The instrument was also sensitive (0.80) and highly specific (0.97) [43]. Questions covered information about asthma symptoms (prevalence of wheezing or whistling in the chest, chest sounding wheezy during or after exercise, dry cough at night), allergic rhinitis symptoms (sneezing, runny or blocked nose) and allergic rhinoconjunctivitis symptoms (itchy-watery eyes accompanied by nose problems), as well as hay fever. Information about the season during which nose problems occurred were also obtained. Among children who experienced wheezing or whistling in the chest, staff enquired about the number of wheezing attacks (1 to 3; 4 to 12; > 12); the frequency of sleep disturbance due to wheeze (none; < 1 night per week; ≥ 1 night per week) and whether speech was limited by wheeze [36]. In addition, primary caregivers were asked whether children had been diagnosed with asthma by a doctor or nurse and, if a diagnosis was reported, whether any medicines were prescribed to treat the condition.

2.4 Outcome definition

Disaggregated outcomes. In the main analysis, we first considered disaggregated outcomes including asthma, allergic rhinitis and rhinoconjunctivitis symptoms; asthma diagnosis; doctor's prescription of asthma medicine; and hay fever based on ISAAC questionnaire, which was previously used and validated in multiple countries including South Africa [37, 44-46].

Suspected asthma. Asthma tends to be under-diagnosed, particularly among younger children [47-50], while wheezing has low positive predictive value and overestimates asthma prevalence [42]. As proposed by Lukfrafka et al. (2010), we defined cases of suspected asthma if children had experienced wheezing and at least one other symptom with high specificity for asthma diagnosis (i.e., wheezing during exercise, dry cough, sleep disturbed due to wheezing, or wheezing severe enough to limit speech).

Seasonal rhinoconjunctivitis. As a proxy for allergic rhinitis and rhinoconjunctivitis, we identified children who experienced rhinitis and rhinoconjunctivitis symptoms during spring or summer, since having such symptoms only during high-pollen seasons has a high predictive value for an allergic origin [51, 52], and their occurrence during other seasons can indicate non-allergic origins. Children who experienced symptoms during other seasons (winter or autumn), along with children without any symptoms were classified as non-cases for this outcome.

Global outcomes. In a secondary analysis, we created new outcomes using a less stringent definition to evaluate the impact of exposure on overall propensity of the different types of allergies. These binary outcomes were defined based on a positive response to any of their respective questions as follows: 1) any asthma symptom – wheezing or whistling in chest or wheezing during exercise or dry cough at night or, 2) any allergic rhinitis/rhinoconjunctivitis symptom – sneezing, runny or blocked nose or itchy watery eyes accompanied by nose problems,

and 3) any respiratory allergy symptom – any of the asthma or allergic rhinitis and rhinoconjunctivitis symptoms mentioned above.

2.5 Statistical Analysis

All analyte concentrations were $\log_{10}$-transformed to reduce the influence of outliers. Thus the relation between a 10-fold increase in maternal urinary pyrethroid metabolite concentrations on the risk of each outcome were estimated using marginal structural models for repeated outcomes assuming a Poisson distribution and applying weights that were formed as the product of inverse probability of censoring weights (IPCWs) to account for potential selection bias due to loss to follow up and inverse probability of treatment weights (IPTWs) to control for confounding [53, 54]. We used separate logistic regression models to estimate the probability of remaining uncensored at 3.5 or 5 years conditional on exposure and other potential predictors of censoring and constructed IPCWs based on the inverse of these probabilities. The IPCWs were stabilized by using the marginal probability of being uncensored as the numerator. Excluding censored individuals at the 3.5- and 5-year visits, we first constructed IPTWs based on the Generalized Propensity Score method [55] by fitting separate multivariable linear regression models to estimate normal density functions for each continuous exposure, conditional on potential confounders. Because model residuals were not normally distributed based on Q-Q plots, we instead used the quantile binning approach [56] which has been shown to provide good bias reduction in continuous exposure settings where the continuous density function is not easily identified, and thus is subject to model mis-specification. We thus categorized each continuous exposure into quintiles and then estimated the predicted probabilities of falling into the observed exposure category by fitting a multinomial regression model [56], conditional on potential confounders identified using directed

acyclic graphs (DAGs) as shown in Figure S4.1. IPTWs were stabilized by using the marginal probability of being in the observed exposure category. The causal relationships assumed under DAGs were based on prior knowledge and current literature [22-25, 57]. In line with DAG theory [58] covariates that were located on non-causal paths were controlled for. We also controlled for strong determinants outcome as this has been shown to increase precision [59]. The following covariates were included in both the censoring and propensity score models: child sex (boy/girl); duration of non-exclusive breastfeeding (months, continuous); maternal age (years, continuous); education (high school/no high school); marital status (married or living-as-married/not married); parity (continuous); energy intake (sufficient/insufficient); HIV status (positive/negative); as well as household wealth index (continuous) and poverty status (yes/no) during pregnancy. In the IPTW models, we also included the following time-varying covariates related to exposure to allergens at 3.5 and 5 years: presence of mold or mildew (yes/no); dogs or cats (yes/no); cockroaches (yes/no); rats, mice or other rodents in the home (yes/no); and child exposure to environmental tobacco smoke (yes/no).

Inverse probability weights are used to generate a pseudo-population in which censoring is independent from exposure and covariates, and exposure is independent of the measured confounders [53, 54]. Whether this was accomplished can be confirmed by assessing if the distributions of confounders are balanced at different levels of exposure in the weighted sample. For this purpose, we estimated absolute standardized differences (comparing proportions or means) and variance ratios (comparing variability) of all covariates across each quintile of exposure versus all other quintiles as well as correlations between each exposure and continuous covariates, as recommended by Austin [60, 61]. As per published guidelines, absolute

standardized differences below 0.2 [60, 61], variance ratios below 2.0 [62] and correlations below 0.1 [60, 61] were used to indicate balance across exposure categories.

Covariates with low rates of missingness (<5%) were imputed at random based on observed univariate probability distributions. We imputed missing breastfeeding data (8.1%) using single predictive mean matching, where we used all outcomes, exposures and covariates as predictor variables. Epidemiological studies strongly suggest that incidence of asthma, wheeze and allergic rhinitis is higher in boys compared to girls [63, 64], in older (5 – 7 years) relative to younger children (2 – 4 years) [65], and in children born by caesarian section compared to those vaginally delivered [66, 67]. However, it has been observed that breastfeeding has a protective effect against asthma and allergies, as demonstrated by various studies [68-70]. Therefore, we investigated effect measure modification by child sex, age, mode of delivery and duration of breastfeeding (which was classified as less than or greater than 18 months) by including the effect modifier as well as a cross-product between each effect modifier and exposure in marginal structural models. For these analyses, we estimated IPTWs as described above except that weights in marginal structural models were stabilized using the probability of being in the observed exposure category conditional on the effect modifier, which has been shown to produce more precise estimates [71]. Each exposure and effect modifier was evaluated in separate models. We considered p-values <0.10 to indicate evidence of effect modification. The 95% confidence intervals (CIs) were calculated based on robust (Huber-White) standard errors.

Sensitivity analysis was conducted to evaluate the robustness of the results. Because a child diagnosed with asthma at the age of 3.5 years is not likely to outgrow the disease at the age of 5

years [72], we performed additional analyses for doctor-diagnosed asthma and suspected asthma by coding all children with a positive value for these variables at 3.5 years as being positive at 5 years. Sensitivity analyses for effect measure modification employed the augmented product term approach, incorporating cross-product terms between each effect modifier and each covariate in the estimation of IPTWs.

All analyses were conducted using R, version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Participant characteristics

All VHEMBE mothers (n=657) were black South Africans. At delivery, the mothers had a mean age of 26.3 (SD, 6.2) years, more than half were unmarried (53.1%) and had less than a high school education (55.7%) (Table 1). Most of the women lived in households below the South African food poverty line (60.6%), and many had low energy intake during pregnancy (68.6%). About 11.7% of women were HIV positive. The mean duration of exclusive breastfeeding was 2.3 months but non-exclusive breastfeeding continued for an average of 15.9 months. A little more than half of the children were boys (51.4%), 7.9% had a low birthweight (<2500 g) and 12.5% were preterm (<37 weeks gestational age at birth). Exposure to allergens was common among study children. At the 3.5-year and 5-year visits, caregivers reported that homes where most of the children had lived had cockroaches, a little less than half had dogs or cats, rats, mice or other rodents, some had mild or mildew but only few homes had tobacco smoke.

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Table 1. Characteristics of participants in the Venda Health Examination of Mothers, Babies and Their Environment, Limpopo Province, South Africa, 2012-2013

	n	Freq	%
Maternal characteristics (at delivery)	657 [a]		
Age, years			
< 25		337	51.3
25 – 35		246	37.4
> 35		74	11.3
Marital status			
Single		349	53.1
Married or living as married		308	46.9
Education			
< 12th Grade		366	55.7
≥ 12th Grade		291	44.3
Parity			
0		287	43.7
1		177	26.9
≥ 2		193	29.4
HIV status			
Negative		580	88.3
Positive		77	11.7
Energy intake during pregnancy [b]			
Insufficient (< x)		451	68.6
Sufficient (≥ x)		206	31.4
Household sociodemographic characteristics	657 [a]		
Food poverty status [c]			
≤ poverty		398	60.6
> poverty		259	39.4
Child characteristics	657 [a]		
Sex			
Female		319	48.6
Male		338	51.4
Low birthweight (<2500 g)			
No		605	92.1
Yes		52	7.9
Gestational age at birth, weeks			
Preterm (< 37)		82	12.5
Full term (≥ 37)		575	87.5
Any breastfeeding, months	652 [a, d]		
≤ 18		382	58.6
> 18		270	41.4

Exclusive breastfeeding, months	653 [a, d]	
≤ 2	370	56.7
> 2	283	43.3
Household characteristics at 3.5 years	647 [e]	
Mold or mildew in the house		
No	462	71.6
Yes	183	28.4
Dogs or cats in the house		
No	367	56.8
Yes	279	43.2
Cockroaches in the house		
No	131	20.3
Yes	515	79.7
Rats, mice or other rodents in the house		
No	441	68.3
Yes	205	31.7
Exposure to environmental tobacco smoke		
No	589	91.6
Yes	54	8.4
Household characteristics at 5 years	620 [f]	
Mold or mildew in the house		
No	467	75.6
Yes	151	24.4
Dogs or cats in the house		
No	384	62.1
Yes	234	37.9
Cockroaches in the house		
No	144	23.3
Yes	473	76.7
Rats, mice or other rodents in the house		
No	380	61.6
Yes	237	38.4
Exposure to environmental tobacco smoke		
No	564	91.1
Yes	55	8.9

3.2 Biomarker concentrations

Table 2 shows the distribution of biomarkers of exposure to pyrethroids insecticides, as well as their detection and quantification frequencies. Concentrations of all four pyrethroids metabolites were above LOD in all maternal samples. Specific gravity-adjusted geometric mean concentrations of *cis*-DBCA, *cis*-DCCA, *trans*-DCCA and 3-PBA were 0.34, 0.48, 0.55 and 1.10 μg/L respectively. The largest range of values was observed for *cis*-DCCA and *trans*-DCCA concentrations, with up to 4,000-fold and 9,000-fold difference in exposure between the highest and the lowest values, respectively.

3.3 Respiratory allergy symptoms and asthma diagnosis

Table 3 summarizes children's respiratory allergy symptoms and asthma diagnosis among 3.5 years old (n=647) and 5 years old (n=620) children as reported by primary caregivers. Approximately one in six children had at least one respiratory allergy symptom (17.2% at 3.5 years and 13.1% at 5 years), one in ten had one allergic rhinitis symptom (12.8% at 3.5 year and 8.9% at 5 years) and a fewer had at least one asthma symptom (6.3% at 3.5 years and 5.7% at 5 years). The most common respiratory allergy symptom was sneezing or having a runny or blocked nose without a cold or the flu (12.8% at 3.5 years and 8.9% at 5 years). About a third of these children also experienced itchy-watery eyes and around half had this symptom specifically during the

Table 2. Distribution of maternal urinary pyrethroid metabolites concentrations (µg/L, specific gravity-corrected) among VHEMBE study participants, Limpopo, South Africa

| | n | ≥ LOD[a], % | ≥ LOQ[b], % | Geometric Mean | Geometric SD | Min | Percentiles | | | |
							25	50	75	Max
cis-DBCA	657	100	99.5	0.34	3.07	0.02	0.15	0.32	0.74	13.39
cis-DCCA	657	100	99.8	0.48	2.58	0.05	0.26	0.45	0.79	209.49
trans-DCCA	657	100	99.5	0.55	3.10	0.03	0.26	0.53	1.05	268.95
3-PBA	656	100	100	1.10	2.41	0.10	0.64	1.05	1.84	102.38

[a]Limits of detection (LOD): 0.0025 (*cis*-DBCA), 0.0045 (*cis*-DCCA), 0.0038 (*trans*-DCCA), and 0.0047 (3-PBA) µg/L. [b]Limits of quantification (LOQ): 0.0082 (*cis*-DBCA), 0.015 (*cis*-DCCA), 0.013 (*trans*-DCCA), to 0.016 (3-PBA) µg/L. Abbreviations: SD, standard deviation; Min, minimum; Max, maximum

Table 3. Respiratory allergy symptoms and asthma diagnosis as reported at the 3.5 (n=647) and 5 years visits (n=620) among VHEMBE children, Limpopo, South Africa

	N	%	N	%
	3.5 years		**5 years**	
Wheezing or whistling in the chest				
No	617	95.4	595	96.1
Yes	30	4.6	24	3.9
If yes, number of wheezing attacks				
1 to 3	26	86.7	21	87.5
4 to 12	1	3.3	2	8.3
> 12	3	10.0	1	4.2
If yes, number of nights sleep disturbed by wheezing per week				
Never	11	36.7	8	33.3
< 1	13	20.0	12	50.0
≥ 1	6	43.3	4	16.7
If yes, speech limited by wheezing				
No	24	80.0	21	87.5
Yes	6	20.0	3	12.5
Chest sounds wheezy during or after exercise				
No	627	97.1	604	97.6
Yes	19	3.0	15	2.4
Dry cough at night				
No	638	98.6	611	98.7
Yes	9	1.4	8	1.3
Doctor-diagnosed asthma				
No	638	98.6	614	99.2
Yes	9	1.4	5	0.8

Doctor prescribed medicine for asthma				
No	641	99.1	613	99.0
Yes	6	0.9	6	1.0
Suspected asthma [a]				
No	622	96.1	599	96.8
Yes	25	3.9	20	3.2
Sneezing, runny or blocked nose				
No	564	87.2	564	91.1
Yes	83	12.8	55	8.9
Itchy-watery eyes accompanied by nose problems				
No	619	95.7	600	97.1
Yes	28	4.3	18	2.9
Hay fever				
No	637	98.6	613	99.2
Yes	9	1.4	5	0.8
Seasonal rhinitis [b]				
No	602	93.0	584	94.3
Yes	45	7.0	35	5.7
Seasonal rhinoconjunctivits [c]				
No	631	97.5	607	98.1
Yes	16	2.5	12	1.9
Any respiratory allergy symptom [d]				
No	536	82.8	539	86.9
Yes	111	17.2	81	13.1

Any asthma symptom [e]				
No	606	93.7	584	94.3
Yes	41	6.3	35	5.7
Any rhinitis symptom [f]				
No	564	87.2	565	91.1
Yes	83	12.8	55	8.9

Note: Totals may not sum to 647 at 3.5-years and 620 at 5-years due to missing data, primarily due to caregivers reporting that they did not know if children experienced symptoms.

[a] Variable combining wheezing and at least one other symptom. Other symptoms: Chest sounds wheezy during or after exercise or dry cough at night or sleep disturbed due to wheezing or wheezing severe enough to limit speech.

[b] Sneezing, runny or blocked nose had they occurred during spring or summer seasons.

[c] Itchy-watery eyes accompanied by nose problems had they occurred during spring or summer seasons.

[d] Respiratory allergy symptoms: Wheezing or whistling in chest, chest sounds wheezy during or after exercise, sneezing, runny or blocked nose, dry cough at night or itchy-watery eyes accompanies by nose problems.

[e] Asthma symptoms: Wheezing or whistling in chest, chest sounds wheezy during or after exercise or dry cough at night.

[f] Rhinitis symptoms: Sneezing, runny or blocked nose or itchy-watery eyes accompanied by nose problems.

pollen season. Wheezing or whistling in chest was the most prevalent asthma symptom (3.9% at 3.5 years and 4.6% at 5 years) with most children experiencing between one to three attacks since their last visit at ages 3.5 and 5 years. Of those children who experienced wheezing, 43.3% had their sleep disturbed for one or more nights per week and 20.0% had their speech limited by wheeze at 3.5 years compared to 16.7% and 12.5% at 5 years. The chest of approximately 3% of children sounded wheezy during or after exercise and less than 2% had dry cough at night, were diagnosed with asthma, hay fever or were prescribed asthma medication. However, a higher percentage (~ 4%) were suspected to have asthma.

3.4 Inverse probability weights and covariate balance diagnostics

The mean of the final inverse probability weights, the product of the IPTWs and IPCWs, was 1.0 for all models and no extreme weights were observed (range: 0.28-5.23; Table S5.1). Inverse probability weighting achieved covariate balance, with all mean absolute standardized differences in potential confounders across exposure quintiles below 0.2, all variance ratios being about 1.0, and correlations between exposures and continuous potential confounders below 0.1 (Figures S5.1 – S5.3).

3.5 Associations between pyrethroid metabolites and respiratory allergy symptoms and asthma diagnosis

Overall, maternal concentrations of multiple pyrethroid metabolites were associated with higher risks of asthma diagnosis and respiratory allergy symptoms. We found that a 10-fold increase in maternal urinary *cis*-DCCA, *trans*-DCCA and 3-PBA concentrations were associated with more

than a doubling in the risk of doctor-diagnosed asthma (*cis*-DCCA: RR=2.1, 95% CI = 1.3, 3.3; *trans*-DCCA: RR=2.1, 95% CI = 1.1, 3.9; 3-PBA: RR=2.4, 95% CI = 1.0, 5.8) and an about 80% increase in the risk of both wheezing or whistling in the chest (*cis*-DCCA: RR=1.8, 95% CI = 1.1, 3.0; *trans*-DCCA: RR=1.7, 95% CI = 1.1, 2.6; 3-PBA: RR=1.8, 95% CI = 1.0, 3.3) and suspected asthma (*cis*-DCCA: RR=1.8, 95% CI = 1.1, 3.1; *trans*-DCCA: RR=1.8, 95% CI = 1.1, 2.8) (Figure 1) (Table S1.1). We also observed that higher concentrations of *cis*-DBCA was related to a 3.5-fold increased risk of dry cough at night (RR=3.5, 95% CI = 1.3, 9.5) and that 3-PBA was associated with a 2-fold increase in the risk of seasonal rhinoconjunctivitis (RR=2.0, 95% CI = 1.1, 3.9). There was suggestive evidence that *cis*-DBCA was also related to seasonal rhinoconjunctivitis (RR=1.9, 95% CI = 1.0, 3.6), that *cis*-DCCA was associated with obtaining a doctor's prescription of asthma medicine (RR=1.4, 95% CI = 1.0, 1.9) and that 3-PBA was related to suspected asthma (RR=1.7, 95% CI = 1.0, 3.2), though confidence intervals crossed the null.

In analyses investigating effect modification, we observed positive associations between 3-PBA and hay fever among 3.5-year-old children (RR=3.8, 95% CI = 1.0, 14.1; p-interaction=0.08) (Table S2.1). Although there was some evidence of effect modification by child age for the associations between *cis*-DBCA and chest sounding wheezy during or after exercise, as well as hay fever, stratum-specific estimates crossed the null. For *trans*-DCCA, positive associations were found with dry cough at night (RR=2.9, 95% CI = 1.1, 7.7; p-interaction<0.01) and doctor-diagnosed asthma (RR=3.7, 95% CI = 1.4, 9.3; p-interaction=0.04) among girls only (Table S2.2). Stratum-specific estimates, crossing the null, were also observed between 3-PBA and both dry cough at night and doctor's prescription of asthma medicine, and between *cis*-DBCA and doctor-diagnosed asthma. Moreover, we found positive associations between 3-PBA and suspected

Figure 1. Associations between a 10-fold increase in maternal urinary pyrethroid metabolite concentrations (µg/L) and respiratory allergies at 3.5 and 5 years of age.
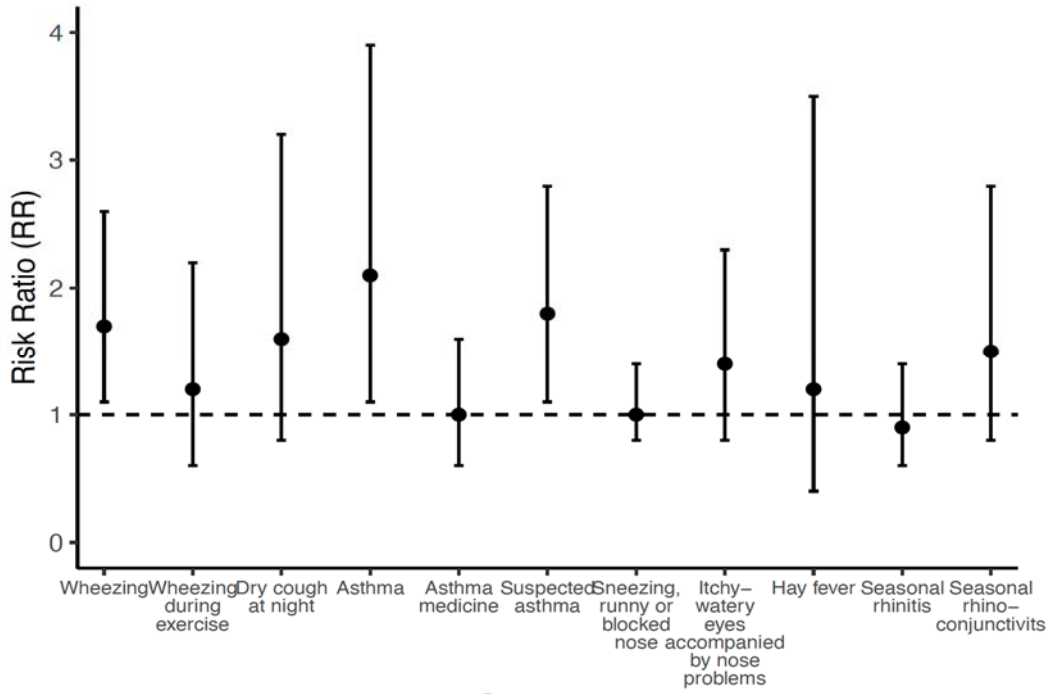
a)
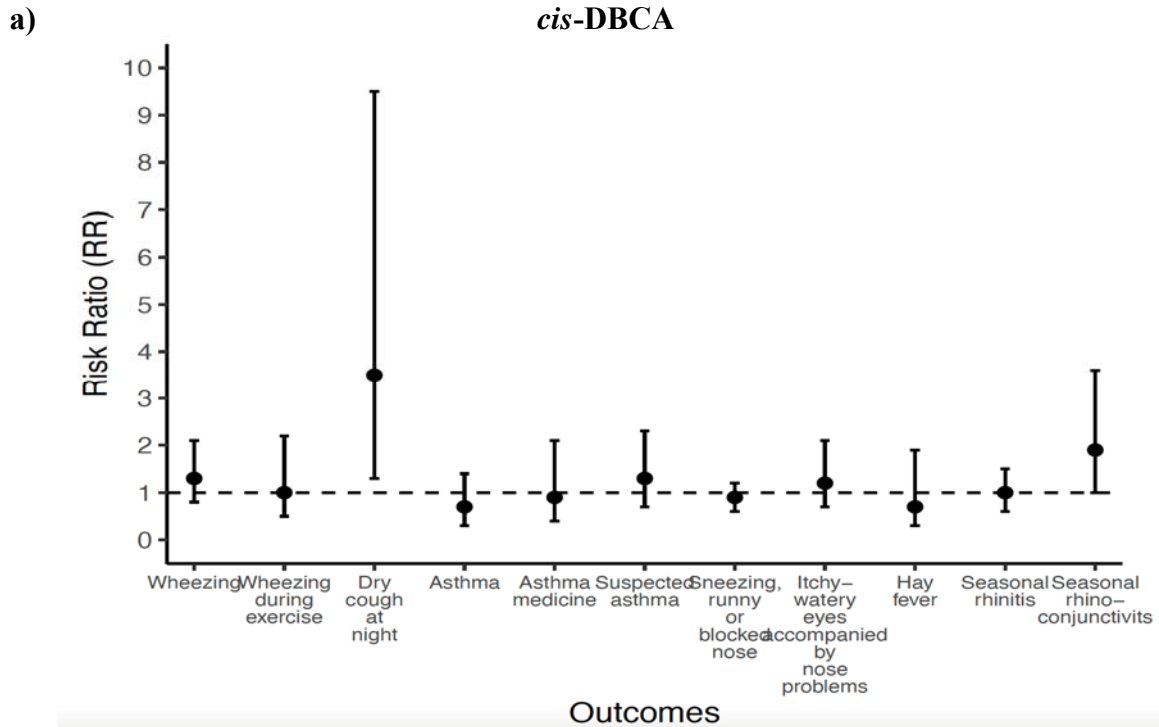
Abbreviations: *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid

b)
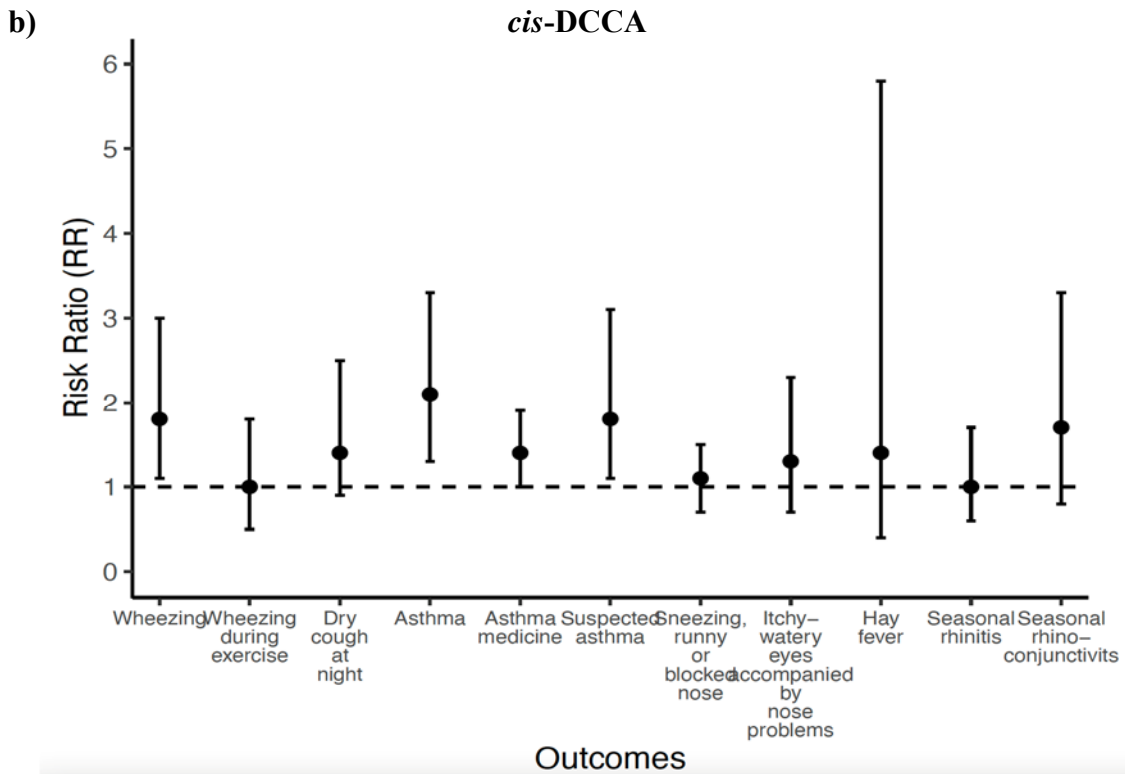
Abbreviations: *cis*-DCCA, *cis*-3-(2,2-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid

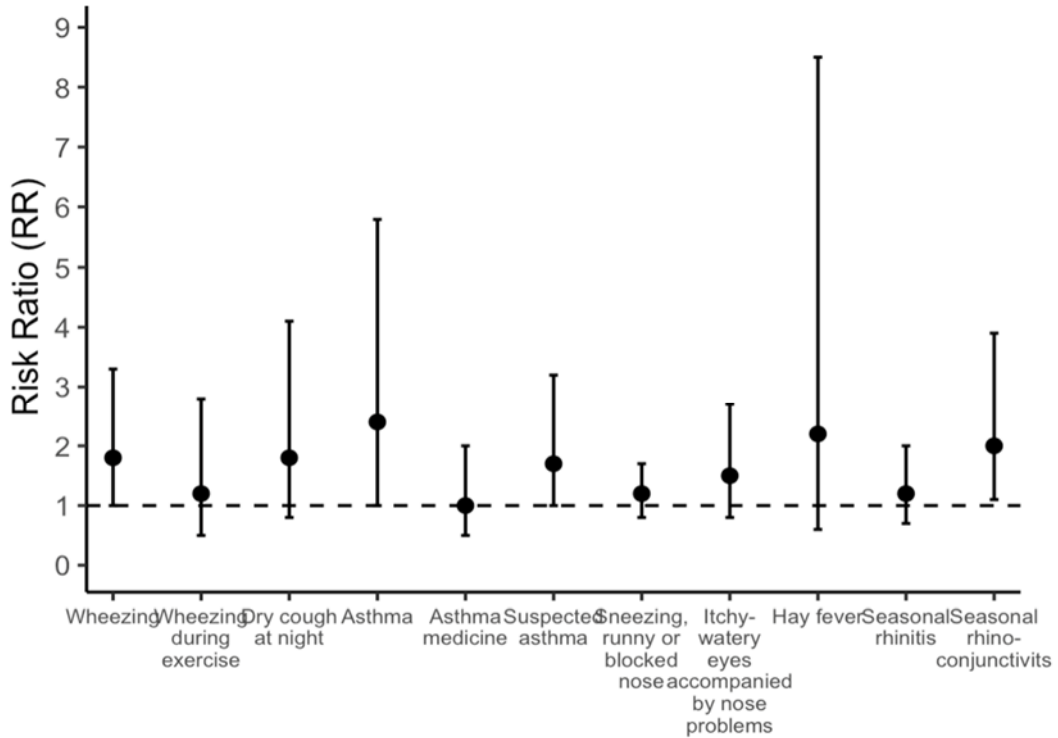**c)** **trans-DCCA**

Abbreviations: *trans*-DCCA, *trans*-3-(2,2-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid

d) **3-PBA**

Abbreviations: 3-PBA, 3-phenoxybenzoic acid

asthma among children who were delivered vaginally (RR=2.3, 95% CI = 1.3, 4.2; p-interaction=0.07); however, stratum-specific estimates, crossing the null, were observed between *cis*-DBCA and doctor-diagnosed asthma. For *cis*-DBCA, positive associations were also found with chest sounding wheezy during or after exercise only among children breastfed for a duration longer than 18 months (RR=2.2, 95% CI = 1.0, 4.9; p-interaction = 0.08) (Table S2.4). Stratum-specific estimates, crossing the null, were also observed between *trans*-DCCA and chest sounding wheezy during or after exercise. Associations between pyrethroids and other symptoms did not vary by child age, child sex, mode of delivery, or duration of breastfeeding.

Results of the sensitivity analyses, where doctor-diagnosed and suspected asthma at 3.5 years were also carried forward to the 5-year visit, were generally similar to those of the main analyses (Table S3.1). For effect measure modification, the results of the augmented product term approach were compared to those of the traditional product term approach, and no substantial changes were observed (results not shown).

4. Discussion

We found that higher maternal urine concentrations of *cis*-DCCA, *trans*-DCCA and 3-PBA were positively associated with asthma diagnosis among 3.5- and 5-year-old South African children participating in the VHMEBE study. These results contrast with those of a prior study, which found negative associations between 3-PBA and DCCA (sum of *cis*- and *trans*-DCCA) and doctor-diagnosed asthma among Costa Rican children aged 5 years [23]. *cis*-DCCA, *trans*-DCCA and 3-PBA are non-specific metabolites that can devolve from different pyrethroids. Consequently, identical levels of metabolites may represent varying levels of exposure to different parent

compounds among the diverse study populations, which may result in contrasting results between studies. Also, discordant results may be due to variations in the distribution of effect modifiers such as age, poverty, malnutrition, and duration of breastfeeding in various populations. Our results also differ from those of a prior study that investigated associations between air concentrations of *cis-* and *trans-* permethrin and doctor-diagnosed asthma among New York African-American and Dominican children 5 to 6 years of age [22]. However, results may not be directly comparable since we measured urinary metabolites, which reflect integrated exposure from the respiratory, dermal and gastric routes relative to the US study that focused exclusively on the respiratory route. Furthermore, detection frequencies were low for *cis-*permethrin (38%) and *trans-* permethrin (40%) in the US study [22] and both the Costa Rican (n=303) and US (n=224) studies had moderate sample sizes, which may have limited their statistical power.

We also observed that *cis-*DBCA, *cis-*DCCA, *trans-*DCCA and 3-PBA were related to an increased risk of wheezing or whistling in chest. Those results are consistent with those of prior studies, which found positive associations between DCCA (sum of *cis-* and *trans-* DCCA), 3-PBA and *cis-*permethrin measured prenatally and cough and/or wheeze among children aged 5 years [22-24].

Experimental data supports our findings as rats injected with lambda-cyhalothrin (a type of pyrethroid) intraperitoneally were reported to develop asthma or respiratory allergy symptoms such as chest congestion and severe coughing while mice injected with saline did not [14], suggesting that pyrethroids may have systemic immune effects. Although, the exact mechanism by which pyrethroids induce these respiratory symptoms has not been fully elucidated, there are

some potential explanations for this association. *In vivo* studies suggest that pyrethroids may interfere with immune function by affecting the synthesis and expression of hormones, specifically estrogens [15, 16], which play an essential role in the etiology of allergies [17, 18]. Another plausible mechanism proposed by *in vitro* studies is through the inhibition of the production of the cytokine interferon (IFN)-gamma [73, 74] by T-helper 2 (Th2) (pro-allergic) cells, thus potentially further decreasing (IFN)-gamma levels, which protect against the development of asthma. An additional possible mechanism of action is through increased sensitization. For instance, an epidemiological study found that workers exposed to cyfluthrin, deltamethrin, tetramethrin, imiprothrin and d-allethrin in a pyrethroid insecticides company complained of some respiratory symptoms like cough, wheeze, shortness of breath and dyspnea. These symptoms coincided with elevated levels of Immunoglobulin (IgE) antibodies, when compared with the control group [75].

Our study has several strengths. This is the first study to examine associations between maternal exposure to pyrethroid insecticides and respiratory allergy symptoms and asthma diagnosis in children from a malaria-endemic area where pyrethroids are used in the context of IRS, addressing a key knowledge gap on health effects of this practice. We had detailed and nearly complete information on multiple confounders and, as opposed to prior studies, we controlled for child exposure to allergens as time-varying confounders. In order to limit the potential for outcome misclassification, we administered questionnaires to the primary caregivers, when mothers reported not living with the child "all of the time" due to reasons such as employment in other cities. In the current study, we also applied inverse-probability weighting methods to address potential selection bias from loss to follow-up and to control for confounding. These methods allowed us to verify that exposures and measured confounders were balanced across the exposure

range in the weighted sample. Nevertheless, unmeasured confounding remains possible and residual confounding or chance could explain our study findings.

A limitation of this study is that exposure was assessed based on a single measurement around the time of delivery, which may have introduced measurement error. We expect this error to be nondifferential with respect to the outcomes, potentially biasing our effect estimates towards the null. Additionally, since urine was collected at the time of delivery, we were unable to identify specific developmental windows of vulnerability. The 'metabolite-by-metabolite' approach used in the models may also be a limiting factor. Given that *cis*-DCCA, *trans*-DCCA and 3-PBA are non-specific, we are unable to identify the parent compounds that may be responsible for the associations. Although the elimination half-life of pyrethroids is short (approximately 5 – 13 hours) [76], the reliability of spot urine concentrations of pyrethroid metabolites in reflecting longer-term exposure is inconsistent in the literature and may vary by population and context. Strong intraclass correlation coefficients (ICC=0.85) [77] were reported among Polish adults whereas weaker reproducibility (ICC ≤ 0.21) was found among US adults [78]. However, in the context of IRS, pyrethroids insecticides are designed to be effective for up to 10 months and throughout the rainy season, therefore elevated exposure to inhabitants may persist for weeks or months from repeated contact with contaminated surfaces, bedding, furniture, and stored food [79]. It has also been previously reported that VHEMBE mothers who stored pesticide containers on the homestead and used pesticides in the yard had higher pyrethroid metabolite concentrations, suggesting that a single measurement may be representative of longer-term exposure in the VHEMBE population [13, 80]. Moreover, because children may have also been exposed to pyrethroids postnatally, it may be difficult to tease apart the effects of prenatal exposure from those

of postnatal exposure. However, weak correlations ($r_s = 0.06 – 0.25$) have been reported between pyrethroid metabolite concentrations measured during pregnancy relative to those measured in 5 year old children, suggesting a limited potential for confounding [23]. In addition, outcomes were based on caregiver report, which may lead to misclassification. However, because participants were blinded to the research questions, such misclassification would expected to be nondifferential and may thus have attenuated our effect estimates and hence would not explain the associations that we observed. Furthermore, wheezing may partly reflect respiratory infections in young children, however, the fact that outcomes were determined based on the ISAAC questionnaire, which was previously used and validated in multiple countries including South Africa [37, 45, 46], strengthens our study results. Finally, we observed a relatively small number of cases for some outcomes and cannot exclude the possibility that our findings may be due to chance.

5. Conclusion

In summary, we found that maternal urinary *cis*-DCCA, *trans*-DCCA and 3-PBA concentrations were positively associated with wheezing or whistling in the chest, doctor-diagnosed and suspected asthma, that *cis*-DBCA was related to an increased risk of dry cough at night and that 3-PBA was positively associated with seasonal rhinoconjunctivitis among 3.5- and 5-year-old South Africa children. Our findings may have important implications due to the increasing prevalence of asthma and wheezing in South Africa and given that more severe respiratory allergy symptoms have been reported among black African children compared to children from high income countries [81]. Consequently, any factor increasing symptoms could have a significant impact on health of African children. However, further research is required to confirm our results.

Declaration of competing interest

Authors have no conflict of interest to declare.

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