THE RELATIONSHIP BETWEEN PESTICIDE METABOLITES AND ASTHMA OUTCOMES AMONG WOMEN FARM WORKERS

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DECLARATION

I, **Dr Hussein Hassan Mwanga** hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Dedication

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Abstract

Background: Various studies have demonstrated an association between exposure to pesticides and adverse respiratory health outcomes including non-specific respiratory symptoms, rhinitis and asthma. Few studies have investigated the relationship between pesticide metabolites and asthma outcomes and only a limited number have explored mechanisms for allergic and non-allergic airway inflammation in individuals exposed to pesticides. A previous sub-study of this group reported an association between allergic airway inflammation as determined by fractional exhaled nitric oxide (FeNO) and low levels of whole blood cholinesterase among women farm workers. The main objective of this study was to investigate the relationship between exposure to different pesticides (ascertained through pesticide metabolites concentrations in urine) and asthma phenotypes (based on respiratory symptoms, cytokine patterns and exhaled nitric oxide profiles) among rural women in the Western Cape Province.

Methods: A cross-sectional study was conducted among rural women working and living on the farms (farm dwellers, n=121) and those residing in the neighbouring towns (town dwellers, n=90). Pesticide exposure was assessed based on urinary metabolite concentrations of organophosphate (OP) and pyrethroid (PYR) residues. Health outcome assessment was ascertained through modified European Community Respiratory Health Survey questionnaire, FeNO concentrations, serum cytokine (Th2 and non-Th2 markers) concentrations, and immunological markers for atopy (Phadiatop) and specific IgE to house dust mite, storage mite and spider mite.

Results: The median age of the study participants was 37 years (interquartile range: 28 - 45 years). The urinary concentration (median and interquartile range) of pesticide residue metabolites for OP (sum of the 6 dialkyl phosphate metabolites) = 134 (42-229) µg/g of creatinine; 3,5,6-trichloropyridinol (TCPY) = 5 (3-10) µg/g of creatinine; and for PYR (sum of the 5 PYR metabolites) = 6 (3-10) µg/g of creatinine. The prevalence of current asthma (asthma attack in the last 12 months or currently taking asthma medication) was 6%; doctor diagnosed asthma was 11%; and adult-onset asthma (history of doctor-diagnosed asthma and first asthma attack at age 16 years or older) was 9%. The proportion of subjects with FeNO above 50 ppb was 7% and between 25-50 ppb was 11%. The proportion of Th2 cytokines (IL-4, IL-5 and IL-13) detected ranged from 18% to 40% while non-Th2 cytokines (IL-6, IL-8, IL-10, IL-17 and interferon-γ) ranged from 35% to 71%. IL-8 was the most commonly detected

(71%) cytokine while IL-5 was the least detected (18%). Most OP metabolites were positively associated with FeNO levels above 50ppb. Both Th2 and non-Th2 cytokines were positively associated with either OP or PYR metabolites. Non-Th2 cytokines showed stronger associations with OP metabolites, the strongest association between diethyl dithiophosphate (DEDTP) and interferon-γ: prevalence odds ratio (95% confidence interval) = 25 (8-78).

Conclusion: OP and PYR urinary metabolite levels in rural women in the Western Cape are higher than in the general population. This study provides evidence suggesting that both OP and PYR pesticides are associated with asthma, which may be due to Th2 and non-Th2 mechanisms, the latter pathway demonstrating consistently stronger relationships.

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Abbreviations

AHS: Agricultural Health Study
BMI: body mass index
ChE: cholinesterase
CI: confidence interval
cis-DCCA: cis-2,2-dichlorovinyl-2,2- dimethylcyclopropane-1-carboxylic acid
DAP: dialkyl phosphate
DEP: diethyl phosphate
DETP: diethyl thiophosphate
DEDTP: diethyl dithiophosphate
DDT: dichlorodiphenyltrichloroethane
DMP: dimethyl phosphate
DMTP: dimethyl thiophosphate
DMDTP: dimethyl dithiophosphate
DBCA: cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid
ED: endocrine disrupters
EPN: O-ethyl O-(4-nitrophenyl) P-phenylphosphonothioate
EPTC: ethyl dipropylthiocarbamate
FeNO: fractional exhaled nitric oxide
FEV1: forced expiratory volume in one second
ID: isotope dilution
IFN-γ: interferon-γ
IgE: immunoglobulin E
IL: interleukin
IMPY: 2-isopropyl- 4-methyl-6-hydroxypyrimidine
LOD: limit of detection
NIOH: National Institute for Occupational Health
NO: nitric oxide
OP: organophosphate
OR: odds ratio
ppb: parts per billion
PYR: pyrethroid
TCPY: 3,5,6- trichloropyridinol
Th1: T helper cells 1
Th2: T helper cells 2
trans-DCCA: trans-2,2-dichlorovinyl-2,2- dimethylcyclopropane-1-carboxylic acid
TRP: transient receptor potential
US: United States of America
WFP: Women on farms
2,4-D: 2,4-dichlorophenoxyacetic acid
2,4,5-T: (2,4,5-trichlorophenoxy)- acetic acid
3PBA: 3- phenoxybenzoic acid
4F3PBA: 4-fluoro-3-phenoxybenzoic acid

Part A: The protocol

1. INTRODUCTION

1.1. BACKGROUD

Pesticides are substances used extensively worldwide to kill or control certain forms of unwanted animal or plant life. Pesticides are widely used in agriculture and in several other settings including households, schools, leisure areas and also in the transportation facilities such as aeroplanes and boats [1,2]. Since pesticides are extensively used in the agricultural sector and a significant number of economically active South African population is employed in this sector, pesticides pose a significant public health problem.

1.2. REVIEW OF THE LITERATURE

Despite their useful effects, pesticides have been associated with various health effects including adverse respiratory health outcomes such as asthma, rhinitis and non-specific respiratory symptoms.

In summary, the following specific pesticides have been associated with various adverse respiratory health outcomes including asthma: organophosphate (OP) insecticides: diazinon, parathion, coumaphos, phorate, malathion, chlorpyrifos, terbufos, dichlorvos and fonofos; carbamate insecticide, carbaryl; organochlorine insecticides: chlordane, heptachlor, lindane and DDT (dichlorodiphenyltrichloroethane); pyrethroid (PYR) insecticide, permethrin; herbicides: 2,4,5-TP (2,4,5-trichlorophenoxypropionic acid), paraquat, diquat, EPTC (ethyl dipropylthiocarbamate), 2,4-D (2,4-dichlorophenoxyacetic acid), glyphosate, atrazine, chlorimuron-ethyl, imazethapyr, metolachlor, metribuzin, pendimethalin, petroleum oil, alachlor and trifluralin; fungicides: captan, metalaxyl and benomyl; and fumigants: ethylene dibromide and 80/20 mix [3–10].

A few other studies have demonstrated an association between different adverse respiratory health outcomes and the exposure to broad groups of pesticides but the specific pesticides were not identified [11–15].

Exposure assessment for pesticides – biomonitoring approaches

Acetylcholinesterase measurements in blood have been used to estimate exposure to organophosphate and carbamate insecticides. Several pesticide metabolites have also been used to estimate exposure to pesticides, mostly measured in the urine

samples. The most commonly measured metabolites for estimating exposure to organophosphates are the six dialkyl phosphate (DAP) metabolites [dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP)] [16]. These DAP metabolites are non-specific, meaning they reflect exposure to organophosphates but they do not identify a specific organophosphate. 3,5,6- trichloropyridinol (TCPY) is a specific metabolite for estimating exposure to a commonly used organophosphate, chlorpyrifos. TCPY is the most common specific organophosphate metabolite measured [17]. Other less commonly measured specific organophosphate metabolites include α and β isomers of malathion monocarboxylic acid and malathion dicarboxylic acid for malathion and 2-isopropyl- 4-methyl-6-hydroxypyrimidine (IMPY) for diazinon [17,18]. 4-nitrophenol has been used for estimating exposure to methyl and ethyl parathion but it is also a metabolite of EPN (O-ethyl O-(4-nitrophenyl) P-phenylphosphonothioate) and other non-organophosphate chemicals [17].

A metabolite of carbaryl, 1-naphthol has been measured to estimate exposure to this commonly used carbamate [17]. For pyrethroid insecticides, 3- phenoxybenzoic acid (3PBA) is a non-specific metabolite, common to about 20 synthetic pyrethroids. There are other more specific metabolites of pyrethroids that have been measured, including 4-fluoro-3-phenoxybenzoic acid (4F3PBA), a metabolite of cyfluthrin; cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA), a metabolite of deltamethrin; and cis- and trans-isomers of 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (cis- and trans-DCCA) which are metabolites of permethrin, cypermethrin and cyfluthrin [17].

Pathophysiological mechanisms

a) Cytokine pathways in asthma

Over the past few years, allergic airway inflammation that occurs in asthmatic individuals has been classified as being either eosinophilic or non-eosinophilic. It is widely accepted that eosinophilic airway inflammation is driven by the Th2 response [19], particularly so in mild and moderate asthma. However, the inflammatory response seen in severe asthma is complex as the Th1 response is also involved [19,20]. Th2 response produces several cytokines including IL-3, IL-4, IL-5, IL-9, IL-10, IL-13 and IL-33 [19,20]; however, the major Th2 cytokines are IL-4, IL-5 and IL-13. The cytokine IL-4 is thought to be very crucial during early stages of the allergic

response (primary sensitisation) since it enhances the differentiation and stimulation of Th2 cells and production of IgE from the B cells. The cytokine IL-13 is considered to play a major role during secondary allergen exposure as it promotes mucus secretion, smooth muscle contraction and airway hyperresponsiveness. Like most other Th2 cytokines, IL-5 promotes eosinophil recruitment, differentiation and activation [19].

b) Pesticides and asthma

Many pesticides are irritants and can induce or aggravate pre-existing asthma through their interaction with functional irritant receptors in the airways thereby inducing neurogenic inflammation [21]. On the other hand, if corrosive, pesticides can also cause direct damage to the airways [21].

Mechanisms by which pesticides act as irritants is explained in detail in a recently published review article [21]. In summary, pesticides like other irritants can activate transient receptor potential (TRP) receptors located in the neuronal cells (sensory C-fibers) and other cells including respiratory epithelia and inflammatory cells leading to the release of inflammatory neuropeptides thereby triggering neurogenic inflammation [21]. An airway neurogenic inflammation is characterized by bronchoconstriction, increased mucus secretion and oedema, which are typical features of asthma [21]. Nonspecific bronchial hyper-responsiveness and consequently asthma symptoms will manifest if this airway inflammation is sustained over time [21].

It has been proposed that organophosphate and carbamate insecticides induce asthma through inhibition of acetylcholinesterase [12]. However, chlorpyrifos (an organophosphate) has been found to cause airway hyperreactivity in guinea pigs through inhibition of the parasympathetic prejunctional muscarinic M2 receptor function at doses below those known to cause acetylcholinesterase inhibition [22]. Inhibition of M2 receptor function can also cause increased mucus secretion [21]. In another animal study, parathion and diazinon (organophosphates) also caused airway hyperreactivity through M2 receptor dysfunction without acetylcholinesterase inhibition [23]. The exact mechanism through which organophosphates decrease M2 receptor function in the airway nerves is unknown. A recent study suggested that the organophosphate inhibition of M2 receptor function is neither through direct

pharmacologic antagonism nor through down regulation of M2 receptor expression [24].

Some pesticides can modulate inflammatory response to common allergens, such as for instance carbaryl has been found to enhance allergic responses to house dust mites in an animal study [25]. Some pesticides may modulate macrophage function. In animal studies, oral administration of malathion resulted in an increased macrophage function in mice, which was postulated to be due to the release of inflammatory mediators (arachidonic acid metabolites and tumour necrosis factor) from mast cells [26]. Interestingly, some pesticides may modulate allergic potential of other pesticides [27]. The allergic potential of phenoxyacetic acid herbicide 2,4-D-butyl and a fungicide, eugenol were increased in mice exposed to parathion and methoxychlor [27]. Eugenol and 2,4-D-butyl are known to be contact allergens. These mice were found to have increased surface antigen expression of T cells and higher numbers of Th1 cytokines (interferon-γ and tumour necrosis factor-α) and interleukin (IL)-17 [27].

Individuals sensitized to common allergens may be more susceptible to the effects of pesticides. This has also been shown in animal studies, in which guinea pigs sensitized to ovalbumin demonstrated a decreased threshold to parathion-induced airway hyperreactivity [28]. Moreover, parathion effects on vagally induced bronchoconstriction were exacerbated in these sensitized guinea pigs [28]. Interestingly, sensitization also changed the mechanism of parathion-induced hyperreactivity from IL-5 independent to IL-5 dependent [28].

Some pesticides such as a carbamate, carbaryl; a fungicide, zineb; and the three herbicides: simazine, alachlor and nitrofen are endocrine disrupters [29]. Endocrine disrupters (EDs) are chemicals that not only interfere with the balance of endocrine system but also influence immune system [29]. Some evidence exists from animal data that demonstrate how EDs can enhance the allergic response. Laboratory studies have demonstrated that some EDs such as nonylphenol and octylphenol can enhance T helper cells 2 (Th2) development and thereby shifting T helper cells 1 (Th1) / Th2 balance towards that favouring the Th2 pathway. Th2 response produces Th2 cytokines such as IL-4, 5 and 13, consequently leading to increased production of immunoglobulin E (IgE) [29].

1.3. JUSTIFICATION

This current analysis will be conducted on data previously collected for a study investigating the health effects of pesticides among rural women in the Western Cape province in South Africa. In this previous sub-study [30] investigating the effects of pesticides on asthma, women with having FeNO levels greater than 50 ppb had an almost 5 fold increased odds (OR = 4.80; 95% CI: 0.80 - 28.00) of cholinesterase (ChE) levels below the laboratory reference standard (6021 IU, Roche Diagnostics®) when compared to women with ChE levels above this reference value. This finding suggested a probable association between exposure to ChE depressing pesticides (organophosphate and carbamate insecticides) and allergic asthma.

In the previous analysis [30] there was however a lack of association between various asthma outcomes and sensitisation to allergens such as house dust mites, spider mites and storage mites encountered on farms known to cause asthma. It was postulated that a possible reason for this was that pesticides to which these women were exposed to may be more important respiratory sensitizers causing allergic airway inflammation than these indoor and outdoor mites. It is anticipated that this current analysis of studying cytokine patterns would shed more light on the pathophysiological mechanisms through which these pesticides may be causing asthma in this group.

Furthermore, in the previous analysis [30], bio-monitoring was limited to only whole blood ChE measurements due to limited resources available in analysing the collected urine samples for the presence of pesticide metabolites. However, in the current analysis, bio monitoring data was available for several pesticide metabolites for characterising the exposure to specific organophosphate and pyrethroid pesticides. These included 3,5,6- trichloropyridinol (TCPY) for chlorpyrifos, 4-fluoro-3-phenoxybenzoic acid (4F3PBA) for cyfluthrin and cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA) for deltamethrin pesticides.

1.4. RESEARCH QUESTIONS

1.4.1. Is pesticide exposure based on objective markers (pesticide metabolites) associated with respiratory symptoms and asthma among women farm workers in the Western Cape Province of South Africa?

1.4.2. What cytokine pathways are present in those with respiratory symptoms and asthma?

1.5. HYPOTHESIS

1.5.1. Pesticide exposure is associated with respiratory symptoms and asthma among these women.

1.5.2. An allergic response involving a Th2 cytokine response is the probable mechanism underlying the airway inflammation suggested by elevated FeNO.

1.6. AIM

To determine the association between pesticides metabolites and respiratory health outcomes among women farm workers in the Western Cape

1.7. OBJECTIVES

i) To determine the urinary concentration of organophosphate and pyrethroid metabolites as markers of short-term pesticide exposure.

ii) To determine serum concentration of Th1 and Th2 cytokines.

iii) To investigate the relationship between exposure to different pesticides (ascertained through pesticide metabolites concentrations in urine) and asthma phenotypes (based on respiratory symptoms, cytokine patterns and exhaled nitric oxide profiles), controlling for known confounders.

2. METHODOLOGY

2.1. Study design

This study involves the analysis of a subset of data that was collected in 2009.The main study was an analytical cross-sectional study of 211 women from farms and towns neighbouring the farms in the Boland region of the Western Cape province of South Africa. The main aim of the larger study was to investigate the respiratory, neurologic and reproductive health effects associated with pesticide exposure.

2.2. Population and sampling

2.2.1. Study population

The study population included women from the farms and towns neighbouring these farms in the Boland region of the Western Cape province of South Africa. There were 5 areas where the study participants were recruited from: Worcester, Paarl,

Stellenbosch, Ceres and Grabouw. The crops grown on the farms were mainly common fruits such as grapes, apples, pears, prunes, berries, lemons and oranges.

2.2.2. Sampling strategy

The recruitment of study participants was accomplished by the non-governmental organisation, Women on farms (WFP). The research team planned to recruit 100 women from farms affiliated to WFP and 100 women not living on farms but from the neighbouring towns in each study area. The targeted sample was 40 women (20 each from farms and towns) from each of the 5 study areas. Due to time constraints and logistical difficulties, the sample was selected by using a non-random sampling method. Farm workers and residents were selected from the 5-10 most accessible farms in each area and town residents from the most accessible houses in each area. One adult female participant per household was selected. WFP recruited a total of 211 women into the study including 113 women currently living on a farm and 98 in towns. Eight women lived in the town but worked in the farm, these were included in the farm worker group. There were a total of 97 farm workers (89 women living in farms and 8 not living in farms). There were 24 women residing but not working on farms. The farm workers and residents (n=121) are referred to as “Farm dwellers”. There were 90 women who neither lived nor worked on a farm and they are referred to as “Town dwellers.

2.2.3. Sample size calculations

Using results from a recently published study [31], the calculated sample size using a two sample comparison of proportions (exposed/control ratio = 1, power = 90%, confidence level = 95%) to detect a 2.25 fold increase in asthma prevalence (20% to 45%) is 160 participants.

2.3. Study instruments

2.3.1. Questionnaire

Trained interviewers administered questionnaires (Appendix A1 and A2) to participants in their language of choice. Briefly, the questionnaire had sections on socio-demographic factors, residential history, household pesticide exposure, environmental pesticide exposure, job history, lifestyle factors and respiratory health. The respiratory health section was a modified version of the European Community Respiratory Health Survey questionnaire [32]. The other sections of the questionnaire

were based on the previous surveys conducted in South Africa [3,33].The questionnaires were translated into Afrikaans and Xhosa and thereafter back translated to ensure accuracy of the translation.

2.3.2. Pesticide metabolite determination

Spot urinary samples (50 ml) were collected in plastic containers topped with a plastic cap and kept on dry ice in the field and during transport and then stored at -20 degrees Celsius before being sent to the laboratory for analysis. The urine samples were couriered to National Institute for Occupational Health (NIOH) laboratory in Johannesburg which has already set up methods for measuring the organophosphate pesticide metabolites, dialkyl phosphates, the chlorpyrifos metabolite, 3,5,6-trichloropyridinol (TCPY) and the pyrethroid metabolites.

An insecticide screen that employs a high performance liquid chromatography/tandem mass spectrometry-based method and quantification using the isotope dilution (ID) calibration [34] was used to screen urine for organophosphate and pyrethroid insecticides. Briefly, using ID calibration, the samples were enriched with isotopically labelled analogues prior to preparation. Chemically the isotope analogue behaves identically to the native analyte, but can be discriminated with a mass filter [35]. This allows complete recovery correction for each sample and improves the sensitivity, accuracy, and selectivity of the analysis. After addition of the labelled standard to urine samples, glucuronide or sulfate-bound urinary metabolites were liberated by enzyme hydrolysis and the analytes were isolated using solid phase extraction. The extractants were concentrated to dryness and reconstituted in solvent for analysis by ID-high performance liquid chromatography-atmospheric chemical ionization-tandem mass spectrometric (MS/MS). Five pyrethroid metabolites [3- phenoxybenzoic acid (3PBA); 4-fluoro-3-phenoxybenzoic acid (4F3PBA); cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA) and cis- and trans-isomers of 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (cis- and trans-DCCA)] and seven organophosphate metabolites [TCPY and the six dialkyl phosphate (DAP) metabolites [dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP)]] were screened. Results were adjusted for concentration by creatinine. The limit of detection (LOD) for all analytes was determined.

2.3.3. Serum cytokines determination

All blood samples were collected during the afternoon and early evening to accommodate those who were working during the day. A blood sample (9 ml) was drawn from each participant using a Becton Dickinson Vacutainer SST tube (with gel medium and clot activator) by the qualified nurse. The blood was allowed to clot for 1-2 hours at room temperature (20-24 degrees Celsius) and then centrifuged at 1350g for 10 minutes at room temperature. The serum was then transferred to another tube and stored at -20 degrees Celsius in a field freezer. The stored serum sample was transported on dry ice to the School of Public Health and Family Medicine, University of Cape Town where it was stored at -80 degrees Celsius until assayed for further measurement. The samples were couriered to the NIOH Immunology laboratory for testing. Some part of the serum was used for other immunology tests in the original study (sensitisation to house dust mite, storage mite and spider mite). The remaining amount of serum was used for cytokine analysis. The BD^{TM} CBA Human Inflammation kit was used to quantitatively measure non-allergic inflammatory interleukin (IL-8, IL-6, IL-10, IL-17, interferon gamma) protein levels in each serum sample. Six bead populations with distinct fluorescence intensities coated with the specific interleukins were multiplexed and resolved in the red channel of the flow cytometer (BD FacsArray^{TM}) (Becton Dickson, Oxford, UK). The inflammatory interleukins associated with allergy (IL-4, IL-5, IL-13) were measured using the BD^{TM} CBA Human Soluble Protein Flex Set assay according to the manufacturer's instructions. The assay allow for multiplexed analysis of multiple proteins from a single sample. The concentrations were determined using the (FacsArray^{TM}) (Becton Dickson, Oxford, UK).

2.3.4. Fractional exhaled nitric oxide (FeNO) determination

Fractional exhaled nitric oxide measurement is a recognised non-invasive method for the assessment of allergic airway inflammation [36,37]. Under the guidance of an experienced nurse, FeNO levels were determined from single-breath exhalations (Appendix B and C). The technique involved inspiration of nitric oxide (NO)-free air via a mouthpiece to total lung capacity, followed immediately by full exhalation at an even rate through the mouthpiece into the apparatus. A hand-held portable nitric oxide sampling device (NIOX MINO® Airway Inflammation Monitor (NIOX MINO); Aerocrine AB, Solna, Sweden) was used. Three technically adequate measurements were performed in line with the current American Thoracic Society /European Respiratory Society recommendations [38]. The FeNO test was done after hours

during the working week. Special instructions were provided to workers to ensure that tested individuals do not smoke tobacco, eat or drink (at least 1 hour before) prior to the test. The participants' height and weight were measured and this information was used to calculate BMI. Ambient NO and temperature were also recorded.

2.4. List and definition of variables

2.4.1. Exposure variables

The exposure variables of interest will be:

(a) Organophosphate metabolite concentrations in urine:

(i) TCPY

(ii) Six DAP metabolites (DMP, DEP, DMTP, DMDTP, DETP and DEDTP)

(b) Pyrethroid metabolites (3PBA; 4F3PBA; DBCA and cis-DCCA and trans-DCCA]

They are all continuous variables but they may be categorised if necessary.

2.4.2. Outcome variables

The following outcome variables will be used for this analysis:

(a) Doctor-diagnosed asthma

(b) FeNO levels:

Two binary variables

- Probable allergic asthma: FeNO > 50ppb
- Possible allergic asthma: FeNO 25-50ppb

(c) Current asthma: Yes to at least one of the following 2 questions: "Have you had an attack of asthma in the last 12 months?" or "Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?"

(d) Adult-onset asthma: doctor-diagnosed asthma and having had the first asthma attack at the age of 16 years or later

(e) Cytokines levels (binary variable categorised as detected vs. non-detected)

Type 1 (non-allergic) and Type 2 (allergic) pattern of elevated cytokines

2.4.3. Covariates

Potential confounders that will be considered include current smoking (binary variable), atopy (binary variable based on Phadiatop test), years of schooling (continuous variable), born on a farm (binary variable), age (continuous variable),

BMI (a continuous variable), employment (binary variable) and household income (continuous variable).

2.5. Pilot study

All study tools were piloted prior to the main study. The fieldwork for the main study was conducted during the period 24 October - 3 December 2009.

2.6. Data management and analysis plan

The database with data input from the questionnaires will be analysed using Stata statistical package version 12 [39]. Exploratory data analysis will be carried out to highlight general features of the data including running descriptive checks to determine the number of observations, the type of variables, presence of outliers, the nature of data cleaning required and the extent of missing data as well as the distributions of the key variables. Descriptive statistics will be calculated to summarize the data. Bivariate analyses will be conducted to assess the nature of the associations between exposures, outcomes and covariates. Multivariate regression analysis will be applied to examine the association between the dependent and independent variables. Confounding by covariates will be considered in the formulation of the models.

3. ETHICS AND COMMUNICATION

The main study was approved by the University of Cape Town's Human Research Ethics Committee (Reference 393/2009) (Appendix D and E). The study was conducted in accordance with the Declaration of Helsinki [40].

3.1. Autonomy

Women were approached to participate in the study on voluntary basis. A written informed consent (Appendix F1 and F2) was obtained from all study participants before participation. Confidentiality of participants' information has been preserved, only the research team have access to the data. The study participants have been assigned unique numbers and only group results will be disseminated.

3.2. Benefit

The study participants were informed of their study findings and those with abnormal results were referred to their medical practitioners. The study findings will provide

more information about the levels of pesticide exposure in these women and their relationship with various respiratory health outcomes including asthma. This information can be used by different stakeholders in the effort to improve health of women on the farms.

3.3. Non-maleficence

There was no harm to the study participants except for the minor discomfort of the needle prick during blood sample collection. There will be no additional risks to the study participants because this current analysis does not require their participation.

3.4. Justice

The reports of respiratory and other health problems among the women in the study site justified the study to be conducted at that particular area.

3.5. Dissemination of research results:

This study is conducted for the partial fulfilment of Master of Medicine (MMED) degree. The final report will be submitted to the University of Cape Town for marking. The study findings will be published in a suitable academic peer-reviewed journal and presented at local and international conferences. A report will be submitted to the stakeholders including the Non-Governmental Organization, Women on Farms Project (WFP).

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Part B: A structured literature review

THE RELATIONSHIP BETWEEN PESTICIDE RESIDUES AND ASTHMA OUTCOMES AMONG WOMEN FARM WORKERS

1. INTRODUCTION

1.1. BACKGROUD

Pesticides are substances used extensively worldwide to kill or control certain forms of unwanted animal or plant life. Pesticides are widely used in agriculture and in several other settings including households, schools, leisure areas and also in the transportation facilities such as aeroplanes and boats [1,2]. Despite their useful effects, pesticides have been associated with various health effects including respiratory health outcomes such as asthma, rhinitis and non-specific respiratory symptoms. Since pesticides are extensively used in the agricultural sector and a significant number of economically active South African population is employed in this sector, pesticides pose a significant public health problem.

1.2. OBJECTIVES OF THE LITERATURE REVIEW

The focus of this literature review was to obtain information on exposure characterisation of pesticides (biomonitoring data), specific pesticides associated with various respiratory health outcomes with a specific focus on asthma, exposure-response relationships, and the pathophysiological mechanisms involved with a particular focus on cytokine pathways.

1.3. SEARCH STRATEGY

Several electronic sources of information were searched for relevant articles including PubMed, Embase and Google Scholar using various key words: allergy, asthma, rhinitis, pesticides, organophosphates, pyrethroids, biological monitoring, dialkyl phosphate, trichloropyridinol, acetylcholinestrase, T helper cells, cytokines, interleukins, farm workers and farmers. Reference lists from the articles obtained were also screened for relevant publications. Information was also sought from non-electronic sources such as dissertations and text books.

2. SPECIFIC PESTICIDES ASSOCIATED WITH ASTHMA

Several epidemiological studies have reported an association between exposure to pesticides and respiratory health effects such as asthma and rhinitis. Exposures to pesticides in these studies have ranged from acute high pesticide exposure events to chronic exposures. Information on the specific pesticides that have been associated with different respiratory health outcomes is summarised in Table 1.

2.1. Insecticides

2.1.1. Organophosphate and carbamate insecticides

Organophosphate and carbamate insecticides inhibit acetylcholinestrase activity. However, while organophosphates irreversibly inhibit acetylcholinesterase, inhibition by carbamates is reversible [3,4]. Organophosphates and carbamates pesticides have shown stronger associations with adverse respiratory health outcomes than other pesticides. In the Agricultural Health Study (AHS) [5], allergic asthma was significantly associated with three organophosphate insecticides: diazinon (odds ratio (OR) = 1.57; 95% confidence interval (CI): 1.05 – 2.35), parathion (OR = 2.05; 95% CI: 1.21 – 3.46) and coumaphos (OR = 2.34; 95% CI: 1.49 – 3.70). On the other hand, phorate (OR = 1.29; 95% CI: 1.01 – 1.65) and malathion (OR = 1.35; 95% CI: 1.04 – 1.75) showed significant association with nonallergic asthma [5]. Another study reported by the AHS demonstrated a significant association between adult onset doctor diagnosed asthma and use of 4 organophosphates; coumaphos (OR = 2.19; 95% CI: 1.02 - 4.69), malathion (OR = 1.60; 95% CI: 1.22 – 2.10), parathion (OR = 2.88; 95% CI: 1.34 – 6.20) and phorate (OR = 2.04; 95% CI: 1.07 – 3.88) [6]. The decrease in plasma cholinesterase of more than 25% was significantly associated (p= 0.018) with a decrease in forced expiratory volume in one second (FEV1) among Spanish agricultural workers, suggesting an association between short-term exposure to acetylcholinesterase inhibiting pesticides and lung dysfunction [7]. Kenyan farm workers with more than 30% inhibition in acetylcholinesterase activity also reported higher rates of respiratory symptoms (prevalence ratio 2.92; 95% CI: 1.12 - 7.61) compared to those without inhibition [8].

Wheeze was strongly associated with reported exposures to two organophosphates; chlorpyrifos (OR = 6.7; 95% CI: 1.6 – 28.0) and terbufos (OR = 5.9; 95% CI: 1.4 – 25.6) among non-smoking farm women in Costa Rica [9]. In the AHS [10], wheeze

was significantly associated with five organophosphates; chlorpyrifos (OR = 1.47; 95% CI: 1.09 - 1.99), dichlorvos (OR = 2.48; 95% CI: 1.09 - 5.64), fonofos (OR = 1.78; 95% CI: 1.07 - 2.98), phorate (OR = 2.87; 95% CI: 1.70 - 4.84), terbufos (OR = 1.66; 95% CI: 1.09 - 2.53). Three organophosphates chlorpyrifos (OR = 1.12; 95% CI: 1.01 - 1.25), malathion (OR = 1.14; 95% CI: 1.02 - 1.28), parathion (OR = 1.50; 95% CI: 1.04 – 2.16), were associated with wheeze in another sub-study of the AHS [11].

Local case reports of two farm workers engaged in pesticides spraying in a large fruit farm in the Western Cape, South Africa who complained of respiratory symptoms had markedly decreased levels of plasma cholinesterase[12]. While one complained of 'asthma', the other one complained of 'chronic bronchitis' [12]. Several chemicals were used in this farm including two organophosphates, chlorpyrifos and azinphos-methyl [12]. A nursery worker from the same farm, also exposed to insecticides suffered from 'asthma' [12].

Organophosphates have also been associated with other respiratory symptoms such as rhinitis. Diazinon was found to be significantly associated (OR = 1.84; 95% CI: 1.23 – 2.75) with current rhinitis in the AHS [13]. Four organophosphate insecticides; dichlorvos (OR = 1.15; 95% CI: 1.03 - 1.28), chlorpyrifos (OR = 1.06; 95% CI: 1.01 - 1.11), diazinon (OR = 1.12; 95% CI: 1.03 - 1.21) and malathion (OR = 1.06; 95% CI: 1.01 - 1.11) were significantly associated with current rhinitis among AHS private pesticide applicators [14].

Use of carbamate insecticides was significantly associated (prevalence odds ratio, POR = 1.9; 95% CI: 1.2 - 3.0) with reported asthma among male farmers in Saskatchewan, Canada [3]. Carbamates used by these farmers include carbofuran, methomyl, and carbaryl [3]. Atopic asthma was significantly associated (OR = 1.41; 95% CI: 1.10 – 1.80) with the use of carbaryl in the AHS [6]. However, there was no significant association between nonatopic asthma and use of the two carbamate insecticides (carbaryl and carbofuran) analysed in this study [6]. Carbamate use was also strongly associated (OR = 2.4; 95% CI: 1.0 – 6.0) with allergic rhinitis among grape farmers in Greece [15].

2.1.2. Organochlorine insecticides

Three organochlorines; chlordane (OR = 1.77; 95% CI: 1.19 – 2.63), heptachlor (OR = 2.01; 95% CI: 1.30 – 3.11) and lindane (OR = 1.57; 95% CI: 1.01 – 2.41) were modestly associated with allergic asthma in the AHS [5]. However in this study [5], nonallergic asthma was statistically significantly associated with only one organochlorine, dichlorodiphenyltrichloroethane (DDT). Not only is DDT use associated with nonallergic asthma, in another sub-study of the AHS [6], DDT use was also associated (OR = 1.79; 95% CI: 1.06 – 3.03) with allergic asthma.

2.1.3. Pyrethroids

Permethrin application on animals has also been associated (OR = 1.71; 95% CI: 1.01 – 2.91) with atopic asthma in the AHS [6]. On the other hand, permethrin use on crops is associated (OR = 2.19; 95% CI: 1.33 – 3.61) with nonatopic asthma [6].Wheeze in the past year is also associated with permethrin use in animals (OR = 1.28; 95% CI: 1.06 – 1.55) [16] and permethrin use in poultry (OR = 1.26; 95% CI: 1.06 – 1.51) [11] in the AHS. Use of permethrin on animals has also been mildly associated (OR = 1.13; 95% CI: 1.03 – 1.23) with current rhinitis in the AHS [14].

2.2. Herbicides

Three herbicides; (RS)-2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) (OR = 1.91; 95% CI: 1.06 – 3.44) , S-ethyl dipropyl(thiocarbamate) (EPTC) (OR = 1.61; 95% CI: 1.06 – 2.43) and paraquat (OR = 1.67; 95% CI: 1.05 – 2.65) were moderately associated with allergic asthma in the AHS [5]. Allergic asthma has also been associated with two herbicides; 2,4-dichlorophenoxyacetic acid (2,4-D) (OR = 1.53; 95% CI: 1.12 – 2.10) and glyphosate (OR = 1.31; 95% CI: 1.02 – 1.67) [6].

Several herbicides have also been associated with wheeze. Wheeze in the past year was associated with the use of 6 herbicides; alachlor (OR = 1.23; 95% CI: 1.06 – 1.41), atrazine (OR = 1.18; 95% CI: 1.05 – 1.32), chlorimuron-ethyl (OR = 1.62; 95% CI: 1.25 – 2.10), EPTC , (OR = 1.37; 95% CI: 1.08 – 1.73) petroleum oil (OR = 1.26; 95% CI: 1.09 – 1.47) and trifluralin (OR = 1.15; 95% CI: 1.02 – 1.30) [16]. In another sub-study of the AHS [10], current use of 8 herbicides (atrazine, chlorimuron-ethyl, glyphosate, imazethapyr, metolachlor, metribuzin, pendimethalin and petroleum oil)

was associated with current wheeze. Furthermore, alachlor, atrazine, chlorimuron-ethyl, EPTC, paraquat, petroleum oil and trifluralin have also demonstrated associations with current wheeze in an earlier study [11]. In a South African study among fruit farm workers [17], long term exposure to paraquat was significantly associated with exercise oxygen desaturation. Interestingly no association was found with the other respiratory outcomes (respiratory symptoms, spirometry, carbon monoxide gas transfer or chest radiography).

Studies of pesticide effects on upper airways have shown that rhinitis was associated with the use of glyphosate and petroleum oil among commercial pesticide applicators [13] as well as among private pesticide applicators [14] in the AHS. Use of 2,4-D was also associated (OR = 1.34; 95% CI: 1.09 – 1.64) with current rhinitis among commercial pesticide applicators [13]. Allergic rhinitis demonstrated associations with glyphosate (OR = 2.5; 95% CI: 1.0 – 6.5) and bipyridyls which includes paraquat and diquat (OR = 4.0; 95% CI: 1.4 - 11.2) [15].

2.3. Fungicides

Allergic asthma has is associated with fungicides; captan (OR = 1.83; 95% CI: 1.15 – 2.94) [5], and metalaxyl (OR = 2.61; 95% CI: 1.35 – 5.04) [6]. Metalaxyl has also been associated (OR = 1.19; 95% CI: 1.02 – 1.38) with current wheeze [11]. Another fungicide, benomyl , demonstrated associations with (OR = 2.35; 95% CI: 1.11 – 4.98) with current rhinitis [13]. Captan use was more prevalent with increased episodes of rhinitis, the strongest association being between 7–12 episodes of rhinitis in past year (OR = 1.32; 95% CI: 1.17 – 1.49) [14]. In a study conducted among workers in Greece, allergic rhinitis was associated with dithiocarbamates (OR = 3.5; 95% CI: 1.2 - 10.2), thiophthalimide (OR = 3.3; 95% CI: 1.2 – 8.7) and triazole (OR = 2.7; 95% CI: 1.0 – 7.0) [15].

2.4. Fumigants

Two fumigants; ethylene dibromide (OR = 2.07; 95% CI: 1.02 – 4.20) and 80/20 mix (carbon tetrachloride and carbon disulfide) (OR = 2.15; 95% CI: 1.23 – 3.76) have been reported with increased odds of with allergic asthma in the AHS [5].

Table 1: Pesticides associated with various respiratory outcomes

Pesticide			Respiratory outcome	Reference
Type	**Chemical Group**	**Active ingredients**		
Insecticide	Organophosphate	Diazinon, parathion, coumaphos	Adult onset allergic asthma (doctor-diagnosed asthma after the age of 19 years with history of doctor-diagnosed eczema or hay fever)	[5]
	Organochlorine	Chlordane, heptachlor, lindane		
Herbicide		2,4,5-TP, paraquat, EPTC		
Fungicide		Captan		
Fumigants		Ethylene dibromide, 80/20 mix		
Insecticide	Organophosphate	Phorate, malathion	Adult onset non-allergic asthma (doctor-diagnosed asthma after the age of 19 years without history of doctor-diagnosed eczema or hay fever)	[5]
	Organochlorine	DDT		
Insecticide	Organophosphate	Coumaphos, malathion, parathion, phorate	Adult onset allergic asthma (doctor-diagnosed asthma after the age of 19 years with history of doctor-diagnosed eczema or hay fever)	[6]
	Carbamate	Carbaryl		
	Pyrethroid	Permethrin (on animals)		
Herbicide		2,4-D, glyphosate		
Fungicide		Metalaxyl		
	Organochlorine	DDT	Adult onset non-allergic asthma (doctor-diagnosed asthma after the age of 19 years without history of doctor-diagnosed eczema or hay fever)	[6]
	Pyrethroid	Permethrin (on crops)		
Herbicide		Paraquat	Exercise oxygen desaturation	[17]
Insecticide	ACHE inhibiting pesticides	Not specified	Forced expiratory volume in one second (FEV1)	[7]
Insecticide	ACHE inhibiting pesticides	Not specified	Respiratory symptoms (chest pain, cough, running nose, wheezing, difficulties in breathing, shortness of breath & irritation of the throat). 3 or more were considered positive	[8]
Insecticide	Organophosphate	Chlorpyrifos, terbufos	Wheezing or whistling sounds in the chest in the past 12 months, without having a cold or flu	[9]
	Organophosphate	Chlorpyrifos, terbufos, dichlorvos, fonofos, phorate	Wheezing or whistling in the chest in the past 12 months	[10]
Herbicide		Atrazine, chlorimuron-ethyl, glyphosate, imazethapyr, metolachlor, metribuzin, pendimethalin, petroleum oil		
Insecticide	Organophosphate	Chlorpyrifos, malathion, parathion	Wheezing or whistling in the chest in the past 12	[11]

	Pyrethroid	Permethrin (on poultry)	months	
Herbicide		Alachlor, atrazine, chlorimuron-ethyl, EPTC, paraquat, petroleum oil, trifluralin		
Fungicide		Metalaxyl		
Insecticide	Pyrethroid	Permethrin (on animals)	Wheezing or whistling in the chest in the past 12 months	[16]
Herbicide		Alachlor, atrazine, chlorimuron-ethyl, EPTC, petroleum oil, trifluralin		
Insecticide	Organophosphate	Not specified	Nursing sister diagnosed asthma and chronic bronchitis	[12]
Insecticide	Carbamate	Not specified	Doctor-diagnosed asthma	[3]
Insecticide	Organophosphate	Diazinon	Stuffy, itchy or runny nose in the past 12 months	[13]
Herbicide		Glyphosate, petroleum oil, 2,4-D		
Fungicide		Benomyl		
Insecticide	Organophosphate	Dichlorvos, chlorpyrifos, diazinon, malathion	Stuffy, itchy or runny nose in the past 12 months	[14]
	Pyrethroid	Permethrin (on animals)		
Herbicide		Glyphosate, petroleum oil		
Fungicide		Captan		
Insecticide	Carbamate	Not specified	Two or more nasal symptoms (rhinorrhoea, sneezing, nasal obstruction and nasal itching) during the last 12 months, apart from a cold PLUS positive skin prick test (SPT) and/or a positive enzyme immunoassay test	[15]
		Glyphosate,		
Herbicide	Bipyridyl	Paraquat, diquat		
Fungicide	Dithiocarbamates, thiophthalimides, triazoles	Not specified		

2,4,5-TP: 2,4,5-trichlorophenoxypropionic acid; EPTC: ethyl dipropylthiocarbamate;

DDT: dichlorodiphenyltrichloroethane; 2,4-D: 2,4-dichlorophenoxyacetic acid

3. DOSE – RESPONSE RELATIONSHIPS

There are few studies that have investigated dose-response relationships between pesticide exposure and respiratory health outcomes [5,6,11,13,14,18]. In the recently published AHS among *male* farmers [5], ten of the 12 pesticides associated with allergic asthma had demonstrable dose-response relationships. Although a history of ever-use of 2,4-D, 2,4,5-T ((2,4,5-trichlorophenoxy)- acetic acid) and permethrin on animals was not significantly associated with allergic asthma, these insecticides demonstrated significant dose-response trends for allergic asthma [5]. Overall, the specific pesticides that showed a significant dose-response relationship with allergic asthma in this study included two organophosphates: coumaphos and parathion; three organochlorines: chlordane, heptachlor and lindane; one pyrethroid: permethrin used on animals; four herbicides: 2,4,5-T, 2,4,5-TP, 2,4-D and EPTC; one fungicide: captan and two fumigants: 80/20 mix and ethylene dibromide [5]. On the other hand, DDT and malathion demonstrated statistically significant dose-response relationships for non-allergic asthma [5]. However, there were no significant dose-response trends observed between pesticide exposure variables and asthma among *women* farmers in the AHS [6].

Studies among South Korean farmers demonstrated significant dose-response trends between restrictive ventilatory defects and paraquat exposure variables: paraquat application years (p=0.015 for trend) and lifetime paraquat application days (p=0.007 for trend) [18]. However, there were no significant dose-response relationships for obstructive ventilatory defects [18].

Pesticide use has also been shown to have significant dose-response relationships with wheeze. The odds of wheeze increased with the duration of pesticide for one herbicide chlorimuron-ethyl (p= 0.012 for trend), and two organophosphates; chlorpyrifos (p= 0.003 for trend) and phorate (p= 0.010 for trend) among commercial pesticide applicators in the AHS [10]. In another sub-study of the AHS [16], all these three pesticides (chlorimuron-ethyl, chlorpyrifos and phorate) demonstrated significant dose-response relationships for wheeze among commercial pesticide applicators, while only chlorimuron-ethyl and chlorpyrifos demonstrated significant dose-response trends among farmers. Parathion, malathion, chlorpyrifos, 2,4-D, atrazine, alachlor, EPTC, paraquat and carbaryl demonstrated significant dose-response relationships with wheeze [11].

The odds of rhinitis increased significantly with the duration of use of petroleum oil and diazinon [13]. Petroleum oil also showed significant dose-response trends with rhinitis in another study among private pesticide applicators [14]. In this latter study [14], the use of permethrin on animals also demonstrated significant dose-response relationships with rhinitis episodes.

4. EXPOSURE ASSESSMENT FOR PESTICIDES – BIOMONITORING APPROACHES

Traditionally, acetylcholinesterase measurements in blood have been used as a proxy for estimating exposure to organophosphate and carbamate insecticides. With increasing laboratory analytical capabilities, several pesticide metabolites, commonly measured in the urine samples, have also been used to estimate exposure to pesticides. The most commonly measured metabolites for estimating exposure to organophosphates are the six dialkyl phosphate (DAP) metabolites [dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP) [19]. These DAP metabolites are non-specific, in that they reflect short-term exposure to organophosphates but are not able to identify a specific organophosphate. The metabolite 3,5,6- trichloropyridinol (TCPY), on the other hand, is a specific metabolite frequently measured for estimating exposure to a commonly used organophosphate, chlorpyrifos [20]. Other less commonly measured specific organophosphate metabolites include α and β isomers of malathion monocarboxylic acid and malathion dicarboxylic acid for malathion, and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY) for diazinon [20,21]. The metabolite 4-nitrophenol has been used for estimating exposure to methyl and ethyl parathion but it is also a metabolite of EPN (O-ethyl O-(4-nitrophenyl) P-phenylphosphonothioate) and other non-organophosphate chemicals [20].

A metabolite of carbaryl, 1-naphthol has been measured to estimate exposure to this commonly used carbamate [20]. For pyrethroid insecticides, 3- phenoxybenzoic acid (3PBA) is a non-specific metabolite, common to about 20 synthetic pyrethroids. There are other more specific metabolites of pyrethroids that have been measured, including 4-fluoro-3-phenoxybenzoic acid (4F3PBA), a metabolite of cyfluthrin; cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA), a metabolite of

deltamethrin; and cis- and trans isomers of 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (cis- and trans-DCCA) which are metabolites of permethrin, cypermethrin and cyfluthrin [20].

5. PATHOPHYSIOLOGICAL MECHANISMS

5.1 Cytokine pathways in asthma

Over the past few years, allergic airway inflammation that occurs in asthmatic individuals has been classified as being either eosinophilic or non-eosinophilic. It is widely accepted that eosinophilic airway inflammation is driven by the Th2 response [30], particularly so in mild and moderate asthma. However, the inflammatory response seen in severe asthma is complex as the Th1 response is also involved [30,31]. Th2 response produces several cytokines including IL-3, IL-4, IL-5, IL-9, IL-10, IL-13 and IL-33 [30,31]; however, the major Th2 cytokines are IL-4, IL-5 and IL-13. The cytokine IL-4 is thought to be very crucial during early stages of the allergic response (primary sensitisation) since it enhances the differentiation and stimulation of Th2 cells and production of IgE from the B cells. The cytokine IL-13 is considered to play a major role during secondary allergen exposure as it promotes mucus secretion, smooth muscle contraction and airway hyperresponsiveness. Like most other Th2 cytokines, IL-5 promotes eosinophil recruitment, differentiation and activation [30]. The predominant functions of different cytokines are summarised in Table 2. Various cytokines associated with different pathophysiological features of asthma are summarised in Table 3.

5.2 Pesticides and asthma

Many pesticides are irritants and can induce or aggravate existing asthma through their interaction with functional irritant receptors in the airways thereby inducing neurogenic inflammation [4]. On the other hand, if corrosive, pesticides can also cause direct damage to the airways [4].

Mechanisms by which pesticides act as irritants is explained in detail in a recently published review article [4]. In summary, pesticides like other irritants can activate transient receptor potential (TRP) receptors located in the neuronal cells (sensory C-fibers) and other cells including respiratory epithelia and inflammatory cells leading to the release of inflammatory neuropeptides thereby triggering neurogenic

inflammation [4]. An airway neurogenic inflammation is characterized by bronchoconstriction, increased mucus secretion and oedema which are typical features of asthma [4]. Nonspecific bronchial hyper-responsiveness and consequently asthma symptoms will manifest if this airway inflammation is sustained over time [4].

It has been proposed that organophosphate and carbamate insecticides induce asthma through inhibition of acetylcholinesterase [3]. However, chlorpyrifos (an organophosphate) has been found to cause airway hyperreactivity in guinea pigs through inhibition of the parasympathetic prejunctional muscarinic M2 receptor function at doses below those known to cause acetylcholinesterase inhibition [22]. Inhibition of M2 receptor function can also cause increased mucus secretion [4]. In another animal study, parathion and diazinon (organophosphates) also caused airway hyperreactivity through M2 receptor dysfunction without acetylcholinesterase inhibition [23]. The exact mechanism through which organophosphates decrease M2 receptor function in the airway nerves is unknown. A recent study has suggested that the organophosphate inhibition of M2 receptor function is neither through direct pharmacologic antagonism nor through down regulation of M2 receptor expression [24].

Some pesticides can modulate inflammatory response to common allergens, for instance carbaryl has been found to enhance allergic responses to house dust mites in an animal study[25]. Some pesticides may modulate macrophage function. In animal studies, oral administration of malathion resulted in an increased macrophage function in mice, which was postulated to be due to the release of inflammatory mediators (arachidonic acid metabolites and tumour necrosis factor) from mast cells [26]. Interestingly, some pesticides may modulate allergic potentials of other pesticides [27]. The allergic potential of phenoxyacetic acid herbicide 2,4-D-butyl and a fungicide, eugenol were increased in mice exposed to parathion and methoxychlor [27]. Eugenol and 2,4-D-butyl are known to be contact allergens. These mice were found to have increased surface antigen expression of T cells and higher numbers of Th1 cytokines (interferon-IFN-γ and tumour necrosis factor-α) and interleukin (IL)-17 [27].

Individuals sensitized to common allergens may be more susceptible to the effects of pesticides. This has also been shown in animal studies, in which guinea pigs sensitized to ovalbumin demonstrated a decreased threshold to parathion-induced

airway hyperreactivity [28]. Moreover, parathion effects on vagally induced bronchoconstriction were exacerbated in these sensitized guinea pigs [28]. Interestingly, sensitization also changed the mechanism of parathion-induced hyperreactivity from IL-5 independent to IL-5 dependent [28].

Some pesticides such as a carbamate, carbaryl; a fungicide, zineb; and the three herbicides: simazine, alachlor and nitrofen are endocrine disrupters [29]. Endocrine disrupters (EDs) are chemicals that not only interfere with the balance of endocrine system but also influence immune system [29]. Some evidence exists from animal data that demonstrate how EDs can enhance the allergic response. Laboratory studies have demonstrated that some EDs can enhance T helper cells 2 (Th2) development and thereby shifting T helper cells 1 (Th1) / Th2 balance towards that favouring the Th2 pathway. Th2 response produces Th2 cytokines such as IL-4, 5 and 13, consequently leading to increased production of immunoglobulin E (IgE) [29].

Table 2: Predominant functions of interleukins [modified from [31]]

Cytokines	Predominant function(s)
IL-2	Eosinophilic recruitment, activation and inflammation
IL-3	Eosinophilic recruitment, activation and inflammation
IL-4	Eosinophilic recruitment, activation and inflammation
IL-5	Eosinophilic recruitment, activation and inflammation
IL-6	Pro- and anti-inflammatory
IL-8	Neutrophil recruitment
IL-9	Eosinophilic recruitment, activation and inflammation
IL-10	Immunomodulation, anti-inflammatory
IL-13	Eosinophilic recruitment, proinflammatory
IL-17	Proinflammatory
IL-33	Eosinophil recruitment, proinflammatory

Table 3: Cytokines associated with pathophysiological features of asthma [modified from [31]]

Aspects of asthma	Interleukins
Airway hyperresponsiveness	IL-2, IL-4, IL-5, IL-9, IL-13, IL-18, IL-33, TNF-α
IgE production	IL-4, IL-9, IL-13, IL-18
Goblet cell metaplasia	IL-4, IL-13
Mucin hypersecretion	IL-9
Mastocytosis	IL-9
Mast cell degranulation/migration	IL-9, IL-33
Eosinophilia	IL-4, IL-5, IL-9, IL-13, IL-17, TNF- α
Neutrophilia	IL-1, IL-2, IL-18, IL-33, TNF-α, IFN-γ
Th2 induction	IL-4, IL-5, IL-9, IL-13, IL-25, IL-33
Airway smooth muscle hypertrophy	IL-13, IL-33
Remodelling epithelial damage/repair	IL-5, IL-9, IL-18, IL-33
Subepithelial fibrosis	IL-13, IL-33
Exacerbations	IL-4, IL-5, IL-6, IL-8, TNF-α

6. CONCLUSION

Several epidemiological studies have demonstrated associations between various specific pesticides and respiratory health outcomes including asthma and rhinitis, some of these showing evidence suggestive of possible dose-response relationships, but the methods for estimating exposures are generally crude and reliant on self-reported exposures rather than objective markers for individual pesticides implicated. Future biological monitoring studies should therefore use standardized units of measurement in order to allow for easier comparison of different levels of pesticide residues between studies.

Furthermore, since the pathophysiological mechanisms of asthma associated with exposure to pesticides is heterogeneous and not clearly understood; more studies are needed, particularly at the molecular level, in order to better understand the mechanisms involved. This will enable the development of improved approaches for exposure monitoring and medical surveillance of workers at increased risk of asthma and rhinitis associated with pesticides.

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Part C: Publication-ready manuscript

This manuscript has been prepared to be submitted for publication in the Journal, International Archives of Allergy and Immunology. The format of the article follows the journal's guidelines for authors (Appendix G) except for the tables which are included in the main text and authors are not mentioned.

Title: The relationship between pesticide metabolites and asthma outcomes among women farm workers

Key words: Asthma, airway inflammation, cytokines, pesticide metabolites, biomonitoring

Short title: Pesticide metabolites and asthma

Abstract

Background: A previous sub-study reported an association between allergic airway inflammation as determined by fractional exhaled nitric oxide (FeNO) and low levels of whole blood cholinesterase. This current study investigated the relationship between pesticide exposure (urinary pesticide metabolites) and asthma phenotypes (based on respiratory symptoms, cytokine patterns and exhaled nitric oxide profiles) among these women.

Methods: A cross-sectional study was conducted among rural women working and living on the farms (farm dwellers, n=121) and those residing in the neighbouring towns (town dwellers, n=90). Pesticide exposure was assessed based on urinary metabolite concentrations of organophosphate (OP) and pyrethroid (PYR) residues. Health outcome assessment was ascertained through modified European Community Respiratory Health Survey questionnaire, FeNO concentrations, serum cytokine (Th2 and non-Th2 markers) concentrations, and immunological markers for atopy (Phadiatop) and specific IgE to house dust mite, storage mite and spider mite.

Results: The median age of the study participants was 37 years (interquartile range: 28 - 45 years). The urinary concentration (median and interquartile range) of OP metabolites (sum of the 6 dialkyl phosphate metabolites) = 134 (42-229) µg/g of creatinine; 3,5,6-trichloropyridinol (TCPY) = 5 (3-10) µg/g of creatinine; and for PYR (sum of the 5 PYR metabolites) = 6 (3-10) µg/g of creatinine. The prevalence of current asthma (asthma attack in the last 12 months or currently taking asthma medication) was 6%; doctor diagnosed asthma was 11%; and adult-onset asthma (history of doctor-diagnosed asthma and first asthma attack at age 16 years or older) was 9%. The proportion of subjects with FeNO above 50 ppb was 7% and between 25-50 ppb was 11%. The proportion of Th2 cytokines (IL-4, IL-5 and IL-13) detected ranged from 18% to 40% while non-Th2 cytokines (IL-6, IL-8, IL-10, IL-17 and interferon-γ) ranged from 35% to 71%. IL-8 was the most commonly detected (71%) cytokine while IL-5 was the least detected (18%). Most OP metabolites were positively associated with FeNO levels above 50ppb. Both Th2 and non-Th2 cytokines were positively associated with either OP or PYR metabolites. Non-Th2 cytokines showed stronger associations with OP metabolites (the strongest association between diethyl dithiophosphate (DEDTP) and interferon-γ: prevalence odds ratio (95% confidence interval) = 25 (8-78)).

Conclusion: OP and PYR urinary metabolite levels in rural women in the Western Cape are higher than in the general population. This study provides evidence suggesting that both OP and PYR pesticides are associated with asthma, which may be due to Th2 and non-Th2 mechanisms, the latter pathway demonstrating consistently stronger relationships.

1. Introduction

Pesticides are substances used extensively worldwide to kill or control certain forms of unwanted animal or plant life. Pesticides are widely used in agriculture and in several other settings including households, schools, leisure areas and also in the transportation facilities such as aeroplanes and boats [1,2]. Since pesticides are extensively used in the agricultural sector and a significant number of economically active South African population is employed in this sector, pesticides pose a significant public health problem.

Despite their useful effects, pesticides have been associated with various health effects including adverse respiratory health outcomes such as asthma, rhinitis and non-specific respiratory symptoms [3]. In summary, the following specific pesticides have been associated with various adverse respiratory health outcomes including asthma: OP insecticides: diazinon, parathion, coumaphos, phorate, malathion, chlorpyrifos, terbufos, dichlorvos and fonofos; carbamate insecticide, carbaryl; organochlorine insecticides: chlordane, heptachlor, lindane and DDT (dichlorodiphenyltrichloroethane); PYR insecticide, permethrin; herbicides: 2,4,5-TP (2,4,5-trichlorophenoxypropionic acid), paraquat, diquat, EPTC (ethyl dipropylthiocarbamate), 2,4-D (2,4-dichlorophenoxyacetic acid), glyphosate, atrazine, chlorimuron-ethyl, imazethapyr, metolachlor, metribuzin, pendimethalin, petroleum oil, alachlor and trifluralin; fungicides: captan, metalaxyl and benomyl; and fumigants: ethylene dibromide and 80/20 mix [4–11]. A few other studies have demonstrated the association between different adverse respiratory health outcomes and the exposure to broad groups of pesticides but the specific pesticides were not identified [12–16].

The pathophysiological mechanism of asthma associated with exposure to pesticides is heterogeneous and not clearly understood. More studies are needed, particularly at the molecular level to better understand the mechanisms involved. Furthermore, the methods for estimating exposures used in previous studies have been generally crude and reliant on self-reported exposures highlighting the need for more objective markers for individual pesticides.

Traditionally, acetylcholinesterase measurements in blood have been used as a proxy for estimating exposure to OP and carbamate insecticides. With increasing laboratory analytical capabilities, several pesticide metabolites, commonly measured

in the urine samples, have also been used to estimate exposure to pesticides. The most commonly measured metabolites for estimating exposure to OPs are the six dialkyl phosphate (DAP) metabolites [dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP)] [17]. These DAP metabolites are non-specific, in that they reflect short-term exposure to OPs but are not able to identify a specific OP. The metabolite 3,5,6- trichloropyridinol (TCPY), on the other hand is a specific metabolite frequently measured for estimating exposure to a commonly used OP, chlorpyrifos [17–19]. Other less commonly measured specific OP metabolites include α and β isomers of malathion monocarboxylic acid and malathion dicarboxylic acid for malathion and 2-isopropyl- 4-methyl-6-hydroxypyrimidine (IMPY) for diazinon [18,20]. The metabolite 4-nitrophenol has been used for estimating exposure to methyl and ethyl parathion but it is also a metabolite of EPN (O-ethyl O-(4-nitrophenyl) P-phenylphosphonothioate) and other non-organophosphate chemicals [18].

For PYR insecticides, 3- phenoxybenzoic acid (3PBA) is a non-specific metabolite, common to about 20 synthetic pyrethroids. There are other more specific metabolites of pyrethroids that have been measured, including 4-fluoro-3-phenoxybenzoic acid (4F3PBA), a metabolite of cyfluthrin; cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA), a metabolite of deltamethrin; and cis- and trans isomers of 2,2-dichlorovinyl-2,2- dimethylcyclopropane-1-carboxylic acid (cis- and trans-DCCA) which are metabolites of permethrin, cypermethrin and cyfluthrin [18].

Over the past few years, allergic airway inflammation that occurs in asthmatic individuals has been classified as being either eosinophilic or non-eosinophilic. It is widely accepted that eosinophilic airway inflammation is driven by the Th2 response [21], particularly so in mild and moderate asthma. However, the inflammatory response seen in severe asthma is complex as the Th1 response is also involved [21,22]. Th2 response produces several cytokines including interleukins (IL) -3, IL-4, IL-5, IL-9, IL-10, IL-13 and IL-33 [21,22]; however, the major Th2 cytokines are IL-4, IL-5 and IL-13. The cytokine IL-4 is thought to be very crucial during early stages of the allergic response (primary sensitisation); it enhances the differentiation and stimulation of Th2 cells and production of IgE from the B cells. The cytokine IL-13 is considered to play a major role during secondary allergen exposure as it promotes mucus secretion, smooth muscle contraction and airway hyperresponsiveness. Like

most other Th2 cytokines, IL-5 promotes eosinophil recruitment, differentiation and activation [21].

This analysis is part of a larger study investigating various health effects of pesticide exposure among rural women in the Western Cape Province in South Africa. In a previous sub-study [3], women having FeNO levels greater than 50 ppb had an almost 5 fold increased odds (OR = 4.80; 95% CI: 0.80 - 28.00) of cholinesterase (ChE) levels below the laboratory reference standard (6021 IU, Roche Diagnostics®) when compared to women with ChE levels above this reference value. This finding suggested a probable association between exposure to ChE depressing pesticides (organophosphate and carbamate insect ides) and allergic asthma. This current study was conducted to determine urinary levels of OP and PYR pesticide metabolites; to examine cytokine pathways among individuals exposed to pesticides; and finally to assess the association between exposure to these pesticides and various asthma phenotypes.

2. Materials and Methods

2.1. Study design, population and sampling

A cross-sectional study of women farm workers and residents living in towns neighbouring the farms, in the Western Cape Province of South Africa was conducted during the period 24 October to 3 December 2009. The recruitment of study participants was accomplished with the assistance of a non-governmental organisation, Women on farms (WFP). The farm areas included Stellenbosch, Ceres, Paarl, Grabouw and Worcester. Farm workers and residents were selected from the 5-10 most accessible but representative farms in each area and town dwellers from the most accessible and representative houses in each area. One adult female participant per household was selected. A total of 211 women were recruited into the study including 113 women currently living on a farm and 98 in towns. Eight women lived in the town but worked on a farm, these were included in the farm worker group. There were a total of 97 farm workers (89 women living in farms and 8 not living in farms). There were 24 women residing but not working on farms. The farm workers and residents (n = 121) are referred to as “farm dwellers”. There were 90 women who neither lived nor worked on a farm and they are referred to as “town dwellers”. The study was conducted in accordance with the Declaration of Helsinki (23). The study was approved by the University of Cape Town’s Human Research Ethics Committee (Reference 393/2009) (Appendix D and E).

2.2. Questionnaire

The questionnaire had sections on socio-demographic aspects (age, schooling, home language, income, employment); residential history (place of residence on a farm or town, period of residence); environmental pesticide exposure (pesticide drift, distance of residence to spraying and other exposures to agricultural spraying); job history (farm worker, non-farm worker, number of years in a job, job title); household pesticide exposure; lifestyle factors (smoking and alcohol consumption) and respiratory health. The respiratory health section incorporated the abbreviated form of the standardised and validated European Community Respiratory Health Survey questionnaire [24]. The non-respiratory questions in the questionnaire were based on those used in previous surveys conducted in South Africa [4,25]. The questionnaire was translated into Afrikaans and Xhosa and thereafter back translated to ensure accuracy of the translation. Trained interviewers administered questionnaires to participants in the language of their choice.

2.3. Urinary pesticide metabolites determination

Spot urinary samples (50 ml) were collected in plastic containers topped with a plastic cap and kept on dry ice in the field and during transport and then stored at -20 degrees Celsius before being sent to the laboratory for analysis. The urine samples were couriered to National Institute for Occupational Health (NIOH) laboratory in Johannesburg which conducted the analyses for organophosphate pesticide metabolites, dialkyl phosphates, the chlorpyrifos metabolite, TCPY and the pyrethroid metabolites.

An insecticide screen that employs a high performance liquid chromatography/tandem mass spectrometry-based method and quantification using the isotope dilution (ID) calibration [26] was used to screen urine for organophosphate and pyrethroid insecticides. Briefly, using ID calibration, the samples were enriched with isotopically labelled analogues prior to preparation. Chemically the isotope analogue behaves identically to the native analyte, but can be discriminated with a mass filter [27]. This allows complete recovery correction for each sample and improves the sensitivity, accuracy, and selectivity of the analysis. After addition of the labelled standard to urine samples, glucuronide or sulfate-bound urinary metabolites were liberated by enzyme hydrolysis and the analytes were

isolated using solid phase extraction. The extractants were concentrated to dryness and reconstituted in solvent for analysis by ID-high performance liquid chromatography-atmospheric chemical ionization-tandem mass spectrometric (MS/MS). Five PYR metabolites (3PBA, 4F3PBA, DBCA, cis-DCCA and trans-DCCA) and seven OP metabolites (TCPY and the six DAP metabolites (DMP, DEP, DMTP, DMDTP, DETP and DEDTP)) were screened. Results were adjusted for concentration by creatinine. The limit of detection (LOD) for the pesticide metabolites were 0.5 µg/l for TCPY; 1 µg/l for DMP; and 0.05 µg/l for DMTP, DMDTP, DEP, DETP, DEDTP, cis-DCCA, trans-DCCA, DBCA, 4F3PBA and 2PBA .

2.4. Serum cytokines determination

All blood samples were collected during the afternoon and early evening to accommodate those who were working during the day. A blood sample (9 ml) was drawn from each participant using a Becton Dickinson Vacutainer SST tube (with gel medium and clot activator) by the qualified nurse. The blood was allowed to clot for 1-2 hours at room temperature (20-24 degrees Celsius) and then centrifuged at 1350g for 10 minutes at room temperature. The serum was then transferred to another tube and stored at -20 degrees Celsius in a field freezer. The stored serum sample was transported on dry ice to the School of Public Health and Family Medicine, UCT where it was stored at -80 degrees Celsius until assayed for further measurement. The samples were couriered to the NIOH Immunology laboratory for testing. Some part of the serum was used for other immunology tests in the original study (sensitisation to house dust mite, storage mite and spider mite). The remaining amount of serum was used for cytokine analysis. The BD^{TM} CBA Human Inflammation kit was used to quantitatively measure non-allergic inflammatory interleukin (IL-8, IL-6, IL-10, IL-17, interferon gamma) protein levels in each serum sample. Six bead populations with distinct fluorescence intensities coated with the specific interleukins were multiplexed and resolved in the red channel of the flow cytometer (BD FacsArray^{TM}) (Becton Dickson, Oxford, UK).

The inflammatory interleukins associated with allergy (IL-4, IL-5, IL-13) were measured using the BD^{TM} CBA Human Soluble Protein Flex Set assay according to the manufacturer's instructions. The assay allow for multiplexed analysis of multiple proteins from a single sample. The concentrations were determined using the (FacsArray^{TM}) (Becton Dickson, Oxford, UK).

Standard curves of the standard serial dilutions, expressed by a 4 parameter logistic model (log CC = D + (A – D) / (1 + (log I / C)^B) (where A=minimum asymptote, B=slope factor, C=inflection point, D=maximum asymptote), were used to determine the limit of detection (LOD) for each specific analyte (IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, IFN-γ) based on the average median fluorescent intensity (MFI) of the negative control. The concentration of analyte was expressed as picogram per millilitre (pg/ml). A regression coefficient (R^2) for the standard curve for each analyte was accepted if ≥ 0.98. The assay detection limit for each analyte was determined by the average fitted concentration of the negative control (0 pg/ml) + 2 standard deviations for each analyte. The LOD for each cytokine were 0.10 pg/ml for IL-4, IL-5, IL-8, IL-13 and IFN-γ; 0.14 pg/ml for IL-6; 0.20 pg/ml for IL-10 and 0.22 pg/ml for IL-17 (Table 3).

2.5. Fractional exhaled nitric oxide (FeNO determination

Fractional exhaled nitric oxide measurement is a recognised non-invasive method for the assessment of allergic airway inflammation [28,29]. Under the guidance of an experienced nurse FeNO levels were determined from single-breath exhalations. The technique involved inspiration of nitric oxide (NO)-free air via a mouthpiece to total lung capacity, followed immediately by full exhalation at an even rate through the mouthpiece into the apparatus. A hand-held portable nitric oxide sampling device (NIOX MINO® Airway Inflammation Monitor (NIOX MINO); Aerocrine AB, Solna, Sweden) was used. Three technically adequate measurements were performed in line with the current American Thoracic Society /European Respiratory Society recommendations [30]. The FeNO test was done after hours during the working week. Special instructions were provided to workers to ensure that tested individuals do not smoke tobacco, eat or drink (at least 1 hour before) prior to the test. The participants' height and weight were measured and this information was used to calculate BMI. Ambient NO and temperature were also recorded.

2.6. Statistical analysis

The exposure variables of interest were the urinary levels of OP metabolites including diakyl phosphates (DMP, DEP, DMTP, DMDTP, DETP and DEDTP) and TCPY and PYR metabolites (3PBA; 4F3PBA; DBCA and cis-DCCA and trans-DCCA). Two additional variables included the sum of all 6 DAP metabolites and the sum of all 5 pyrethroid metabolites. These metabolites levels were also dichotomised as above the 75th percentile of the detected values.

The main outcome variables defined were doctor-diagnosed asthma, adult-onset asthma, current asthma, FeNO levels between 25 and 50ppb, FeNO levels above 50ppb and the cytokine levels detected. Adult-onset asthma was defined as history of doctor-diagnosed asthma and having had the first asthma attack at the age of 16 years or later. Current asthma was defined as having had an attack of asthma in the last 12 months or currently taking medicines for asthma. Cytokine levels were dichotomised as individually detected and non-detected as well as any Th2 or non-Th2 detected in serum.

The data was analysed using Stata statistical package version 12 [31]. Frequency distributions, Chi-square test and simple logistic regressions were used to assess the association between categorical variables. Continuous variables were summarised using median and interquartile range since they were not normally distributed. Wilcoxon sum rank test was used to assess the association between binary variables and continuous variables. Multivariate regression analyses were used to examine the association between the outcomes of interest and pesticide metabolites controlling for confounders. Confounders selected to be included in the models were current smoking, atopy, born on a farm, and level of education as demonstrated in the previous sub-study [3].

3. Results

3.1. Demographic characteristics, allergic and asthma outcomes (n=211)

The demographic characteristics and the prevalence of asthma and allergic outcomes are presented in Table 1. Town dwellers were significantly older and with significantly higher body mass index (BMI) than farm dwellers. Level of education was generally low in both town and farm dwellers.

Overall, half of the study population were current smokers with a higher proportion (57%) being among farm dwellers (Table 1). The majority of farm dwellers were employed unlike the town dwellers. A large proportion (69%) of women who were born on a farm were still living and working on a farm although a minority (14%) of town dwellers reported being born on a farm.

Town dwellers had on average significantly higher FeNO levels compared to farm dwellers (Table 1). Almost half (44%) of the study population were atopic. Interestingly, the prevalence of all asthma and allergic outcomes was higher among town dwellers, although this did not reach statistical significance. The levels of IgE to storage mite and spider mite were not significantly different between farm and town dwellers.

Table 1: Demographic characteristics, allergic and asthma outcomes among rural women in the Western Cape (n=211)

Characteristics	Farm dwellers (n = 121)	Town dwellers (n = 90)	Overall (n = 211)
Demographic characteristics: (median, interquartile range)			
Age (years)	33(27-40)***	40.5(31-49)	37(28-45)
BMI (kg/m2)	25.18(21.57-30.81)**	28.58(23.78-35.71)	26.44(22.52-32.87)
Education (years of schooling)	9(7-10)	9 (7-11)	9(7-10)
Length of stay in current residence (years)	15(8-24)**	22(12-41)	17(9-29)
Born on a farm: n (%)	83 (69)***	13 (14)	96 (46)
Current smoker: n (%)	69 (57)*	36 (40)	105 (50)
Currently employed: n (%)	101 (84)***	25 (28)	126 (60)
Asthma phenotypes: n (%)			
Asthma attack in the last 12 months	2 (2)	1 (1)	3 (1)
Currently taking medicines for asthma	4 (3)	8 (9)	12 (6)
Current asthma	4 (3)	8 (9)	12 (6)
Doctor-diagnosed asthma	11 (9)	12 (13)	23 (11)
Adult-onset asthma	9 (7)	10 (11)	19 (9)
FeNO (ppb): median (interquartile range)	9.17(5.67-14)**	12.33(8.33-22.33)	10.33(6-17.33)
FeNO=25ppb-50ppb	11 (9)	12 (14)	23 (11)
FeNO>50ppb	7 (6)	8 (9)	15 (7)
Allergic sensitisation: n (%)			
Atopy (positive Phadiatop)	46 (38)	44 (51)	90 (44)
Storage mite sensitisation (*Lepidoglyphus destructor)*	23 (19)	21 (24)	44 (21)
Spider mite sensitisation (*Tetranychus urticae)*	18 (15)	17 (20)	35 (17)

*p < 0.05; **p < 0.01; ***p < 0.001; BMI: body mass index
Current smoker: having smoked at least 20 packs of cigarettes or 30 grams of tobacco in a lifetime or at least one cigarette per day for one year AND having smoked tobacco in the last month or more
Current asthma: asthma attack in the last 12 months or currently taking medicines for asthma;
Adult-onset asthma: history of doctor-diagnosed asthma and having had the first asthma attack at the age of 16 years or later

3.2. Urinary pesticide metabolite concentrations

Table 2 summarises the results of the urinary pesticide metabolites (adjusted for creatinine) detected in the study population. The descriptive statistics presented in Table 2 are only for the detected values. However, when the data for the values below LOD were transformed to the LOD divided by the square root of 2 and the descriptive statistics recalculated (Supplementary Table 1), the median and interquartile range did not change significantly from those estimated using the former method. Urine samples from 18 participants were outside the WHO recommended creatinine concentration range of 0.3×10^6µg/L – 3.0×10^6µg/L [32]. For some participants, urine samples were not sufficient to measure concentrations of the pesticide metabolites (7 for TCPY, 15 for dialkyl phosphates and 10 for pyrethroid metabolites). Only 10 individuals had undetected levels of pesticide metabolites (8 for TCPY, one for DMTP and one individual for all the pyrethroid metabolites). Pesticide metabolites measured were detected in the majority of samples in both farm and town dwellers. Most of the pesticide metabolites detected were higher among farm dwellers (range of difference: 0.03 to 6.72 µg/g of creatinine) except for the DMTP, DMDTP and DETP which were in contrast higher among town dwellers (Table 2). TCPY and trans-DCCA were statistically significantly higher among farm dwellers. DMP and DMTP were the predominant organophosphate metabolites detected while 3PBA was the predominant pyrethroid metabolite detected.

Table 2: Urinary pesticide metabolites levels detected among rural women in the Western Cape

Pesticide metabolites	Farm dwellers	Town dwellers	Overall
	Median (interquartile range)		
	Corrected for creatinine (µg/g creatinine)		
I. Organophosphate metabolites			
Dialkyl phosphates (n = 178)			
∑DAP	141.42(37.4-249.83)	132(45.64-204.45)	133.59(41.86-229.09)
DMP	32.91(13.50-55.75)	26.19(14.33-52.36)	29.63(14.06-53.22)
DMTP (n=177)	13.41(3.05-62.45)	37.86(6.55-77.20)	22.04(4.53-65.85)
DMDTP	5.70(0.83-51.51)	9.57(0.87-66.22)	6.87(0.85-61.77)
DEP	5.01(1.37-12.90)	4.13(0.59-9.47)	4.27(1.08-10.04)
DETP	3.70(1.15-26.98)	3.94(1.35-26.18)	3.87(1.20-26.98)
DEDTP	1.99(0.55-5.10)	1.70(0.60-8.02)	1.89(0.58-6.44)
Chlorpyrifos metabolite (n = 178)			
TCPY	6.35(3.67-10.95)*	4.26(2.72-8.27)	5.38(3.25-9.45)
II. Pyrethroid metabolites (n = 182)			
Pyrethroids	6.60(3.61-9.96)	5.26(2.74-8.42)	6.01(3.24-9.67)
cis-DCCA	0.72(0.27-1.28)	0.56(0.23-1.13)	0.63(0.26-1.24)
trans-DCCA	0.85(0.48-1.29)**	0.59(0.28-1.02)	0.70(0.37-1.22)
DBCA	0.33(0.05-0.63)	0.30(0.04-0.60)	0.31(0.05-0.62)
4F3PBA	0.76(0.35-1.32)	0.70(0.33-1.30)	0.73(0.33-1.32)
3PBA	3.85(2.13-6.25)	3.34(2.27-5.92)	3.41(2.21-6.00)

*p < 0.05; **p < 0.01; TCPY: 3,5,6- trichloropyridinol; ∑DAP: sum of the 6 dialkyl phosphate metabolites; DMP: dimethyl phosphate
DMTP: dimethyl thiophosphate; DMDTP: dimethyl dithiophosphate; DEP: diethyl phosphate; DETP: diethyl thiophosphate
DEDTP: diethyl dithiophosphate; Pyrethroids: sum of the 5 pyrethroid metabolites;
cis-DCCA: cis- 2,2-dichlorovinyl-2,2- dimethylcyclopropane-1-carboxylic acid
trans-DCCA: trans- 2,2-dichlorovinyl-2,2- dimethylcyclopropane-1-carboxylic acid
DBCA: cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid;
4F3PBA: 4-fluoro-3-phenoxybenzoic acid; 3PBA: 3- phenoxybenzoic acid

3.3. Serum cytokine concentrations (n=201)

Table 3 summarises the results of the cytokine concentrations measured and the proportion of each cytokine detected. Cytokine data were available for 201 out of 211 participants. Blood samples could not be obtained from 5 participants and for another 5 participants their serum samples were insufficient for analysis. Th2 cytokine levels were on average higher among town dwellers with IL-13 reaching a statistically significant higher level ($p<0.05$) in this group. There was no consistent pattern among non-Th2 cytokines with some (IL-6 and IL-8) being higher among town dwellers while others (IL-10, IL-17 and IFN- γ) being higher among farm dwellers. The proportion of Th2 cytokines detected ranged from 18% to 40% while non-Th2 cytokines ranged from 35% to 71%. The proportion of cytokines detected was not significantly different between town and farm dwellers but, in general, IL-8 was the most dominantly detected (71%) cytokine while IL-5 was the least detected (18%).

3.4. Host and other potential confounders associated with asthma outcomes

Table 4 summarises the unadjusted logistic regression models of the relationship between potential confounders and the asthma outcomes. Age, BMI, current employment and currently living in a farm were not associated with any asthma outcome. Atopy, current smoking, level of education and being born on a farm were associated with some asthma outcomes. None of the potential confounders was associated with the cytokines, notably atopy was not associated with Th2 cytokines.

3.5. Association between pesticide metabolites and asthma outcomes using multiple regression models

Table 5 and 6 summarise the results of adjusted multiple logistic regression models of the association between pesticide metabolites and different asthma phenotypes including cytokines. TCPY was weakly and positively associated with virtually all asthma, FENO and cytokine outcomes. Most organophosphate metabolites were associated with higher odds of FeNO levels above 50ppb, the strongest association being with DEP (OR = 2.54; 95% CI: 0.62 - 10.37). However, there was no consistent association between pesticide metabolites and other asthma outcomes. Both non-Th2 and Th2 cytokines were positively associated with pesticide metabolites although non-Th2 cytokines especially IL-8 showed stronger associations with ethyl phosphate metabolites (DEP, DETP and DEDTP). There was no consistent

association between cytokine levels (continuous variables) and pesticide metabolites in multiple linear regression models (data not shown).

Table 3: Serum cytokine levels detected among rural women in the Western Cape

Cytokines	Distribution of detected values median (interquartile range) in pg/ml			Limit of detection (pg/ml)	Proportion detected n (%)		
	Farm dwellers	**Town dwellers**	**Overall**		**Farm dwellers**	**Town dwellers**	**Overall**
Th2 cytokines							
IL-4	4.68(3.20-6.92)	5.09(3.52-8.12)	4.84(3.52-7.25)	0.10	51 (43)	30 (37)	81 (40)
IL-5	2.06(1.66-2.31)	2.23(1.82-3.16)	2.07(1.68-2.60)	0.10	23 (19)	13 (16)	36 (18)
IL-13	4.80(2.98-6.37)*	6.06(4.90-7.58)	5.55(3.39-7.23)	0.10	39 (33)	28 (34)	67 (33)
Any Th2		N/A		N/A	61 (51)	44 (54)	105 (52)
Non-Th2 cytokines							
IL-6	5.16(3.61-7.50)	5.27(3.75-10.26)	5.16(3.62-8.69)	0.14	63 (53)	38 (46)	101 (50)
IL-8	16.49(10.56-36.22)	19.47(13.08-37.30)	18.93(11.45-36.90)	0.10	85 (71)	57 (70)	142 (71)
IL-10	4.47(2.80-5.60)	4.22(2.74-6.36)	4.39(2.77-5.87)	0.20	45 (38)	26 (32)	71 (35)
IL-17	10.34(6.20-15.61)	9.13(7.09-19.02)	9.76(6.40-16.10)	0.22	52 (44)	28 (34)	80 (40)
IFN-γ	10.53(7.41-17.80)	9.14(6.56-17.30)	10.30(7.41-17.30)	0.10	60 (50)	31 (38)	91 (45)
Any non-Th2		N/A		N/A	85 (71)	57 (70)	142 (71)

IL: interleukin; IFN- γ: interferon gamma; * p-value<0.05; Any Th1: any of the Th1 cytokines (IL-6, IL-8, IL-17, IL-10 or IFN-γ); Any Th2: any of the Th2 cytokines (IL-4, IL-5 or IL-13)
N/A: Not applicable

Table 4: Unadjusted logistic regression models of the association between potential confounders and asthma outcomes among rural women in Western Cape

	Asthma outcomes. Odds Ratio (95% Confidence Interval)						
	Doctor diagnosed asthma	**Adult onset asthma**	**Current asthma**	**FeNO=25ppb-50ppb**	**FeNO>50ppb**	**Any of non-Th2 cytokines detected**	**Any of Th2 cytokines detected**
Prevalence, (%) (n=211)	**11%**	**9%**	**6%**	**11%**	**7%**	**71%**	**52%**
Age (years)	1.01(0.98-1.05)	1.02(0.99-1.06)	1.04(0.99-1.09)	1.00(0.95-1.03)	0.99(0.95-1.04)	1.00(0.97-1.02)	1.00(0.97-1.02)
BMI (kg/m2)	1.02(0.96-1.08)	1.03(0.97-1.10)	1.01(0.93-1.10)	1.05(0.98-1.11)	1.02(0.95-1.10)	1.02(0.98-1.07)	1.01(1.00-1.05)
Education (years of schooling)	0.85(0.74-0.96)***	0.85(0.74-0.97)*	0.78(0.66-0.92)**	0.95(0.83-1.09)	1.18(0.96-1.46)	1.02(0.93-1.13)	0.98(0.89-1.07)
Born on a farm	0.61(0.25-1.50)	0.67(0.25-1.79)	0.10(0.01-0.79)*	0.29(0.10-0.81)*	0.27(0.07-0.99)*	1.17(0.63-2.15)	1.07(0.62-1.87)
Current smoker	1.66(0.68-4.02)	1.43(0.55-3.72)	1.44(0.44-4.70)	0.62(0.25-1.49)	0.23(0.06-0.84)*	0.77(0.42-1.41)	0.68(0.39-1.19)
Currently employed	0.86(0.36-2.07)	0.73(0.28-1.87)	0.46(0.14-1.50)	0.56(0.24-1.34)	0.55(0.19-1.58)	1.36(0.74-2.53)	0.90(0.51-1.59)
Atopy (Positive Phadiatop)	3.37(1.32-8.58)*	3.10(1.13-8.5)*	7.12(1.52-33.40)*	2.20(0.90-5.34)	21.28(2.74-165.23)***	1.32(0.71- 2.45)	1.09(0.62-1.90)
Farm vs. town dwellers	0.65(0.27-1.55)	0.64(0.25-1.65)	0.35(0.10-1.20)	0.63(0.26-1.51)	0.61(0.21-1.76)	1.10(0.59-2.03)	0.91(0.52-1.60)

*p < 0.05; **p < 0.01; ***p < 0.001; Each OR represents a separate unadjusted logistic regression model;
Any Th1: any of the Th1 cytokines (IL-6, IL-8, IL-17, IL-10 or IFN-γ); Any Th2: any of the Th2 cytokines (IL-4, IL-5 or IL-13)

Table 5: Adjusted multiple logistic regression models of the association between pesticide metabolites and asthma outcomes among rural women in Western Cape

Pesticide metabolites	Asthma outcomes: Odds Ratio (95% Confidence Interval)						
	Doctor-diagnosed asthma	**Adult-onset asthma**	**Current asthma**	**FeNO: 25ppb–50ppb**	**FeNO > 50ppb**	**Any non-Th2 cytokine detected**	**Any Th2 cytokine detected**
I. Organophosphate metabolites							
Dialkyl phosphates							
∑DAP	0.68(0.23-2.05)	0.66(0.20-2.20)	1.38(0.33-5.76)	0.66(0.22-1.92)	2.53(0.74-8.64)	1.77(0.81-3.87)	1.77(0.90-3.46)
DMP	0.16(0.02-1.29)	0.22(0.03-1.72)	0.54(0.06-4.83)	0.17(0.02-1.33)	1.77(0.40-7.88)	4.23(1.54-11.65)**	1.69(0.83-3.46)
DMTP	0.12(0.02-0.94)*	0.16(0.02-1.29)	¥	1.03(0.33-3.23)	0.47(0.10-2.15)	1.34(0.60-3.00)	0.85(0.42-1.72)
DMDTP	1.52(0.53-4.33)	1.91(0.62-5.82)	2.47(0.60-10.13)	1.78(0.60-5.29)	1.80(0.45-7.23)	0.46(0.22-0.98)*	0.79(0.39-1.62)
DEP	0.78(0.23-2.62)	1.08(0.31-3.71)	0.74(0.13-4.31)	1.26(0.40-3.97)	2.54(0.62-10.37)	2.71(1.05-7.00)*	1.99(0.95-4.19)
DETP	1.45(0.46-4.60)	2.03(0.61-6.73)	1.53(0.26-8.97)	0.65(0.17-2.49)	1.06(0.23-4.87)	23.25(3.08-175.49)**	2.75(1.27-5.92)*
DEDTP	1.19(0.38-3.78)	1.67(0.51-5.45)	0.77(0.13-4.51)	0.80(0.24-2.72)	0.97(0.22-4.33)	23.84(3.15-180.74)**	7.70(3.00-19.74)***
Chlorpyrifos metabolite							
TCPY	1.35(0.47-3.92)	1.41(0.45-4.44)	1.26(0.27-5.76)	1.56(0.52-4.65)	1.50(0.35-6.38)	1.93(0.83-4.45)	1.56(0.57-2.34)
II. Pyrethroid metabolites							
Pyrethroids	0.62(0.19-2.02)	0.84(0.26-2.77)	2.04(0.48-8.59)	0.13(0.02-0.98)*	0.35(0.07-1.90)	2.18(0.98-4.88)	1.32(0.69-2.55)
cis-DCCA	0.11(0.01-0.93)*	0.16(0.02-1.29)	0.23(0.02-2.62)	0.33(0.07-1.58)	0.59(0.10-3.53)	2.10(0.92-4.80)	1.47(0.73-2.93)
trans-DCCA	0.14(0.02-1.19)	¥	0.28(0.02-3.47)	0.78(0.20-2.96)	0.81(0.12-5.31)	1.21(0.56-2.63)	1.04(0.52-2.10)
DBCA	0.17(0.02-1.30)	0.22(0.03-1.74)	¥	0.97(0.29-3.29)	0.94(0.20-4.36)	1.74(0.78-3.88)	1.33(0.66-2.67)
4F3PBA	1.01(0.30-3.40)	1.40(0.40-4.90)	1.74(0.29-10.56)	0.69(0.18-2.62)	1.07(0.23-5.04)	4.32(1.58-11.82)**	2.51(1.20-5.22)*
3PBA	1.06(0.35-3.19)	1.15(0.35-3.76)	2.64(0.61-11.47)	0.14(0.02-1.10)	0.40(0.07-2.31)	1.63(0.72-3.69)	1.30(0.64-2.64)

Adjusted for current smoking, atopy, born on a farm and level for education; Each OR represents a separate adjusted logistic regression model
Pesticide residues categorized as above 75 percentile of the detected values; ¥ OR not calculable; Any Th1: any of the Th1 cytokines (IL-6, IL-8, IL-17, IL-10 or IFN-γ);
Any Th2: any of the Th2 cytokines (IL-4, IL-5 or IL-13)

Table 6: Adjusted multiple logistic regression models of the association between pesticide metabolites and cytokines among rural women in Western Cape

Pesticide metabolites	Cytokines: Odds Ratio (95% Confidence Interval)							
	Th2 cytokines					Non-Th2 cytokines		
	IL-4	IL-5	IL-13	Il-6	IL-8	IL-10	IL-17	IFN-γ
I. Organophosphate metabolites								
Dialkyl phosphates								
∑DAP	1.60(0.83-3.11)	2.92(1.33-6.43)**	1.49(0.76-2.93)	1.77(0.91-3.44)	1.77(0.81-3.87)	1.83(0.93-3.60)	2.25(1.16-4.37)*	1.70(0.88-3.27)
DMP	2.34(1.14- 4.82)*	2.00(0.82-4.87)	1.38(0.65-2.92)	3.69(1.72-7.92)**	4.23(1.54-11.65)**	2.65(1.28-5.52)**	3.58(1.71-7.48)**	3.25(1.57-6.72)**
DMTP	1.59(0.77- 3.29)	0.84(0.31-2.31)	0.49(0.21-1.13)	1.76(0.86-3.61)	1.34(0.60-3.00)	1.06(0.50-2.26)	1.53(0.74-3.14)	0.85(0.42-1.73)
DMDTP	0.39(0.17-0.88)*	0.28(0.08-1.01)	1.39(0.66-2.94)	0.21(0.09-0.47)***	0.46(0.22-0.98)*	0.27(0.11-0.71)**	0.25(0.10-0.59)**	0.39(0.18-0.83)
DEP	3.50(1.65-7.44)**	7.76(3.15-19.11)***	1.87(0.89-3.95)	3.43(1.57-7.49)**	2.71(1.05-7.00)*	7.79(3.40-17.83)***	4.59(2.13-9.89)***	3.63(1.70-7.73)**
DETP	4.09(1.90-8.77)***	8.15(3.27-20.30)***	2.35(1.11-4.99)*	5.85(2.49-13.79)***	23.25(3.08-175.49)**	4.18(1.95-8.96)***	8.07(3.51-18.53)***	4.92(2.24-10.80)***
DEDTP	10.28(4.29-24.64)***	13.26(5.16-34.08)***	4.69(2.16-10.16)***	14.87(4.94-44.72)***	23.84(3.15-180.74)**	19.13(7.30-50.14)***	17.05(6.46-45.02)***	25.12(8.07-78.17)***
Chlorpyrifos metabolite								
TCPY	1.20(0.58-2.48)	0.48(0.15-1.47)	1.03(0.48-2.22)	2.56(1.23-5.34)*	1.93(0.83-4.45)	0.77(0.35-1.68)	1.83(0.89-3.74)	1.40(0.69-2.83)
II. Pyrethroid metabolites								
Pyrethroids	2.32(1.19-4.52)*	1.07(0.46-2.50)	0.74(0.36-1.50)	2.95(1.48-5.90)**	2.18(0.98-4.88)	2.23(1.14-4.37)*	3.78(1.91-7.50)***	2.66(1.36-5.19)**
cis-DCCA	2.26(1.12-4.58)*	1.44(0.59-3.49)	0.72(0.33-1.57)	2.72(1.32-5.58)**	2.10(0.92-4.80)	1.62(0.78-3.34)	2.96(1.45-6.03)**	2.12(1.06-4.24)*
trans-DCCA	1.52(0.75-3.11)	0.93(0.36-2.43)	0.65(0.29-1.47)	2.51(1.21-5.20)*	1.21(0.56-2.63)	2.28(1.10-4.73)*	2.12(1.04-4.33)*	1.57(0.78-3.17)
DBCA	1.60(0.79-3.24)	1.03(0.40-2.65)	0.76(0.35-1.67)	2.40(1.18-4.91)*	1.74(0.78-3.88)	1.80(0.87-3.70)	2.09(1.03-4.24)*	1.63(0.82-3.25)
4F3PBA	2.53(1.23-5.19)*	2.50(1.04-6.00)*	1.48(0.70-3.13)	4.97(2.24-11.04)***	4.32(1.58-11.82)**	2.94(1.41-6.13)**	3.38(1.63-7.02)**	2.74(1.34-5.61)**
3PBA	2.68(1.29-5.57)**	0.95(0.37-2.48)	0.83(0.38-1.81)	2.18(1.05-4.51)*	1.63(0.72-3.69)	2.50(1.17-5.33)*	3.79(1.80-7.97)***	2.17(1.06-4.42)*

Adjusted for current smoking, atopy, born on a farm and level for education; Each OR represents a separate adjusted logistic regression model
Pesticide residues categorized as above 75 percentile of the detected values; Cytokines categorised as detected vs. non-detected

3.6. Association between cytokines and other asthma outcomes

Table 7 summarises the results of adjusted multiple logistic regression models of the association between cytokines and other asthma outcomes in this study population. Allergic airway inflammation (FeNO>50ppb) was positively associated with all Th2 (IL-4, IL-5 and IL-13), Th1 (IFN-γ), Th17 (IL-17) cytokines and IL-10 (Table 7). All Th2 cytokines were also positively associated with doctor-diagnosed and adult-onset asthma.

Table 7: Adjusted multiple logistic regression models of the association between cytokines and asthma outcomes among rural women in Western Cape

Cytokines	Asthma outcomes: Odds Ratio (95% Confidence Interval)				
	Doctor-diagnosed asthma	**Adult-onset asthma**	**Current asthma**	**FeNO: 25ppb–50ppb**	**FeNO > 50ppb**
Th2 cytokines					
IL-4	1.04(0.39-2.75)	1.59(0.56-4.46)	0.64(0.14-3.00)	0.41(0.15-1.15)	1.13(0.31-4.04)
IL-5	2.17(0.69-6.88)	2.92(0.89-9.57)	0.36(0.02-5.41)	0.64(0.17-2.40)	1.62(0.34-7.64)
IL-13	1.34(0.51-3.55)	1.15(0.40-3.31)	1.04(0.23-4.79)	0.45(0.15-1.33)	1.34(0.37-4.87)
Any Th2	1.70(0.63-4.55)	1.87(0.64-5.54)	0.75(0.16-3.45)	0.44(0.17-1.14)	0.70(0.19-2.60)
Non-Th2 cytokines					
IL-6	1.20(0.46-3.12)	1.60(0.56-4.57)	0.59(0.13-2.71)	0.53(0.21-1.36)	0.62(0.17-2.31)
IL-8	0.85(0.30-2.36)	1.05(0.33-3.28)	0.58(0.12-2.78)	0.34(0.13-0.89)*	0.50(0.12-2.16)
IL-10	1.09(0.39-3.03)	1.53(0.52-4.47)	0.45(0.06-3.22)	0.37(0.12-1.17)	1.25(0.33-4.71)
IL-17	0.96(0.37-2.53)	1.12(0.40-3.14)	0.89(0.20-3.92)	0.53(0.20-1.41)	1.08(0.30-3.82)
IFN-γ	0.77(0.30-1.99)	0.70(0.25-1.98)	0.33(0.07-1.67)	0.36(0.13-0.98)*	1.33(0.38-4.68)
Any non-Th2	0.85(0.30-2.36)	1.05(0.33-3.28)	0.58(0.12-2.78)	0.34(0.13-0.89)*	0.50(0.12-2.16)

*p<0.05; Any Th2: any of IL-4, IL-5 or IL-13; Any non-Th2: any of IL-6, IL-8, IL10, IL-17 or IFN-γ;
Cytokines categorised as detected vs. non-detected

4. Discussion

This study demonstrated that OP and PYR metabolite levels in rural women in the Western Cape are higher than in the general population. Using these exposure proxy markers, the findings of this study suggests that both OP and PYR pesticides are associated with asthma, which may be due to Th2 and non-Th2 mechanisms, the latter pathway demonstrating consistently stronger relationships.

The DAP levels detected in this study were higher than levels found among general population in the US [33] and in the Netherlands [34], and also generally higher than concentrations among pesticide applicators in Peru [35]. However, the levels of DAP detected in this study were generally lower compared to levels among applicators and general farm workers in a previous study in the Western Cape province of South Africa [17] as well as pesticide applicators in Japan [36] and Italy [37]. The possible reason for the lower levels of DAP found in this current study compared to these studies in other settings [17,36,37] could be due to the very low number (only 4) of pesticide applicators among the total number of study participants, a large proportion who were general farm labourers.

TCPY is a short-term marker of exposure to both chlorpyrifos and chlorpyrifos-methyl [1,37]. TCPY levels in this current study were higher than levels found among the general population in the Netherlands [34] and in the US [38]. Furthermore, the proportion of TCPY detected in this study was higher than in US Latino farm workers [39]. However, when TCPY levels found in this study are compared to farm workers (pesticide applicators and other workers) in Italy, participants in this study had lower median levels than their Italian counterparts [37]. Interestingly, TCPY levels in our study, which were measured from spot urine samples, were comparable to the 24 hour urine TCPY concentrations reported for the general population in Italy [40]. To our knowledge, no previous study has measured TCPY levels in farming or non-farming communities in South Africa.

DBCA is a specific metabolite of deltamethrin while 4F3PBA is a specific metabolite of cyfluthrin [18] . Cis- and trans-DCCA are the metabolites of permethrin, cypermethrin and cyfluthrin [18]. On the other hand, 3PBA is a non-specific metabolite, common to about 20 synthetic pyrethroids including permethrin, cypermethrin, cyfluthrin and deltamethrin [18,41]. In our study, DBBA and 4F3PBA were the highest PYR metabolites detected in urine samples implying that the study

participants were more exposed to deltamethrin and cyfluthrin than the other pyrethroids. The levels of PYR metabolites detected in this study were higher than levels reported among individuals from the general population in Germany [42], Poland [43], Italy [44], US [45] and Japan [46]. Furthermore, the proportion of PYR metabolites detected in our study were substantially higher than in US Latino farm workers [39]. The levels of 3PBA in our study were higher than levels reported among Japanese workers involved with farming activities including spraying pesticides [46] but similar to US forestry workers exposed to pesticides [47]. When compared to flight attendants exposed to pyrethroids, 3PBA and trans-DCCA levels in our study were lower while DCBA and 4F3PBA levels were higher and cis-DCCA levels similar to the levels found in flight attendants [41].

In this study, individuals who lived in the surrounding towns near the farms were also exposed to OP and PYR pesticides based on the results of the urinary determinations of pesticide metabolites. It is likely that these workers who do not live on the farms may be exposed to pesticides either through pesticide drift as a result of living in towns close to farming activities or other environmental exposures such as contaminated surfaces, food and water or through household use of pesticides [17]. This suggests that characterising exposure according to the place of residence or work (whether farm or town dwellers) in rural populations living near farms may not always be accurate and other means of validating exposure through more objective markers such as those used in this study may provide an alternative approach.

In this current study, IL-13 levels were higher among town dwellers compared to farm dwellers. Moreover, town dwellers were more likely to be atopic, and more likely to be sensitised to storage and spider mites than farm dwellers. Furthermore, the Th2 cytokines were positively associated with allergic airway inflammation (FeNO > 50 ppb), with a larger proportion (9%) having such high levels compared to farm dwellers. These findings suggest that the allergic asthma phenotype is more dominant entity in this group probably associated with indoor and outdoor mite allergens [48].

In this study, a greater proportion of non-Th2 cytokines (71%) were detected than Th2 cytokines (52%). Furthermore, while OP and PYR metabolites were positively associated with both Th2 and non-Th2 cytokines, stronger associations were observed between non-Th2 cytokines and pesticide metabolites. This suggests that non-Th2 pathways may be playing a dominant role in the airway inflammation

observed in individuals exposed to pesticides. This is consistent with recent studies that have implicated non-Th2 pathways such as Th1 and Th17 in the pathogenesis of asthma especially in severe, persistent asthma [21,48]. Non-Th2 cytokines such as IL-8 and IL-17 have also been linked to neutrophilic asthma [49,50], suggesting that this may be an important phenotype associated with OP and PYR pesticide exposures.

A more detailed analysis of cytokine patterns in the Th2 pathway, also demonstrated that both OP and PYR metabolites were positively associated with Th2 cytokines. Furthermore, most of the OP metabolites were also positively associated with allergic airway inflammation (FeNO > 50 ppb). Additionally, Th2 cytokines were also consistently positively associated with allergic airway inflammation (FeNO > 50 ppb). Together, these findings suggest an involvement of Th2 mechanisms in the airway inflammation observed in individuals exposed to these OP and PYR pesticides. While the few case reports of asthma due to these pesticides previously reported in the literature [51–54] could not identify the pathophysiological mechanisms that were responsible, a recently published study [55] reported the case of a 29 year old woman who developed anaphylaxis after exposure to pyrethroids, implying that these pesticides may induce an allergic response. An association between pesticide exposure and allergic asthma has also been reported in other epidemiological studies [9,56].

The positive association observed between most of the OP metabolites and allergic airway inflammation (FeNO > 50 ppb) in our study is consistent with the findings from a previous sub-study [3] of this group, which demonstrated an association between low levels of ChE and FeNO. However, the association with FeNO in this current study was weaker than in the previous study. The latter could be due to the fact that the low ChE levels measured in the previous study also indicate carbamate exposure in addition to OP exposure. Carbamates which were not measured in the current study, have also been associated with asthma in the literature [9,13].

To our knowledge, no previous epidemiological studies have investigated the effect of pesticide exposure ascertained through urinary pesticide metabolite concentrations on allergic airway inflammation as determined by FeNO. Furthermore, the relationship between pesticide metabolites and cytokine pathways has also not been previously studied, suggesting the use of novel insights into the mechanisms underlying asthma associated with pesticides. However, there are some limitations of

using this approach. The lack of strong association between the measured pesticide metabolites and some asthma outcomes in our study could have been due to the cross-sectional study design. This may have resulted in a healthy worker effect whereby individuals who developed adverse health effects from pesticides may have left their employment on the farms to work in other alternative jobs. The short half-life of these OP and PYR metabolites, markers of short-term exposure, may have also contributed to the lack of strong association being observed, especially when these were related to long term asthma outcome variables such as doctor-diagnosed and adult-onset asthma. This is suggested by the fact that stronger associations were found with short-term outcomes such FeNO and current serum cytokine levels. Another possible explanation for the lack of strong associations observed between pesticide metabolites and some asthma outcomes could be due to the low number of pesticide applicators in our study, which may have contributed to a muted exposure gradient. Although there was no standardisation of blood sampling for some factors such as food intake, use of medications and sleep patterns; it is unlikely that this created a systematic error.

In conclusion, this study has shown that OP and PYR metabolite levels in rural women in the Western Cape are higher than in general population. Furthermore, it provides evidence suggesting that both OP and PYR pesticides are associated with asthma. The cytokine patterns suggest that while non-Th2 cytokines play a major role, Th2 cytokines are also associated with airway inflammation in individuals exposed to pesticides. Apart from these immunological (Th2 and non-Th2) mechanisms, pesticides can also act as irritants and interact with their functional irritant receptors resulting in asthma [57].

The study findings suggest that greater efforts should be made to protect farm workers and the surrounding farming communities from the respiratory health risks associated with pesticides. Farm workers, managers and owners should be educated about the proper use of pesticides and their adverse respiratory health effects. Furthermore, workers at high risk should undergo regular medical surveillance including biomonitoring for urinary pesticide metabolites to assess that adequate control measures are in place to protect their health.

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5. APPENDICES

Appendix A1

English Questionnaire

Health effects due to pesticide exposure amongst rural women in the Western Cape

UNIVERSITY OF CAPE TOWN

Study Number _____

Date _____

Area _____

Farm Name _____

Name of Interviewer _____

GENERAL INSTRUCTIONS

Thank you for agreeing to take part in this study.

We will work through the questionnaire as follows: I will ask the questions and give you the answer choices and tick or circle the answers you give me in the questionnaire. Choose the answer that is the closest to how you feel.

Please note that there are no right or wrong answers to the questions asked. Please feel free to answer just what you think. You may stop at any time if you do not want to carry on with these questions. Your answers are confidential and will not be shared with anyone. Only the research staff will have access to the questionnaire once it has been completed.

Section 1: DEMOGRAPHIC CHARACTERISTICS

We would like to ask you a few questions about yourself.

1.1 How old are you? _____ (years**)**

Date of birth ____/_____/_____

1.2 What is the highest level of education you have passed?

Less than one year completed	1
Sub A/Class 1/Grade 1	2
Sub B/Class 2/Grade 2	3
Standard 1/Grade 3	4
Standard 2/Grade 4	5
Standard 3/Grade 5	6
Standard 4/Grade 6	7
Standard 5/Grade 7	8
Standard 6/Grade 8	9
Standard 7/Grade 9	10
Standard 8/Grade 10	11
Standard 9/Grade 11	12
Standard 10/Grade 12	13
Further studies – incomplete	14
Diploma/other post school – complete	15
Degree	16

1.3 Which main language do you speak at home? _____

Section 2: HOUSEHOLD FACTORS

2.1 Is the house you live in:

Owned by your family	1
Rented	2
Owned by the owner of the farm	3
Other (please specify)	4

Specify _____

2.2 Does your house have:

		Yes	No
A	Electricity		
B	A radio		
C	A television		
D	A landline telephone		

E	A fridge		
F	A computer		
G	A washing machine		
H	A cell phone (anybody)		

2.3 How many people usually live and sleep in your household?

	Number of people

Section 3: ECONOMIC FACTORS

Now we would like to ask a few questions about you and the work that you do.

3.1 What kind of work do you do? (If working, please tell me your occupation. For example, Farmer, Street Trader, Primary School Teacher, Domestic Worker)

Not working	No
Working	Yes
If working, specify	

3.2 Please indicate which of the following are your sources of income. Please answer this question whether or not you are working.

		Yes	No
A	Work		
B	Spouse/partner		
C	Parents		
D	Brothers and/or sisters		
E	Children		
F	Child Support Grant		
G	State Old Age Pensions		
H	Disability Grant		
I	Care Dependency Grant		
J	Foster Care Grant		
K	Grants-in-Aid		
L	Workman's Compensation Fund		
M	Other (Please specify)		

3.3 What is your household income? _____

3.4 How often do the people in your family go hungry or have no food to eat?

Never	0
Seldom	1
Sometimes	2
Often	3

3.5 During which months of the year do you go hungry? _____ (months of year).

Section 4. RESIDENTIAL HISTORY

Now I'd like to ask you a few questions about the places where you have lived in your lifetime:

4.1 Where do you currently live (Town, city, farm)? _____

How long have you lived here? _____ (Years/Months)

If on a farm,

4.2 What kind of farm is this? (what is grown here?) _____

4.2.1 Is this an export farm? _____ (Yes, No)
If yes, where are crops exported to? _____ (countries)

4.2.2 Is this a Tesco farm _____ (Yes, No)

4.3 How far from your house is the nearest vineyard/orchard? _____ (meters)

4.4 Are pesticides sprayed on the vineyard/orchard during the year? ____ (Yes/No)

4.5 When last was pesticides applied in the vineyard/orchard? _____ (number of days)

IF YES, complete the following:

4.5 How many months a year are pesticides applied on the farm _____

How many days per month are pesticides applied during the spraying months? _____

Number of days per year _____

4.6 Does the pesticides spraying come into the house? _____ (Yes/No)

4.7 Do you come into contact with pesticides outside the house while spraying occurs (e.g. hanging your washing? . _____ (Yes/No)

4.8 Who apply pesticides on this farm _____(Men, Women, Both)

4.9 Does the farmer provide you with protective clothes and equipment (including gloves,
masks, overalls, etc? _____
If yes, is it free of charge? _____ (Yes, No)

4.10 Are shower/washing rooms provided for workers coming into contact with pesticides?

_____ (Yes, No)

4.11 When spraying happens, are workers expected to work in sprayed blocks? ___(Yes, No)

4.12 How soon after spraying/application of pesticides do you return to the vineyard/orchard? _____ (number of days)

4.13 What is the method of pesticide application? _____ (Tractor, backpack or other
methods)

4.14 What are the sources of drinking water at your house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

4.15 What are the sources of water for recreational use (bathing, washing of clothes) at your house? _____ (municipal water, storage dam on mountain, borehole/spring, river water, farm dam, rain water tank, etc)

4.16 Did you live elsewhere before? _____ (Yes/No)

If YES,

Please provide the details about the places where you have lived **PREVIOUSLY** in the following table:

	Places lived previously										
	1	2	3	4	5	6	7	8	9	10	11
Number of years											
Was pesticides sprayed on the farm?											

4.12 Were you born on a farm where pesticides were applied? ___ (Yes/No)

Section 5. WORK HISTORY

Current job

5.1 What is your current occupation? _____

5.2 What is your job title? _____

5.3 For how many years have you worked in this job? _____ (years)

5.4 Do you currently work on a farm? ___ (Yes/No)

If you work on a farm,

5.5 Are you a permanent or seasonal farm worker? _____

5.6 If you do not live on the farm you work at:

5.6.1 Which crops are produced on the farm
_____

5.6.2 Is the farm you work on an export farm? _____ (Yes, No)
If yes, where are crops exported to? _____
(countries)

5.6.3 Is the farm you work on a Tesco farm? _____ (Yes, No)

5.7.1 Do you work in the field? _____ (Yes/No)

5.7.2 Do you apply (spray/mix) pesticides _____ (Yes/No)

5.7.3 If YES which pesticides do you use _____

_____

5.7.4 When last did you apply pesticides? _____ (number of days)

5.7.5 How many months a year do you apply pesticides? _____

How many days per month do you apply pesticides in the spraying months? _____

Total number of days per year _____

5.7.6 Do you drive a tractor while others spray pesticide? _____(Yes/No)
If yes, how many times per year? _____

5.7.7 Which Personal Protective Equipment do you use? _____
(Indicate with A = Apron, B = Boots, G = Gloves, M = Mask, O = Overalls, Gls = Goggles)

5.78 Is PPE provided free of charge? _____ (Yes, No)

Previous jobs

Please provide the details about your **PREVIOUS** work in the following table

	Previous jobs										
	1	2	3	4	5	6	7	8	9	10	11
Number of years											
Work on a farm (Yes, no)											
Occupation											
Job Title											
If on farm:											
Do you work in the field? (Yes, No)											
Do/did you apply (spray or mix) pesticides (Yes, No)											
How many days per year do/did you apply pesticides?											
Were you the tractor driver ? (Yes, No)											
How many days per year were you the tractor driver?											
Which PPE did you use?*											
*Indicate with A = Apron, B = Boots, G = Gloves, M = Mask, O = Overalls, Gls = Goggles											

Section 6. ALCOHOL USE

6.1 Do you drink alcohol or did you drink before _____? (Yes/No)

If yes,

6.2 Have you ever felt that you should drink less alcohol? ____
(Yes/No)

6.3 Have people ever angered you by criticising your drinking habits? ____
(Yes/No)

6.4 Have you ever felt guilty or bad because you drink alcohol? ____
(Yes/No)

6.5 Have you ever had a drink early in the morning to make you feel better or to get over a 'babalaas'? ____
(Yes/No)

Section 7. SMOKING AND OTHER DRUG USE

7.1 Have you ever smoked tobacco (cigarettes or pipe) for as long as a year? ____ (Yes/No)

('Yes' means at least 20 packs of cigarettes or 30 grams of tobacco in a lifetime or at least one cigarette per day for one year)

If Yes,

7.1.1 How old were you when you started smoking? ____ (years)

7.1.2 Do you smoke currently? ___ (Yes/No)
('Yes' means smoking tobacco in the last month or more)

7.1.3 If no, how old were you when you stopped smoking? _____
7.1.4 How much do/did you now smoke on average?

Number of cigarettes per day ____

Pipe tobacco in grams/week _____

7.1.5 Do you or did you inhale the smoke? ____ (Yes/No)

7.2 Have you been regularly exposed to tobacco smoke from other people smoking cigarettes or pipe in the last 12 months?
('Regularly' means on most days or nights)

7.3 Do you take drugs or have taken drugs before? _____ (Yes/No)

7.3.1 If YES, please state for how many years _____ (years)

Section 8. HOUSEHOLD PESTICIDE USAGE

8.1 Do you or any one in your house use pesticides in the garden or in your home?____ (Yes/ No)

If yes, what do you use? _____

8.2 Do pesticide contaminated clothes get washed at home? ____ (Yes/ No)

8.4 If yes, does it get washed with the rest of the washing? _____ (Yes/ No)

8.5 Do you eat fruit or vegetables from your garden ? _____ (Yes / No)

8.6 Do you use empty pesticide containers at home for domestic purposes? ____(Yes/ No)

8.7 **If yes,** what do you use them for? _____

Section 9 MEDICAL, REPRODUCTIVE AND RESPIRATORY HISTORY

9.1 Do you suffer from :
Asthma _____ (Yes/No)
Bronchitus _____ (Yes/No)
TB _____ (Yes/No)
Eczema _____ (Yes/No)
Hayfever _____ (Yes/No)
Farmers Lung _____ (Yes/No)
Other diseases: _____ (Yes/No) **if yes,** specify _____

9.2 What was your weight at birth _____
9.3 At what age did you reach puberty? _____
9.4 Did you ever experience pesticide poisoning that was confirmed by a doctor? ____(Yes, No)

If yes, how many times_____

9.5 Do you frequently feel/have :
Dizzy _____ (Yes/No)
Nauseas _____ (Yes/No)
Headaches _____ (Yes/No)
Skin, nose and/or eye irritation _____ (Yes/No)
Skin rashes _____ (Yes, No)
Nauseas and want to vomit (Yes, No)
Cold or open sores _____ (Yes, No)

Section 10 (Q16)

10.1. Are you abnormally tired? ____ (Yes / No)

10. 2. Do you have palpitations of the heart when you do not exert yourself? ____ (Yes/No)

10. 3. Do you often have painful tingling in some part of your body? ____ (Yes/No)

10. 4. Do you often feel irritated without any particular reason? ____ (Yes/No)

10. 5. Do you often feel depressed without any particular reason? ____ (Yes/No)

10.6. Do you often have problems concentrating? ____ (Yes/No)

10. 7. Do you have a short memory? ____ (Yes/No)

10. 8. Do you often perspire without any particular reason? ____ (Yes/No)

10. 9. Do you have any problems with buttoning and unbuttoning? ____ (Yes/No)

10.10 Do you generally find it hard to get the meaning from reading newspapers and books? ____ (Yes/No)

10. 11.Have your relatives told you that you have a short memory? ____ (Yes/No)

10. 12. Do you sometimes feel a heavy feeling on your chest? ____ (Yes/No)

10. 13. Do you often have to make notes about what you must remember? ____ (Yes/No)

10. 14. Do you often have to go back and check things you have done such as locking the door? ____
(Yes/No)

10. 15. Do you have a headache at least once a week? ____ (Yes/No)

10. 16. How many times do you have sex per week? ____ (Yes/No)

10. 16a. Do you think that this is less than most persons of your age? ____ (Yes, No)

Section 11. Time to pregnancy

11. 1. Have you ever been pregnant? _____ (Yes/No)

11. 2. If yes, how many times? _____

11. 3. List how many pregnancies ended in

Live birth ____
Stillbirth ____
Miscarriage ____
Ectopic/Tubal pregnancy ____
Other ____

11.4 FOR LIVE BIRTHS AND STILLBIRTHS ONLY (omit twins) Fill in the following
Table:

	Pregnancy										
	1	2	3	4	5	6	7	8	9	10	11
Weight of baby (kg)											
During the month this pregnancy was conceived, were you or your husband using any form of birth control? (Yes, No)											

Method of birth control*											
Were you using birth control all the time, nearly all the time, or only sometimes?											
If NO BIRTH CONTROL or ONLY SOMETIMES: How many months did it take you to get pregnant?											
*oral (the pill), intrauterine device (coil, loop), condoms, diaphragm (cap), rhythm or withdrawal, other											

Section 12. ALLERGIC HEALTH PROBLEMS

12.1 Have you had wheezing or whistling in your chest at any time in the last 12 months? _____ (Yes/No)

If yes, go on to Question 12.2
If no, go on to Question 12.4

12.2 Have you been short of breath when the wheezing noise was present? _____ (Yes/No)

12.3 Have you had this wheezing or whistling when you did not have a cold or flu? ___ (Yes/No)

12.4 Have you been woken up with a feeling of tightness in your chest at any time in the last 12 months? _____ (Yes/No)

12.5 Have you had an attack of shortness of breath that came on during the daytime when you were at rest at any time in the last 12 months? ____ (Yes/No)

12.6 Have you been woken by an attack of coughing at any time in the last 12 months? _____ (Yes/No)

12.7 Have you ever had asthma? ____ (Yes/No)

If Yes, go on to Question 12.
If No, skip to next Question

12.8 If yes, was this confirmed by a doctor?

12.9 How old were you when you were told you have asthma? _____ (years)

12.10 Have you had an attack of asthma in the last 12 months? _____ (Yes/No)

12.11 Are you using any medicines, including inhalers/pumps, nebulizers,

syrups or tablets, for asthma or breathing problems? ____ (Yes/No)

12.12 When you are near animals, feather or in a dusty part of the house, do you ever get a feeling of
tightness in your chest? ____ (Yes/No)

12.13 Do you get a tight chest or wheeze when you work in the:
12.13.1 Vineyard/Orchard ____ (Yes/No)
12.13.2 Packing room ____ (Yes/No)
12.13.3 Other ____ (Yes/No) If yes, specify _____

12.14 Have you had any nasal allergies including hay fever or itchy and watery eyes/nose in the last 12 months? ____ (Yes/No)

12.15 Do you get itchy/watery eyes or nose when you work in the:
12.14.1 Vineyard/Orchard ____ (Yes/No)
12.14.2 Packing room ____ (Yes/No)
12.14.3 Other ____ (Yes/No) If yes, specify _____

12.16 Have you had any skin problems in the last 12 months? ____ (Yes/No)

12.17 Do you get red, itchy pimples when you work in the:
12.17.1 Vineyard/Orchard ____ (Yes/No)
12.17.2 Packing room ____ (Yes/No)
12.17.3 Other ____ (Yes/No) If yes, specify _____

Thank you for taking part in this study

Appendix A2

Afrikaans Questionnaire

Gesondheids gevolge weens blootstelling aan gifstowwe op landlike vrouens in die Weskaap

UNIVERSITEIT VAN KAAPSTAD

Vraelysnommer _____

Datum _____

Area _____

Naam van plaas _____

Naam van Onderhoudvoerder _____

ALGEMENE INSTRUKSIES

Dankie dat jy ingestem het om aan hierdie studie deel te neem.

Ons gaan soos volg deur die vraelys werk: Ek sal die vrae vra en aan jou die moontlike antwoordkeuses gee en ek sal jou antwoorde merk en omsirkel in die vraelys. Kies die antwoord wat die naaste is aan hoe jy voel.

Let asseblief op dat daar geen regte of verkeerde antwoorde op die vrae is nie. Antwoord asseblief soos jy voel. Jy kan enige tyd ophou as jy nie wil voortgaan met die vrae nie. Jou antwoorde is vertroulik en sal aan niemand anders bekend gemaak word nie. Slegs die navorsingspersoneel sal toegang tot die vraelys hê nadat dit voltooi is.

Afdeling 1: DEMOGRAFIESE BESONDERHEDE

Ons wil jou graag ’n paar vrae oor jouself vra.

1.1 Hoe oud is u? _____(jaar)

Geboortedatum ____/_____/_____

1.2 Wat is die hoogste vlak van onderrig wat jy geslaag het?

Minder as een jaar voltooi	1
Sub A/Klas 1/Graad 1	2
Sub B/Klas 2/Graad 2	3
Standerd 1/Graad 3	4
Standerd 2/Graad 4	5
Standerd 3/Graad 5	6
Standerd 4/Graad 6	7
Standerd 5/Graad 7	8
Standerd 6/Graad 8	9
Standerd 7/Graad 9	10
Standerd 8/Graad 10	11
Standerd 9/Graad 11	12
Standerd 10/Graad 12	13
Verdere onderrig – onvoltooid	14
Diploma/ander naskools – voltooid	15
Graad	16

1.3 Wat is die taal wat die meeste tuis gepraat word? _____

Afdeling 2: INLIGTING OOR HUISHOUDING

2.2 Is die huis waarin jy woon:

Die eiendom van jou gesin	1
Gehuur	2
Die eiendom van die plaaseienaar	3
Ander (spesifiseer asb.)	4

Spesifiseer asseblief _____

2.2 Is die volgende in jou huis:

		Ja	Nee
A	Elektrisiteit		

B	’n Radio		
C	’n Televisie		
D	’n Landlyntelefoon		
E	’n Yskas		
F	’n Rekenaar		
G	’n Wasmasjien		
H	’n Selfoon (enige iemand)		

2.3 Hoeveel mense woon en slaap gewoonlik in jou huishouding?

	Aantal mense

Afdeling 3: EKONOMIESE FAKTORE

Nou wil ons graag ’n paar vrae oor jou en die werk wat jy doen, vra.

3.2 Watter soort werk doen jy? (Indien jy werk, wat is jou beroep? Byvoorbeeld boer, straathandelaar, laerskoolonderwyser, huishulp)

Werk nie	Nee
Werk	Ja
Indien u werk, spesifiseer	

3.2 Dui asseblief aan watter van die volgende is jou bronne van inkomste. Antwoord asseblief hierdie vraag – of jy werk of nie.

		Ja	Nee
A	Werk		
B	Eggenoot/lewensmaat		
C	Ouers		
D	Broers en/of susters		
E	Kinders		
F	Kinderonderhoudstoelae		
G	Staatsouderdomspensioen		
H	Ongeskiktheidstoelae		
I	Sorgafhanklikheidstoelae		
J	Pleegsorgtoelae		
K	Hulptoelae		
L	Vergoeding vir beroepsbeserings		
M	Ander		

Indien ander, spesifiseer asseblief _____

3.3 Wat is u totaal huishoudelike inkomste? _____

3.4 Hoe gereeld ly die mense hier honger of het nie kos om te eet nie?(please tick**)**

Nooit	
Selde	
Soms	
Dikwels	

3.5 Gedurend watter maande van die jaar, ly u honger?
_____

_____ (maande van die jaar)

Afdeling 4. LEWENSGESKIEDENIS

Nou wil ek jou graag ’n paar vrae vra oor die plekke waar u al in jou leeftyd gewoon het:

4.1 Waar woon jy nou? (Dorp, stad, plaas)? _____

Hoe lank woon jy al hier? _____(jare/maande)

Indien op ‘n plaas woon nie, skip na vraag 4.15

4.2 Watter soort plaas is hierdie (waarmee word hier geboer)?
_____

4.2.1 Is hierdie plaas ‘n uitvoerplaas? ____ (Ja/Nee)
Indien ja, waarnatoe uitvoer hierdie plaas hul gewasse?
_____

_____ (lande)

4.2.2 Is hierdie ,n Tesco plaas? ____ (Ja/Nee)

4.3 Hoe ver is jou huis van die naaste wingerd/lande? _____(meters)

4.4 Word gifstowwe gedurende die jaar op die wingerd/lande gespuit? ____ (Ja/Neee)

4.5 Wanneer laas was daar gifstowwe aangewend op die wingerd/boord. _____ (aantal dae)

Indien Ja, Voltooi die volgende:

4.6 Hoeveel maande ’n jaar word gifstowwe op die plaas aangewend? _____

Hoeveel dae in die maand word gifstowwe aangewend gedurend die bespuiting maande? _____

Aantal dae in ‘n jaar _____

4.7 Kom die gifstowwe in die huis in? ____ (Ja, Nee)

4.8 Kom u in kontak met gifstowwe buite die huis terwyl daar gespuit word? (b.v. wanneer u wasgoed buitekant gaan op hang)? ____(Ja, Nee)

4.9 Wie wend gifstowwe aan op die plaas? _____ (Mans, vrouens, albei)

4.10 Voorsien die plaas eienaar/bestuurder u vir klere van beskerming en Toerusting?(b.v. handskoene, oorpakke en maskers ens.) ____ (Ja/Nee)
Indien ja, is dit gratis? ____ (Ja/Nee)

4.11 Het die plaas ’n stort vir plaaswerkers wie in aanraking kom met gifstowwe ____ (Ja/Nee)

4.12 Wanneer bespuiting plaasvind, word dit verwag van die werkers om in hierdie blokke te werk wat kortliks gespuit was? ____ (Ja/Nee)

4.13 Nadat hulle die gifstowwe aangewend het, hoeveel dae daarna gaan u terug wingerd/boorde toe? _____ (aantal dae)

4.14 Dui aan hoe u die gifstowwe aanwend:

Trekker met balkspuit _____ (Ja/Nee)
Trekker sonder balkspuit _____ (Ja/nee)
Rugsak _____ (Ja/Nee)
Quad bike _____ (Ja/Nee)
Ander _____ (Ja/Nee) Indien ja, spesifiseer _____

4.15 Waar kom die drinkwater in jou huis vandaan? _____
(Munisipale water, opgaardam op berg, boorgat/fontein, rivierwater, plaasdam, reënwatertenk, ens.)

4.16 Waar kom die water vir gebruiksdoeleindes in jou huis vandaan (b.v. bad of klere was)?_____ (munisipale water, opgaardam op berg, boorgat/fontein, rivierwater, plaasdam, reënwatertenk, ens.)

4.17 Het u in die verlede erens anders gewoon? _____ (Ja/Nee)

Indien Ja,

Gee asseblief besonderhede van die plekke waar u **IN DIE VERLEDE** gewoon het in die volgende tafel

	Plekke in die verlede gewoon										
	1	2	3	4	5	6	7	8	9	10	11
Waar het u gewoon? (plaas, dorp, stad)											
Antal jare											
Was gifstowwe aangewend op hierdie plaas? (Ja/Nee)											

4.18 Was u gebore op ’n plaas waar hulle gifstowwe aangwend het? _____ (Ja/Nee)

Afdeling 5. WERKSGESKIEDINIS

Huidige werk

5.1 Wat is u huidige beroep?
_____

5.2 Wat is u werkstitel? _____

5.3 Hoeveel jare doen u die werk? _____ (jare)

5.4 Is u ’n lid van ’n vakbond? ____ (Ja/Nee)

5.5 Werk u huidiglik op ’n plaas? ____ (Ja/Nee)

Indien u op’n plaas werk, gaan voort van vraag 5.6 af
Indien u nie op ’n plaas werk nie, skip na vraag 5.12

5.6 Is u ‘n permanent of seisoen plaaswerker? _____

5.7 Indien u nie op die plaas woon waar u werk:

5.7.1 Met watter soort gewasse boer hierdie plaas _____
5.7.2 Is hierdie plaas ‘n uitvoerplaas? ____ (Ja/Nee)
Indien ja, waarnatoe uitvoer hierdie plaas hul gewasse?
_____

_____ (lande)

5.7.3 Die plaas waar u werk, is dit ’n Tesco plaas? ____ (Ja/Nee)

5.8 Werk u in die wingerd/boord? ___ (Ja/Nee)

5.9 Wend u gifstowwe aan? (mend/spuit) ___ (Ja/Nee)

5.9.1 Indien Ja, watter gifstowwe gebruik u?
_____

_____ (name van die gifstowwe)

5.9.2 Wanneer laas het u gifstowwe aangewend? _____ (aantal dae)
5.9.3 Hoeveel maande ’n jaar wend u gifstowwe aan? _____(aantal maande)

Hoeveel dae in die maand word gifstowwe aangewend gedurend die bespuiting maande? _____

Aantal dae in ‘n jaar _____

5.10 Ry u ’n trekker terwyl anders, van agter die trekker, spuit? ____ (Ja/Nee)
Indien ja, hoeveel keer in ’n jaar? _____

5.11 Watter klere van beskerming dra u? _____ (Dui aan met V = Voorskoot, S =
Steuwels, H = Handskoene, M = Masker, GM = Gasmasker, O = Oorpak, SB = Skermbril)

5.12 U klere van beskerming en toerusting, is dit gratis? ___ (Ja/Nee)

Vorige werk

Gee asseblief die besonderhede oor jou **VORIGE** werk met gifstowwe in die volgende tabel

	Vorige werk										
	1	2	3	4	5	6	7	8	9	10	11
Aantal jare											
Op ‘n plaas gewerk (Yes, Nee)											
Beroep											
Werkstitel											
Indien op ‘n plaas:											
het u in die wingerd /boord gewerk (Ja/Nee)											
Het u (spuit of meng) gifstowwe aangewend? (Ja/Nee)											
Hoeveel dae ‘n jaar het u gifstowwe aangewend?											
Het u trekker gery?											

(Ja/Nee)											
Hoeveel dae ‘n jaar het u trekker gery?											
Watter klere van berskerming het u gedra*											
*Dui aan met V = Voorskoot, S = Steuwels, H = Handskoene, M = Masker, GM = Gasmasker, O = Oorpak, SB = Skermbril											

Afdeling 6. ALKOHOLGEBRUIK

6.1 Drink jy alkohol of het u al voorheen alkohol gedrink? ____(Ja/Nee)

Indien Ja,

6.2 Het jy al gevoel dat jy minder alkohol moet gebruik? ____(Ja/Nee)

6.3 Het jy al kwaad geword as mense jou drinkgewoontes kritiseer? ____(Ja/Nee)

6.4 Het jy al ooit sleg of skuldig gevoel oor jy alkohol gebruik? ____(Ja/Nee)

6.5 Het jy al ooit vroeg in die oggend gedrink om beter te voel of om jou babelas beter te maak? ____ (Ja/Nee)

Afdeling 7. ROOK EN ANDER DWELM MIDDEL GEBRUIK

7.1 Het u al ooit al oor ‘n jaar tabak, sigarette of pyp gerook ? ____ (Ja/Nee)
(‘Ja’ beteken ten minste 20 pakke sigarette of 30 gramme van tabak in ‘n leeftyd of ten minste een sigaret ‘n dag vir een jaar)

Indien Ja,

7.1.1 Hoe oud was u toe u begin rook? ____ (jaar oud)

7.1.2 Rook u op die huidige oomblik? ___ (Ja/Nee)
(‘Ja’ beteken rook in die afgelope maand of meer)

7.1.3 Indien nee**,** hoe oud was u toe u ophou? _____ (jaar oud)
7.1.4 Hoeveel rook u of het u ongeveer gerook?

Aantal sigarette ‘n dag ____
Pyp tabak in gramme/week _____

7.1.5 Haal u of het u die rook ingehaal? ____ (Ja/Nee)

7.2 In die afgelope 12 maande, was u gereeld bloodgestel aan tabak rook van ander mense
wie sigarette en pyp rook? ____ (Ja/Nee)
('Gereeld' beteken op meeste dae en aande)

7.3 Neem u dwelmmiddels of het enige dwelmmiddels voorheen gebruik? ____ (Ja/Nee)

7.3.1 Indien Ja, dui asseblief aan vir hoeveel jare _____ (jare)

Afdeling 8. GEBRUIK VAN HUISHOUDELIKE GIFSTOWWE

8.1 Gebruik jy enige gifstowwe in jou tuin of in jou huis? ____ (Ja / Nee)
(bv. Target of Doom)

Indien JA – watter gifstowwe gebruik u?
_____

_____
_

8.2 Werk enige ander persoon in die huis met gifstowwe? ____ (Ja/Nee)

8.3 Word klere wat met gifstowwe besmet is, by die huis gewas? ____ (Ja/Nee)

8.4 Indien JA, word dit saam met ander wasgoed gewas? _____ (Ja/ Nee)

8.5 Eet jy vrugte of groente uit jou tuin? _____ (Ja/ Nee)

8.6 Gebruik jy leë plaagdoderhouers tuis vir huishoudelike doeleindes? ____ (Ja/Nee)

8.7 Indien JA, waarvoor gebruik jy dit? _____

Afdeling 9. MEDIESE, VOORPLANTING EN ASEMHALING GESKIEDINIS

9.6 Lei u aan:
Asma _____ (Ja/Nee)
Brongitis _____ (Ja/Nee)
TB _____ (Ja/Nee)
Ekseem _____ (Ja/Nee)
Hooikoors _____ (Ja/Nee)
Boer se longe _____ (Ja/Nee)
Ander siekte: _____ (Ja/Nee) **indien ja,** spesifiseer _____

_____

9.7 Wat was u geboorte gewig? _____

9.8 Op watter ouderdom het u puberteit bereik? _____
9.9 Was u al ooit vergif deur gifstowwe wat bevestig was deur ‘n dokter? ____(Ja, Nee)

Indien ja, hoeveel keer_____

9.10 Het u of voel u dikwels :
Duiselig ____ (Ja/Nee)
Mislik(naar) ____(Ja/Nee)
Hoofpyn ____ (Ja/Nee)
Prikkeling in u vel, neus of/en oog _____ (Ja/Nee)
Vel uitslag _____ (Ja/Nee)
Mislik (naar) en u wil opgooi ____ (Ja/Nee)
Verkoue of wonde wat oop is _____ (Ja/Nee)

Adeling 10 (Q16)

10.1 Voel u buitengewoon moeg ? (JA/NEE)

10.2 Het u hartkloppens al het u nie geoefen nie ? (JA/NEE)

10.3 Het u dikwels pynvolle prikkel sensasies in ‘n gedeelte van jou liggaam ? (JA/NEE)

10.4 Voel u dikwels geirriteerd sonder enige rede ? (JA/NEE)

10.5 Voel u dikwels teneergedruk sonder enige rede ? (JA/NEE)

10.6 Het u dikwels probleme met konsentrasie ? (JA/NEE)

10.7 Is u kort van gedagte ? (JA/NEE)

10.8 Sweet u dikwels sonder enige rede ? (JA/NEE)

10.9 Het u enige probleme om u knope vas en los te maak ? (JA/NEE)

10.10 Vind u dit oor die algemeen moeilik om koerante en boeke te verstaan ? (JA/NEE)

10.11 Het u familie al vir u gese dat u kort van gedagte is ? (JA/NEE)

10.12 Voel u soms ‘n swaar drukking op u bors ? (JA/NEE)

10.13 Moet u dikwels notas maak oor dinge wat u moet onthou ? (JA/NEE)

10.14 Moet u dikwels teruggaan om seker te maak dat u sekere dinge gedoen het bv. Of die deur gesluit is ? (JA/NEE)

10.15 Het u ‘n hoofpyn ten minste een keer per week ? (JA/NEE)

10.16a. Dink u dat dit minder is as ander persone van u ouderdom ?
(JA/NEE)

Afdeling 11. TYD VAN SWANGERSKAP

11. 1. Was u al ooit swanger? _____ (Ja/Nee)

11. 2. Indien ja, hoeveel keer? _____

11. 3. Lys hoeveel keer toe u swanger was, het u swangerskap op ge-eindig in:

Lewendige geboortes ____
Dood geboortes ____
Miskraam ____
Ectopic/Swangerskap in die eierstok ____
Ander ____

11.4 VIR LEWENDIGE GEBOORTES EN DOOD GEBORTES ALLENLIK(nie tweelings
nie) Voltooi die volgende tafel: (gee ’n antwoord vir elke baba)

	Swangerskap										
	1	2	3	4	5	6	7	8	9	10	11
Gewig van baba (kg)											
Gedurend die maand van u swangerskap, Het u of u man enige vorm van geboortebeperkings gebruik? (Ja/Nee)											
Metode van geboortebeperking*											
Het u geboortebeperkings al die tyd, amper al die tyd of net somtyds gebruik?											
Indien NIKS GEBOORTEBEPERKING OF NET SOMTYDS: Hoe lank het ‘n probeer om swanger te word											
*mondeling(die pil), gekronkel of lissie, Kondome, diafragma (kap), ritme of ontrekking, ander											

11.5 Is gesonheidsdienste toeganklik vir u om die volgende by te woon:

Swangerskap ____ (Ja/Nee)
Indien ja, watter dienste (hospitaal, kliniek) _____

Geboorte aan u kinders ____ (Ja/Nee)
Indien ja, watter dienste (hospitaal, kliniek) _____

Ginekologiesesorg ____ (Ja/Nee)
Indien ja, watter dienste (hospitaal, kliniek) _____

Seksuele oorsending siekte ____ (Ja/Nee)
Indien ja, watter dienste (hospitaal, kliniek) _____

Ander voorplantingsdienste ____ (Ja/Nee)
Indien ja, spesifiseer watter probleeme en watter dienste (hospitaal, kliniek)

_____

Adeling 12. ALLERGIESE GESONDHEIDSPROBLEEME

12.1 In die afgelope 12 maande, het u ‘n asemfluit of ‘n fluit van keel op u bors al ooit gehad al? _____ (Ja/Nee)

Indien ja, gaan voort met 12.2
Indien nee, gaan voort met 12.4

12.2 Was u kort van asem toe die geluid van die asemfluit teenwoordig was? ____ (Ja/Nee)

12.3 Het u die asemfluit/asemhyg gehad terwyl u nie griep of verkoue gehad het nie ____ (Ja/Nee)

12.4 Het u al ooit wakker kom word deur ‘n gevoel van u bors wat toe trek ? _____ (Ja/Nee)

12.5 In die afgelope 12 maande, het u al ooit ‘n aanval gehad deur kort van asem wees gedurend die dag terwyl u rustig gewees het? ____ (Ja/Nee)

12.6 In die afgelope 12 maande, het u al ooit wakker kom word deur ‘n aanval van hoes ? _____ (Yes/No)

12.7 Het u al ooit aan asma gelei? ____ (Ja/Nee)

Indien ja, gaan voort met 12.7.1
Indien nee, skip na vraag 12.8

12.7.1 Indien ja, was dit bevestig deur ‘n dokter?

12.7.2 How oud was u toe u ingelig was dat u aan asma lei? _____ (jare oud)

12.7.3 In die afgelope 12 maande, het u ‘n aanval van asma gehad? ____ (Ja/Nee)

12.7.4 Gebruik u enige medisyne, ingesluit met pompe/opsnuifers, nebulizers,

stroop of pille vir asma of asemhalingsprobleeme? ____ (Ja/Nee)

12.8 Wanneer u naby diere of in stowwerige gedeeltes is van die huis, kry u ooit ’n gevoel van toetrek in u bors? _____ (Ja/Nee)

12.9 As u op ‘n plaas werk, trek u bors toe of ‘n asemfluit wanneer u in die:
12.9.1 Wingerd/boord werk ____ (Ja/Nee)
12.9.2 Pakstoor werk ____ (Ja/Nee)
12.9.3 Ander ____ (Ja/Nee) Indien ja, spesifiseer asseblief _____

12.10 In die afgelope 12 maande, het u al ooit nasaal allergies probleeme saam met hooikoors of kraperige en waterige oe en neus gehad? ____ (Ja/Nee)

12.11 As u op ‘n plaas werk, kry u kraperige/waterige oe of neus wanneer u in die:
12.11.1 Wingerd/boord werk ____ (Ja/Nee)
12.11.2 Pakstoor werk ____ (Ja/Nee)
12.11.3 Ander ____ (Ja/Nee) Indien ja, spesifiseer asseblief _____

12.12 In die afgelope 12 maande, het u enige vel probleeme gehad? _____ (Yes/No)

12.13 As u op ‘n plaas werk, kry u rooi kraperige puisies wanneer u in die:
12.13.1 Wingerd/boord werk ____ (Ja/Nee)
12.13.2 Pakstoor werk ____ (Ja/Nee)
12.13.3 Ander ____ (Ja/Nee) Indien ja, spesifiseer asseblief _____

12.4 In die afgelope 12 maande, apart van u werk, was u blootgestel aan enige gifstowwe? ____ (Ja/Nee)

DANKIE DAT U AAN HIERDIE STUDIE DEELGENEEM HET

Appendix B

Exhaled Nitric Oxide Pre-test Data Collection Sheet

UCT OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG VINEYARD WORKERS IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA - 2009

EXHALED NITRIC OXIDE PRE-TEST DATA COLLECTION SHEET

Survey Number _____

A. IDENTIFICATION DATA

1.Surname _____

2. First name/s _____

3. Work number _____

4. Date of birth: Day_____Month_____Year____

5. Gender: Male (1)
Female (2)

8. Interviewer's initials _____

9. Date of interview:
Day____Month_____Year_____

10. Farm: _____

B.HEALTH PROBLEMS

Recent chest infections

1. Have you had the flu or sinusitis in the past 3 weeks?
Yes (1)
No (2)

2. Are you being treated for Tuberculosis (TB)?
Yes (1)
No (2)

2.1 If yes, for how long? _____months _____weeks

If YES, to next question, indicate to person that the tests will not be

done today. Schedule another appointment in three months time since the start of TB medication.

Nose and eye symptoms

4. Have you ever had any nose or eye problems due to allergies and/or hay fever?

Yes (1)
No (2)

C. SMOKING HISTORY

1. Do you smoke?

Yes (1)
No (2)

1.1 If yes, have you smoked (cigarettes/tobacco) in the last hour?

Yes (1)
No (2)

D. ALCOHOL CONSUMPTION

1. Do you drink alcohol?

Yes (1)
No (2)

1.1 If yes, when have you last consumed alcohol?

1-2 hours ago (1)
1 day ago (2)
1 week ago (3)

1.2 How much alcohol did you consume?

_____

E. MEDICATION USAGE (show booklet)

1. Are you taking any medicine/s from a doctor or clinic at the moment for asthma, and or hayfever?

Yes (1)
No (2)

1.1 If yes, what are you taking and when last did you take them?

Names No. of hours since last dose

_____ _____
_____ _____ _____
_____ _____ _____

F. PHYSICAL ACTIVITY

1. Do you exercise?

Yes	(1)
No	(2)

2. When was the last time you exercised?

1-2 hours ago	(1)
1 day ago	(2)
1 week ago	(3)

G. RECENT FOOD INTAKE

1. Did you have anything to eat or drink in the last hour?

Yes	(1)
No	(2)

If YES to above question, reschedule test for at least 1 hour later the same day or another date.

Appendix C

Exhaled Nitric Oxide Data Collection Sheet

UCT OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG WOMEN ON FARMS PROJECT IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA 2009

EXHALED NITRIC OXIDE DATA COLLECTION SHEET

Date: _____

Time _____

Card 1

Ambient NO concentration (ppb)	_____	☐☐☐	1-3
Ambient temperature (degrees celcius)	_____	☐☐	4-5
Survey Number	_____	☐☐☐	6-8
1. Subject's blood pressure	systolic _____	☐☐☐	9-11
	diastolic _____	☐☐☐	12-14
2. Subject's age (in years)	_____	☐☐	15-16
3.1 Subject's height (in centimetres)	_____	☐☐☐	17-19
3.2 Subject's weight (in kilograms)	_____	☐☐☐	20-22
4. Gender:	Male (1)	☐	23
	Female (2)		
5. Effort number (start)	_____	☐☐☐	24-26
6.1 FENo measurement (ppb) 1st effort	_____	☐☐☐	27-29
6.2 FENo measurement (ppb) 2nd effort	_____	☐☐☐	30-32
6.3 FENo measurement (ppb) 3rd effort	_____	☐☐☐	33-35

Appendix D

Letter of Approval from Human Research Ethics Committee

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

13 October 2009

REC REF: 393/2009

Dr MA Dalvie
Public Health

Dear Dr Dalvie

PROJECT TITLE: HEALTH EFFECTS DUE TO PESTICIDE EXPOSURE AMONGST RURAL WOMEN IN THE WESTERN CAPE.

Thank you for addressing the queries raised by the Research Ethics Committee.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study including the following documentation:

Approval is granted for one year till the 20th October 2010.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

S Thomas

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix E

Human Research Ethics Committee Annual Progress Report

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Annual Progress Report

Date	25/2/2013
HREC REF Number	393/2009
Protocol number (if applicable) & Protocol title	Title of full study: Health effects due to pesticide exposure amongst rural women residents in the Western Cape Title of sub-study: Asthma and allergy due to pesticide exposure amongst rural women residents in the Western Cape/ Relationship between pesticide residues and asthma outcomes among women farm workers
Principal Investigator	M A Dalvie
Department / Office Internal Mail Address	School of Public Health and Family Medicine

List of documentation

N/A

HUMAN RESEARCH ETHICS COMMITTEE
26 FEB 2013
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

HREC office use only (FWA00001637; IRB00001938)			
☑ Approved	This serves as notification of annual approval, including all documentation described above.		
☐ Not approved	See attached comments.		
Type of review	☑ Expedited	☐ Full committee	
Expiry date	15 MARCH 2014		
Signature		Date	

Appendix F1

English Consent Form

Consent to participate in a survey investigating health effects due to pesticide exposures on women from the rural Western Cape

1. **Title of research project**

Health effects due to pesticide exposure amongst rural women residents in the Western Cape

2. Names of the researchers

Mohamed Aqiel Dalvie (BSc, Honours, MSc, PhD)
Algernon Africa (BTech)
Vicky Major (MSc)
Lungiswa Giwane
Jean May

3. Purpose of research

This study is being conducted by The University of Cape Town to investigate the health effects of pesticides on women in the Western Cape. We would like to conduct measurements on you. The study will be of benefit to women living in farming areas and who are exposed to pesticides in the environment.

4. Description of the research project

Your son will be required to produce a urine and 2 blood samples and undergo a respiratory test and you will complete a questionnaire.

a) **Questionnaire:** A member of our study team will interview you in privacy to complete the questionnaire. You will be asked questions about general personal information, your general medical health, and lifetime environmental exposure to pesticides.

b) **Urine sample:** Your will produce a urine sample (in privacy) in a plastic container and give it to the nurse. The sample will be analysed for pesticides.

c) **Blood sample:** A nurse will draw 14 ml blood from a vein on your arm. The blood will be analysed for to test your allergy status and for pesticide residues.

d) **Respiratory test:** A nurse will perform a respiratory test.

5. Risks and discomforts of the research

a) From the blood tests. A single needle stick will be felt when the blood is taken. Sometimes a small bruise may occur from the needle stick, but this is minor and will heal quickly. The total amount of blood taken is quite small and the body will quickly replace it. Blood samples will be used only to measure allergy and will be destroyed at the end of the study.

b) From the questionnaire.

There are minimal risks associated with completing the questionnaire. The only risk is a loss of confidentiality about personal information but the data will be seen only by study personnel. All reports will present aggregate data in which individuals will not be identifiable.

6. Expected benefits to you and others

Your health will be assessed for free.

Refreshments will be provided as compensation for time in participating in the study.

This study on the health effects of pesticides will benefit women living in farming areas and who are exposed to pesticides in the environment. Steps can be taken to reduce or prevent exposure to the pesticides or the pesticide can be banned. The blood and urine results can be used to develop ways in which the amount of pesticides in your body can be monitored.

7. Costs to you resulting form participation in the study

The study is offered at no cost to you.

8. Confidentiality of information collected

Study participants will not be personally identified in any reports on this study. The records will be kept confidential to the extent provided by law. The records, including any identification information, will be destroyed after the results have been fully analysed.

9. Documentation of the consent

One copy of this document will be kept together with our research records on this study. A second copy will be given to you to keep.

10. Contact person.

You may contact the following person for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

Name of person: MA Dalvie (The principal investigator) telephone 021 4066610

Name of person: Lamees Emjedi (Ethics administrator) telephone 021 4066492

11.Voluntary nature of participation

Your participation in this project is voluntary. Subsequent to your consent, you may refuse to participate in or withdraw from the study at any time without penalty or loss of benefits to which you may otherwise be entitled.

12. Consent of the participant

I have read the information given above. I understand the meaning of this information. I hereby consent to participate in the study.

_____
_____
Printed name of participant **signature**

_____
_____
Date

_____ _____
_____
Interviewers (print) **signature**
Date

_____ _____
_____

Witness (print) **signature**
Date

Date:_____

Study Number _____

Appendix F2

Afrikaans Consent Form

Toestemming om deel te neem in ‘n opname van ondersoek oor Gesondheids gevolge weens blootstelling aan gifstowwe op landlike vrouens in die Weskaap

2. **Titel van navorsingsprojek**

Gesondheids gevolge weens blootstelling aan gifstowwe op landlike vrouens in die Weskaap

2. **Name van navorsers**
Mohamed Aqiel Dalvie (BSc, Honours, MSc, PhD)
Mohamed Jeebhay (MbChB, MMED, PhD)
Leslie London (MbChB, MMED, PhD)

3. Doel van navorsing

Die Universiteit van Kaapstad is besig met ‘n studie oor die gesondheids gevolge weens
blootstelling aan gifstowwe op landlike vrouens in die Weskaap. Ons wil graag vir u toets.
Daar is voordele van hierdie studie vir vrouens wie op plase woon en wie bloodgestel aan gifstowwe is in die algemene omgewing.

4. Beskrywing van die navorsingsprojek

Daar word van u verwag om ’n urien en 2 bloedmonsters te voorsien en u sal ’n asemhalingstoets ondergaan en u moet ’n vraelys voltooi.

a) **Vraelys: :** ’n Lid van ons studie-span sal ’n ondervhoud in privaat met u voer om die vraelys te voltooi. Daar sal vrae gestel word i.v.m. u algemene persoonlike inligting, u algemene mediese gesondheid en lewenslange omgewings- blootstelling aan gifstowwe.

b) **Urien monster:** U moet ’n urien-monster (in privaat) in ’n plastiese houer doen en dit aan die verpleegster gee.

c) **Bloed monster:** ’n Verpleegster sal 10ml bloed uit een van u are in u arm trek. Die bloed sal getoets word vir u allergiese status

d) **Asemhalingstoets:** U sal ‘n asemhalingstoets ondergaan met ‘n verpleegster.

11. Gevare(risikos) en ongemaklikhede van die navorsing

a) **Van die bloed toets:** ’n Enkel steek(prik) van die naald sal gevoel word wanneer die bloed getrek word. Somtyds sal daar ’n klein kneusplek op die arm wees a.g.v. die naald, maar dit is nie so erg nie en dit sal baie gou gesond raak. Die hoeveelheid bloed wat getrek word is baie min en die liggaam sal dit gou weer vervang. Bloedmonsters sal slegs gebruik word om allergiese probleeme te meet en sal aan die einde van die studie vernietig word.

b)**Van hierdie vraelys.**

Daar is minimale risikios geassosieer met die voltooing van die vraelys. Die enige risiko is die verlies van vertroulikheid of werkoefening informasie maar die data sal alleenlik by die studie personeelgesien word. Alle uitslae bevat gesamentlike data waar individuale nie geidentifiseer sal word nie

12. Verwagte voordele aan u en andere

U gesondheid sal gratis bepaal word en ons sal u verwys na ’n publieke gesondheids fasiliteit indien ons enige gesondheidsprobleeme geidentifiseer het.

Verversings sal voorgesit word as vergoeding vir u tyd en deelname aan die navorsing (toetsing)

Die studie oor die gesondheids gevolge weens blootstelling aan gifstowwe sal baat vind by vrouens wie woonagtig is op plase waar daar blootstelling van gifstowwe by die werk en in die omgewing is.Stappe kan geneem word om die bloot-stelling van gifstowwe te verminder of die gifstowwe kan verban word. Diebloed en die urine uitslae kan gebruik word om maniere te ontwikkel waarin die aantal gifstowwe in die liggaam gemonitor kan word.

13. Koste aan u as gevolg van u deelneming in die studie

Die studie word aangebied teen geen koste aan u.

14. Vertroulikheid van inligting wat gekollekteer word.

U naam sal nie op enige van die studie verslae verskyn word nie. Die verslae sal vertroulik gehou word tot die uitgestrektheid wat deur die wet voorsien word. Die

uitslae, uitsluitende enige identiteits informasie sal vernietig word nadat dit heeltemal geanaliseer was.

15. Dokumentasie van toestemming

Een afskrif van die document sal saam met ons navorsingsrekords oor die studie gehou word.

Die tweede afskrif sal aan u gegee word om te hou.

16. Kontak persoon.

U mag een van die volgende persone kontak vir antwoorde tot verder vrae in verband met die navorsing, u regte of enige besering wat u voel met die studie verband hou.

Naam van persoon: MA Dalvie (Die hoof navorser) telefoon 021 4066610

Naam van persoon: Lamees Emjedi (Etiese administrateur) telefoon 021 4066492

17. **Vrywilligheid geaardheid van deelname**

U deelname aan die projek is vrywillig. U kan teen enige tyd weier om deel te neem of onttrek van

die studie sonder om beboet te word of aan verlies te lui van voordele wat u toekom.

13. Toestemming van deelnemer

I have read the information given above. I understand the meaning of this information. I hereby consent to participate in the study.

_____ _____ _____

Naam in drukskrif van ouer/ deelnemer (volwassene) **handtekening**

_____

Datum

_____ _____ _____

Verslagewer (drukskrif) **handtekening** **Datum**

_____ _____ _____

getuie (drukskrif) **handtekening** **Datum**

Datum:_____

Studie Nommer _____

Naam _____

Kontak nommer _____

Appendix G

International Archives of Allergy and Immunology

Guidelines for authors

International Archives of Allergy and Immunology

Guidelines

Guidelines for Authors
www.karger.com/iaa_guidelines

Introduction

'International Archives of Allergy and Immunology' appears monthly and provides a forum for publication of work from all aspects of modern allergology and immunology. Papers considered of special interest will be treated as 'hot topics' and every attempt will be made to publish these especially fast.

The Editor-in-Chief, the Managing Editor and a team of internationally established Associate Editors competently covering all fields of allergology and immunology are responsible for the scientific quality of this international core journal.

'International Archives of Allergy and Immunology' publishes original work in the fields of allergy, immunopathology and transplantation, cellular immunology, immunogenetics, immunopharmacology and immunoendocrinology, mucosal immunity, immunology of infectious diseases and immunology of connective tissue diseases. In addition, reviews, minireviews, commentaries and opinions on controversial subjects are published regularly.

A new system of manuscript submission, reviewing and processing guarantees competent and fair reviewing as well as fast and high quality publication.

Submission

Only papers written in English are considered and should be submitted online:

Online Manuscript Submission

If you have any problems with submission, please contact:

S. Karger AG
Editorial Office 'International Archives of Allergy and Immunology'
P.O. Box
CH–4009 Basel (Switzerland)
Tel. +41 61 306 1360
Fax +41 61 306 1434
E-mail iaa@karger.com

Types of Papers

Original research papers: Submitted manuscripts should be fully documented reports of original research. They must describe significant and original observations to be critically evaluated and, if necessary, repeated.

Short communications: These manuscripts should not contain more than 2 printed pages (i.e. 5 manuscript pages), including an abstract, essential references and not more than 3 tables or figures. Such communications should represent complete, original studies and should be arranged in the same way as full length manuscripts.

Novel insights from clinical practice (formerly case reports): the publication space available for case reports is very limited. The journal only considers case reports with significant new insights or with an extremely unusual and memorable course. Highlighted boxes containing one or two bullet points on '**Established facts**' (what is already known) and '**Novel insights**' (what new information has been gained) are required and should be placed on the first page of the report. These should be selected so as to reinforce the novelty of the clinical observation.

Example

Established Facts

- Already known fact 1 ...
- Already known fact 2 ...
- ...

Novel Insights

- New information 1 ...
- New information 2 ...
- ...

The manuscript should be presented with an abstract (unstructured, max. 200 words), followed by introduction, case report and discussion. Maximum 3 figures.

Review articles, minireviews and commentaries: Authors, who wish to contribute a manuscript to one of these categories should contact the Editor-in-Chief. Minireviews should be focused, brief reports on topics of current interest. Commentaries should offer a more personalized perspective on a topic that will be of interest to the general readership. All contributions to these categories will be subject to Editorial review.

Letters to the Editor concerning work published in the journal may be submitted. They should not exceed 2 manuscript pages including one table or figure.

Conditions

All manuscripts are subject to editorial review. Manuscripts are received with the explicit understanding that they are not under simultaneous consideration by any other publication.

A cover letter with the name, address, and telephone and telefax numbers of the corresponding author must accompany each manuscript. This letter must include a statement that affirms that all authors concur with the submission.

Submission of an article for publication implies the transfer of the copyright from the author to the publisher upon acceptance. Accepted papers become the permanent property of 'International Archives of Allergy and Immunology' and may not be reproduced by any means, in whole or in part, without the written consent of the publisher.

It is the author's responsibility to obtain permission to reproduce illustrations, tables, etc. from other publications.

One of the criteria considered in reviewing manuscripts is the proper treatment of animals. In particular, the use of painful or otherwise noxious stimuli must be carefully and thoroughly justified. Papers that do not meet these criteria will not be accepted for publication.

Plagiarism Policy

Whether intentional or not, plagiarism is a serious violation. We define plagiarism as a case in which a paper reproduces another work with at least 25% similarity and without citation.
If evidence of plagiarism is found before/after acceptance or after publication of the paper, the author will be offered a chance for rebuttal. If the arguments are not found to be satisfactory, the manuscript will be retracted and the author sanctioned from publishing papers for a period to be determined by the responsible Editor(s).

Arrangement

Manuscripts should be written in English and be as concise as possible.

Be sure to display line numbers (1, 2, 3, and so forth) in the left margin of the manuscript (line numbering can be added from the Page Setup or Format menu of word processing programs). The line numbering should be continuous throughout the entire manuscript, from the title page through to the final page (i.e., do not begin numbering from 1 again at the top of each page).

Title page: The first page of each paper should indicate a concise title, the authors' full names, affiliations and e-mail addresses, the institute where the work was conducted, and a short title for use as running head.

Full address: The exact postal address of the corresponding author complete with postal code must be given at the bottom of the title page. Please also supply phone and fax numbers, as well as e-mail address.

Key words: Please supply 3–10 key words in English that reflect the content of the paper.

Abstract: Provide on a separate page an abstract of not more than 250 words. This abstract should consist of four paragraphs, labeled Background, Methods, Results, and Conclusions. They should briefly describe, respectively, the problem being addressed in the study, how the study was performed, the salient results, and what the authors conclude from the results. *The text* should be arranged in the following sections: Introduction, Material and Methods, Results, Discussion, Acknowledgement, References.

Footnotes: Avoid footnotes. When essential, they are numbered consecutively and typed at the foot of the appropriate page.

Tables and illustrations: Tables and illustrations (both numbered in Arabic numerals) should be prepared on separate pages. Tables require a heading and figures a legend, also prepared on a separate page. For the reproduction of illustrations, only good drawings and original photographs can be accepted; negatives or photocopies cannot be used. Due to technical reasons, figures with a screen background should not be submitted. When possible, group several illustrations on one block for reproduction (max. size 180 x 223 mm) or provide crop marks. Each illustration must be labeled with its number and the author's name. Electronically submitted b/w half-

tone and color illustrations must have a final resolution of 300 dpi after scaling (final size), line drawings one of 800–1,200 dpi. Figure files must not be embedded in a document file but submitted separately (for detailed instructions, see Online Submission Instructions).

Abbreviations: Avoid overuse of abbreviations. Introduce an abbreviation only when the same term occurs three or more times.

Color Illustrations

Online edition: Color illustrations are reproduced free of charge. In the print version, the illustrations are reproduced in black and white. Please avoid referring to the colors in the text and figure legends.

Print edition: Up to 6 color illustrations per page can be integrated within the text at CHF 800.00 per page.

References

In the text identify references by Arabic numerals [in square brackets]. Material submitted for publication but not yet accepted should be noted as 'unpublished data' and not be included in the reference list. The list of references should include only those publications which are cited in the text. Do not alphabetize; number references in the order in which they are first mentioned in the text. The surnames of the authors followed by initials should be given. There should be no punctuation other than a comma to separate the authors. Preferably, please cite all authors. Abbreviate journal names according to the Index Medicus system. Also see International Committee of Medical Journal Editors: Uniform requirements for manuscripts submitted to biomedical journals (www.icmje.org).

Examples

(a) Papers published in periodicals: Sun J, Koto H, Chung KF: Interaction of ozone and allergen challenges on bronchial responsiveness and inflammation in sensitised guinea pigs. Int Arch Allergy Immunol 1997;112:191–195.

(b) Papers published only with DOI numbers: Theoharides TC, Boucher W, Spear K: Serum interleukin-6 reflects disease severity and osteoporosis in mastocytosis patients. Int Arch Allergy Immunol DOI: 10.1159/000063858.
(c) Monographs: Matthews DE, Farewell VT: Using and Understanding Medical Statistics, ed 3, revised. Basel, Karger, 1996.

(d) Edited books: Parren PWHI, Burton DR: Antibodies against

HIV-1 from phage display libraries: Mapping of an immune response and progress towards antiviral immunotherapy; in Capra JD (ed): Antibody Engineering. Chem Immunol. Basel, Karger, 1997, vol 65, pp 18–56.

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Appendix H
Supplementary Materials

Supplementary Table 1: Urinary pesticide metabolites levels among rural women in the Western Cape

Pesticide metabolites	Farm dwellers	Town dwellers	Overall
Median (interquartile range)			
Corrected for creatinine (µg/g creatinine)			
I. Organophosphate metabolites			
Dialkyl phosphates (n = 178)			
DAP	141.42(37.4-249.83)	132(45.64-204.45)	133.59(41.86-229.09)
DMP	32.91(13.50-55.75)	26.19(14.33-52.36)	29.63(14.06-53.22)
DMTP	13.41(3.05-62.45)	36.44(6.11-71.85)	21.87(4.03-65.85)
DMDTP	5.70(0.83-51.51)	9.57(0.87-66.22)	6.87(0.85-61.77)
DEP	5.01(1.37-12.90)	4.13(0.59-9.47)	4.27(1.08-10.04)
DETP	3.70(1.15-26.98)	3.94(1.35-26.18)	3.87(1.20-26.98)
DEDTP	1.99(0.55-5.10)	1.70(0.60-8.02)	1.89(0.58-6.44)
Chlorpyrifos metabolite (n = 186)			
TCPY	6.15(3.50-10.64)*	4.14(2.70-7.57)	5.16(2.84-9.24)
II. Pyrethroid metabolites (n = 183)			
Pyrethroids	6.60(3.61-9.96)	5.26(2.74-8.42)	6.01(3.24-9.67)
cis-DCCA	0.71(0.27-1.28)	0.56(0.23-1.13)	0.62(0.26-1.24)
trans-DCCA	0.85(0.47-1.29)**	0.59(0.28-1.02)	0.70(0.37-1.22)
DBCA	0.31(0.05-0.63)	0.30(0.04-0.60)	0.30(0.04-0.62)
4F3PBA	0.73(0.31-1.32)	0.70(0.33-1.30)	0.73(0.32-1.32)
3PBA	3.61(2.11-6.25)	3.34(2.27-5.92)	3.40(2.18-6.00)

*p < 0.05; **p < 0.01; TCPY: 3,5,6- trichloropyridinol; DAP: sum of the 6 dialkyl phosphate metabolites; DMP: dimethyl phosphate
DMTP: dimethyl thiophosphate; DMDTP: dimethyl dithiophosphate; DEP: diethyl phosphate; DETP: diethyl thiophosphate
DEDTP: diethyl dithiophosphate; Pyrethroids: sum of the 5 pyrethroid metabolites;
cis-DCCA: cis- 2,2-dichlorovinyl-2,2- dimethylcyclopropane-1-carboxylic acid
trans-DCCA: trans- 2,2-dichlorovinyl-2,2- dimethylcyclopropane-1-carboxylic acid
DBCA: cis-2,2-dibromovinyl-2,2-dimethylcyclopropane-1-carboxylic acid;
4F3PBA: 4-fluoro-3-phenoxybenzoic acid; 3PBA: 3- phenoxybenzoic acid
Values below LOD were substituted by LOD divided by square root of 2